Phase II Study of Combined Tretinoin and Arsenic Trioxide For Patients with Newly Diagnosed Acute Promyelocytic Leukemia Followed by Risk-Adapted Postremission Therapy

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

1.1 Study Title: Phase II study of combined tretinoin and arsenic trioxide for patients with newly diagnosed acute promyelocytic leukemia followed by risk adapted postremission therapy.

1.2 Objectives: (1) To determine the rate of molecular remission after induction with combined tretinoin and ATO (along with idarubicin in patients with high-risk disease or who develop leukocytosis) in acute promyelocytic leukemia (APL); (2) to determine the rate of clinical complete remission (CR) and the time to remission after induction with tretinoin and ATO (with idarubicin in patients with high-risk disease or who develop leukocytosis); (3) to determine the proportion of patients in molecular remission after each course of postremission therapy; (4) to determine the disease-free and event-free survival of patients treated with this program; (5) to determine the toxicity of this treatment program, including the early death rate (within 30 days), the incidence of APL differentiation syndrome, the number and length of hospitalizations, the incidence of secondary myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML), and the effects of treatment on left ventricular ejection fraction (LVEF); (6) to characterize the differentiation of APL cells during treatment with combined tretinoin and ATO using serial immunophenotyping studies of peripheral blood, and (7) explore the *in vivo* induction of telomerase-dependent cell death by ATRA and ATO.

1.3 Patient Population: Patients with untreated APL $\geq$18 years of age will be eligible.

1.4 Study Design: Risk groups will be assigned according to established criteria at the time of diagnosis: (1) low-risk patients have a presenting WBC ≤10,000/μL and platelet count >40,000/μL; (2) intermediate-risk patients have a presenting WBC ≤10,000/μL and platelet count ≤40,000/μL, and (3) high-risk patients have a presenting WBC >10,000/μL. Induction for all patients will consist of tretinoin 45 mg/m^2 po daily (rounded to the nearest 10-mg increment) in two divided doses (25 mg/m^2 in patients <20 years of age due to a higher incidence of pseudotumor cerebri) for 35 days and ATO 0.15 mg/kg IV daily for 35 doses, given 5-7 days per week. The drugs will then be discontinued, and the patient will be followed until a clinical complete remission is achieved. Idarubicin 12 mg/m^2 IV for 4 doses will be added during induction for patients with high-risk disease or who develop leukocytosis during therapy, because of the increased risk of APL differentiation syndrome and relapse. Dexamethasone 10 mg twice daily with be given on days 1-14 of induction as prophylaxis for the APL differentiation syndrome. All patients will then receive four courses of consolidation with tretinoin for 15 days and ATO for 25 doses. During consolidation, high-risk patients or patients who have received Idarubicin during induction will receive central nervous system prophylaxis with 6 doses of 70mg intrathecal cytarabine, at the discretion of the site PI. Patients who have not achieved a molecular remission after the completion of Consolidation 4 will be removed from study. Following consolidation, high-risk patients will receive 8 cycles of maintenance therapy consisting of tretinoin for 15 days and ATO for 10 doses every 3 months. Low- and intermediate-risk patients will not receive maintenance therapy. The differentiating effects of therapy will be characterized by serial flow cytometric analyses of peripheral blood. Effects of combined ATRA and ATO on telomerase activity, telomere length, and *TERT* expression will be assessed by analyses of serial blood and bone marrow samples. Disease status will be monitored with serial analyses of blood samples using RT-PCR for PML-RARα mRNA. Patients will be followed until relapse, death, loss to follow-up, or removal from study.

1.5 Time to Completion: Up to 39 patients will be entered on this study. With an expected accrual of 10-15 patients per year, this study will take approximately 3-4 years to complete.

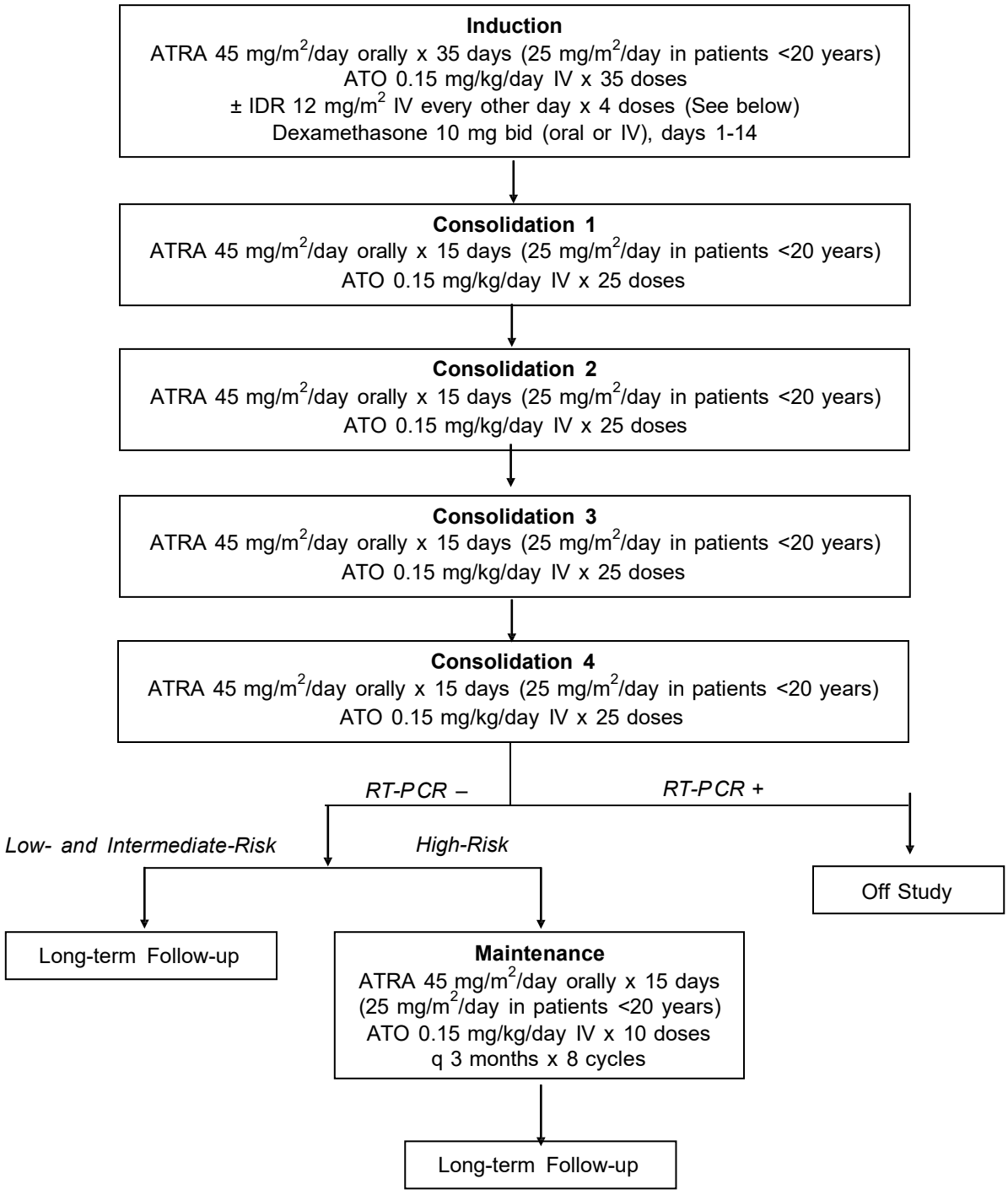

Fig. 1. Treatment Schema. See Section 1.4 for details. Risk assessment will be performed at the time of diagnosis. Idarubicin will be added during induction on day 2 if the presenting WBC is > 10,000/μL or if WBC is >5,000/μL on day 5, >10,000/ μL on day 10, or >15,000/μL on day 15. Low- and intermediate-risk patients with a molecular remission after Consolidation 4 will go on to long-term follow-up, where as high-risk patients in molecular remission will receive maintenance therapy. Patients failing to achieve a molecular remission after Consolidation 4 will be removed from study. Patients with high-risk disease or patients that receive Idarubicin during Induction will also receive a

total of 6 doses of cytarabine 70 mg intrathecally every 2 to 4 weeks during consolidation, at the discretion of the site PI. Abbreviations: ATRA, all-*trans* retinoic acid; ATO, arsenic trioxide; IDR, idarubicin;

2.1 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objective:

- To determine the rate of molecular remission after induction with combined tretinoin and ATO (along with idarubicin in patients with high-risk disease or who develop leukocytosis) in APL.

Secondary Objectives:

- To determine the rate of clinical complete remission (CR) and the time to remission after induction with tretinoin and ATO (with idarubicin in patients with high-risk disease or who develop leukocytosis).
- To determine the proportion of patients in molecular remission after each course of postremission therapy.
- To determine the disease-free and event-free survival of patients treated with this program.
- To determine the toxicity of this treatment program, including the early death rate (within 30 days), the incidence of APL differentiation syndrome, the number and length of hospitalizations, the incidence of secondary myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML), and the effects of treatment on left ventricular ejection fraction (LVEF).
- To characterize the differentiation of APL cells during treatment with combined tretinoin and ATO using serial immunophenotyping studies of peripheral blood.
- Explore the *in vivo* induction of telomerase-dependent cell death by ATRA and ATO.

3.0 BACKGROUND AND RATIONALE

3.1 Disease Background. APL is a subtype of AML with a distinct biology and clinical presentation marked by cytopenias and severe coagulopathy. Although it accounts for only 10% of all cases of AML, it has become a model for therapies targeted to molecular abnormalities that produce a neoplastic phenotype. The vast majority of APL is characterized by a specific translocation that fuses the promyelocytic leukemia gene (*PML*) located on chromosome 15 with a gene encoding a retinoic acid receptor (*RAR*α) on chromosome 17 [1-4]. PML, which is predominantly localized within multi-protein nuclear body structures (PML bodies), normally exhibits growth suppressor and pro-apoptotic activity. RARα at physiologic levels of its ligand, retinoic acid, acts as a transcriptional activator at retinoid response elements and is implicated in myeloid differentiation [5, 6]. The presence of the PML-RARα fusion protein disrupts these functions, leading to the development of leukemia.

3.2 Induction with ATRA. Early studies showed that, as a single agent, ATRA produces complete remissions in up to 90% of patients by inducing terminal differentiation of APL cells [7-9]. Furthermore, the use of ATRA followed by consolidation chemotherapy

significantly reduces relapse rates and improves survival [8, 10]. In studies conducted at Memorial Sloan Kettering Cancer Center (MSKCC), ATRA induction followed by three courses of idarubicin and cytarabine produced five-year disease-free and overall survival rates of 65% and 68%, respectively [11]. In the North American Intergroup study I0129, 346 patients with APL were randomized to receive either ATRA or daunorubicin and cytarabine for remission induction followed by two courses of consolidation chemotherapy with or without ATRA maintenance. While there was no difference in the remission rate between the ATRA and chemotherapy groups (72% vs. 69%, respectively), the three-year DFS was significantly improved in the ATRA-treated patients compared with those treated with standard chemotherapy (72% vs. 32%; $p < 0.001$) [12].

3.3 Induction with Concomitant ATRA and Chemotherapy. Fenaux *et al.* compared the use of concomitant ATRA and chemotherapy with ATRA alone for induction. Patients under age 65 years with presenting leukocyte counts below 5,000/μL were randomized to receive either ATRA alone (n = 109) or with daunorubicin and cytarabine (n = 99). Chemotherapy was added in patients receiving ATRA alone if leukocytosis developed, and concomitant ATRA-chemotherapy was given to patients presenting with an elevated white blood count (WBC), since these patients were recognized to be at greater risk for the development of APL differentiation syndrome. Patients then went on to receive two courses of consolidation therapy, followed by a second randomization to receive several different maintenance regimens or observation. Relapse at two years was estimated at 6% in the ATRA-chemotherapy group compared with 16% in the group receiving ATRA alone ($p = 0.04$). The two-year event-free survival was 84% in the ATRA-chemotherapy group and 77% in the ATRA alone group ($p = 0.1$) [13]. Exploiting the sensitivity of APL to anthracyclines when used as single agents for remission induction [14], Mandelli *et al.* similarly reported one- and two-year DFS rates of 83% and 79% for patients treated with ATRA and idarubicin followed by three cycles of consolidation therapy (the "AIDA" regimen) [15]. Moreover, combination ATRA and idarubicin produced molecular remissions in 60.5% of patients, whereas ATRA alone produces molecular remissions in only 5-15% of patients [16].

3.4 Management and Prevention of APL Differentiation Syndrome. Some APL patients treated with ATRA or ATO have experienced a syndrome characterized by fever, dyspnea, weight gain, leukocytosis, pulmonary infiltrates, and pleural or pericardial effusions. This syndrome can be fatal. Corticosteroids given at the first suspicion of the APL differentiation syndrome appear to mitigate signs and symptoms and reduce mortality. If patients develop unexplained fever, dyspnea, weight gain, abnormal chest auscultatory findings, or chest radiographic abnormalities, treatment with dexamethasone 10 mg intravenously q 12 hours should be initiated immediately. Therapy should be continued for at least 3 days or longer until signs and symptoms have resolved [17, 18]. In uncontrolled trials, a very low mortality and morbidity rate was reported as a result of differentiation syndrome after ATRA treatment when corticosteroids were administered prophylactically in patients presenting with WBC counts >5,000/μL [19, 20].

3.5 Consolidation with Anthracyclines Alone. The administration of cytarabine during consolidation therapy is not required for long-term remissions in APL. Estey *et al.* reported a one-year disease-free survival rate of 87% in patients initially treated with ATRA and idarubicin followed by two courses of idarubicin consolidation and maintenance therapy [21]. Similarly, Sanz *et al.* found that patients treated with a regimen identical to AIDA, except for the omission of cytarabine and etoposide during consolidation, had an overall estimated three-year relapse-free survival of 90% [22]. In this study, three risk groups were identified: (1) low-risk patients have a presenting WBC ≤10,000/μL and platelet count >40,000/μL; (2) intermediate-risk patients have a presenting WBC

≤10,000/μL and platelet count ≤40,000/μL, and (3) high-risk patients have a presenting WBC >10,000/μL. Differences in relapse-free survival among these three groups were highly significant (*P* <0.0001). In a subsequent trial, the addition of ATRA to idarubicin or mitoxantrone during consolidation significantly reduced the relapse rate (20.1% vs. 8.7%; *p* = 0.004) and improved disease-free and overall survival compared to consolidation chemotherapy without ATRA in patients with intermediate- or high-risk disease [20].

3.6 Maintenance Therapy. The role of maintenance therapy in APL remains controversial. In the North American Intergroup trial I0129, of the 94 patients assigned to maintenance with ATRA, 29 relapsed, whereas 60 of the 105 patients assigned to observation relapsed. The three-year DFS was 65% for patients receiving ATRA maintenance compared to 40% for those randomized to observation, regardless of induction regimen (p < 0.001) [12]. Fenaux et al. randomized patients to maintenance with intermittent ATRA (15 days every 3 months for two years), continuous low-dose 6-mercaptopurine and methotrexate, both ATRA and chemotherapy, or observation. The two-year relapse rate was 11% in patients receiving maintenance chemotherapy compared to 27% in patients randomized to no chemotherapy (*P* = 0.0002) and 13% in patients receiving intermittent ATRA compared to 25% in patients not receiving ATRA (*P* = 0.02). Overall survival was improved in patients who received maintenance chemotherapy (*P* = 0.01), and there was a trend towards improved survival in patients treated with maintenance ATRA (*P* = 0.22) [13]. In contrast, two more recent reports included a Japanese [23] and Italian study [24] showed no benefit to maintenance therapy. These discrepancies are likely attributable to the intensity of prior induction and consolidation.

3.7 RT-PCR Testing for PML-RARα. Molecular detection of PML-RARα fusion mRNA by RT-PCR amplification has become an important tool in the management of APL. This assay is useful in the diagnosis of APL, in predicting response to ATRA, and in the detection of minimal residual disease after remission induction [25-28]. Additional studies also indicate that RT-PCR monitoring can predict relapse and identify patients who require further antileukemic therapy [29-31]. In studies conducted at Memorial Sloan Kettering Cancer Center (MSKCC), the bone marrow of 82 patients who received ATRA induction followed by postremission chemotherapy, RA, and other biologic agents was serially evaluated using RT-PCR. Forty of 47 patients (85%) with newly diagnosed APL who were induced using ATRA alone had residual disease detectable by RT-PCR before additional therapy. After three cycles of consolidation therapy, residual disease was found in only four of 40 evaluable patients (10%). Among newly diagnosed patients who had two or more negative RT-PCR assays, only three of 41 (7%) relapsed, whereas all four patients (100%) who had two or more positive results relapsed. These data indicate that two or more negative RT-PCR assays on bone marrow, performed at least one month apart after completing therapy, are strongly associated with long-term remissions. Conversely, a confirmed positive test is highly predictive of relapse [16].

Similar results were reported by Diverio *et al.*, in which bone marrow samples of 163 patients with APL in first remission were prospectively analyzed every three months using RT-PCR. Of the 21 patients who converted to RT-PCR-positivity after the completion of therapy, 20 relapsed clinically at a median of 3 months (range, 1-14 months) after the first positive result. The remaining patient subsequently tested negative and continued in clinical remission. Among 142 patients who tested negative in two or more assays after consolidation therapy, only eight (6%) relapsed [32]. Quantitative real-time RT-PCR was also used retrospectively to assess PML-RARα mRNA before treatment and at various post-treatment time points in APL patients. Results were reported as a normalized quotient (NQ), obtained by dividing the copy number of PML-RARα by that of the reference gene GAPDH. NQs greater than 10^{-5} were found to be associated with a 17-fold

increase in the relapse risk [33]. Based on these data, the goal of therapy in APL is molecular remission.

3.8 ATO for Relapsed APL. The mechanism of action of ATO against APL is complex and remains poorly understood. *In vitro* studies showed that NB4 cells expressing PML-RARα underwent apoptosis associated with down regulation of bcl-2 and modulation of PML-RARα [34]. These effects occurred in a dose-dependent manner with partial differentiation of leukemic promyelocytes at low concentrations (0.1-0.5 µmol/l) and apoptosis at higher concentrations (0.5-2 µmol/l) [35]. ATO also induced apoptosis in myeloid leukemia cell lines lacking the PML-RARα rearrangement, suggesting that the mechanism of action may be independent of PML-RARα expression [36].

An initial report from Chinese investigators showed that among 10 patients with a molecularly confirmed diagnosis of relapsed APL, nine achieved complete remission after treatment with single-agent ATO [37]. This striking antileukemic activity was confirmed in a pilot study at MSKCC, in which low-dose ATO induced remissions in 11 of 12 patients with relapsed APL. Clinical response was associated with incomplete differentiation and induction of apoptosis with caspase activation in leukemic cells [38]. In a larger, multicenter trial, ATO produced clinical complete remissions in 34 of 40 patients (85%) with relapsed APL. Fourteen of the 29 patients (48%) evaluable for RT-PCR conversion became negative after an initial induction course, and 25 patients (86%) were RT-PCR-negative after subsequent consolidation courses. Ten patients developed signs or symptoms of APL differentiation syndrome and were effectively treated with dexamethasone. Electrocardiographic QT prolongation was seen in 63% of patients. One patient with an absolute QT interval of over 500 msec developed asymptomatic torsades de pointe [39].

3.9 Single-Agent ATO for Newly Diagnosed APL. Mathews et al. treated 72 patients with ATO 10 mg/day in adults and 0.15 mg/kg in pediatric patients for up to 60 days in induction. This was followed by a 28-day consolidation. Patients then received ATO for 10 days/month for 6 months [40]. Sixty-two patients (86%) achieved hematologic remission. There were seven early deaths. With median follow-up of 58 months, the estimated 5-year overall, event-free, and disease-free survival rates for the entire group were 74%, 69%, and 80%, respectively. Five-year overall and event-free survival for good-risk (presenting WBC <5,000/µL and platelets >20,000/µL) and high-risk groups (all others) were 100% vs. 63% and 90% vs. 60%, respectively [41]. This study demonstrates that single-agent ATO can produce durable remission in newly diagnosed APL, particularly for patients with low- or intermediate-risk disease.

3.10 Concomitant ATRA and ATO for Remission Induction. Although some early reports suggested potential antagonistic effects of ATRA and ATO, based on differentiation parameters obtained from *in vitro* culture systems [42], more recent studies have shown additive or synergistic effects against NB4 cells in *in vitro* and *in vivo* systems [43, 44]. Using syngeneic grafts of leukemic blasts from PML-RARα transgenic mice, Lallemand-Breitenbach *et al.* demonstrated that concomitant ATRA-ATO accelerates tumor regression through enhanced differentiation and apoptosis. While treatment with ATRA or ATO alone prolonged survival of these animals by two- to three-fold, combining these agents led to prolonged tumor-free survival [45]. Similarly, Rego *et al.* showed that combination ATRA and ATO prolonged survival in leukemic PML-RARα transgenic mice or nude mice transplanted with PML-RARα leukemia cells, compared with either drug alone [46].

Induction with combination ATRA and ATO (n = 21) has been compared to single-agent ATRA (n = 20) and ATO (n = 20) in patients with newly diagnosed APL [47]. One of three different chemotherapy regimens was added in patients who had a WBC >10,000/μL. All patients then received three cycles of consolidation followed by one of three maintenance regimens. The complete remission rates for ATRA, ATO, and combined ATRA and ATO were 95%, 90%, and 95%, respectively. The time to achieve complete remission, however, differed significantly among the three groups, with a median time of 40.5 days (range, 25-65) for ATRA, 31 days (range, 28-38) for ATO, and 25.5 days (range, 18-35) for the combination. The decrease in PML-RARα transcripts determined by quantitative real-time RT-PCR was significantly greater for combination therapy as compared to ATRA or ATO monotherapy ($P < 0.01$). All patients in the combination group remained in remission, whereas seven of the 37 patients treated with single-agent ATRA or ATO relapsed after a follow-up duration of 8-30 months (median, 18 months).

Estey *et al.* have also studied combined ATRA and ATO for remission induction in APL. Patients began ATO on day 10 of therapy and continued both drugs until attaining a marrow remission. This was followed by three courses of consolidation with ATRA and ATO. Targeted chemotherapy with gemtuzumab ozogamicin (Mylotarg®) was added if the initial WBC was over 10,000/μL or if patients had not achieved a molecular remission within 3 months of a documented clinical CR. Thirty-nine of 44 patients (89%) achieved clinical complete remissions after a median of 28 days. Although 35 of 37 patients (95%) remained RT-PCR-positive for PML/RARα at the time of clinical remission, all 29 evaluable patients achieved molecular remission within three months of clinical remission. Three patients with high-risk disease have relapsed [48]. In a follow-up report, results from this trial were reported for 82 patients. In the initial cohort of 65 patients, ATO was added to ATRA on day 10 of therapy. A second cohort of 17 patients received ATRA and ATO concomitantly on day 1. As above, GO was given to high-risk patients on day 1. There were seven early deaths, and 75 patients (92%) attained remission. Molecular remission was documented at the time of CR in 5 of 13 patients (38%) and by three months in 47 of 50 patients (94%). The estimated 3-year survival was 85% [49].

3.11 Reducing Standard Postremission Chemotherapy. While there has been dramatic improvement in long-term survival for patients with APL, the development of therapy-related MDS and AML is an emerging problem. In one study, of 77 patients with APL treated with chemotherapy with or without ATRA during induction followed by consolidation chemotherapy, five (6.5%) developed secondary MDS or AML [50]. Moreover, multiple courses of anthracycline-based chemotherapy can lead to late cardiac toxicity [51]. Among 141 patients treated with doxorubicin-based therapy for lymphoma, 39 patients (28%) developed subclinical cardiomyopathy [52]. The introduction of newer agents for the treatment of APL provides the basis for reducing the amount of standard chemotherapy required for long-term remission, thereby decreasing the morbidity and long-term complications of treatment.

In a study conducted at MSKCC, 31 patients with newly diagnosed APL received an unconjugated humanized anti-CD33 monoclonal antibody HuM195 after achieving a clinical complete remission with ATRA-based induction followed by standard consolidation chemotherapy [53]. Twelve of the 24 patients (50%) who were evaluable for conversion of a positive RT-PCR assay for PML-RARα became negative after HuM195, without additional therapy. All patients achieved a molecular remission after one cycle of consolidation chemotherapy. In contrast, previous studies at MSKCC showed that ATRA induction followed by three cycles of intensive chemotherapy produced molecular remissions in 90% of patients [16]. After a median follow-up of 5 years, relapse-free survival was 93% [54].

Based on these data, a subsequent study tested a risk-adapted approach to postremission therapy based on molecular monitoring of residual disease [55]. Fifteen patients with newly diagnosed APL who achieved a clinical CR following ATRA-based induction were treated with HuM195 (3 mg/m^2 twice weekly for 3 weeks) followed by ATO (0.15 mg/kg 5 times weekly for 5 weeks). Patients in molecular remission after ATO received one consolidation course of idarubicin. If patients remained RT-PCR-positive after ATO, up to three cycles of idarubicin could be given, with one cycle beyond documentation of molecular remission. Minimal residual disease was detectable by RT-PCR analysis in seven patients prior to enrollment. Four of these patients converted to RT-PCR negativity after treatment with HuM195, and three achieved molecular remission after ATO. No patient required more than one course of consolidation with idarubicin. Thirteen of the 15 patients are alive after 15+ to 64+ months (median follow-up, 43 months). Two patients relapsed with extramedullary disease (CNS and kidney) after 6 and 56 months. One of these patients died in remission due to thrombotic stroke and encephalopathy resulting from treatment for isolated CNS relapse, and one died of relapsed leukemia. One patient had a molecular relapse after 8 months but is now RT-PCR-negative following allogeneic bone marrow transplantation. Using this risk-adapted approach, the mean number of hospital days during consolidation therapy was 16 (range, 0-52). In the previous study, the mean number of hospital days was 55 (range, 24-82) ($P < 0.001$) for patients who received HuM195 and three cycles of idarubicin and cytarabine.

Using a similar strategy, Estey *et al.* treated 19 patients with APL with combination ATRA and gemtuzumab ozogamicin (GO). Idarubicin was added in three patients because of hyperleukocytosis. Postremission therapy consisted of 8 courses of ATRA and GO. Idarubicin could be administered for persistent or recurrent RT-PCR-positivity. Sixteen patients achieved clinical complete remission. All 12 evaluable patients were RT-PCR-negative within 2 to 4 months of clinical remission. After a median follow-up of 5 months (range 1-14+), all patients remained in molecular remission [56]. Taken together, these studies suggest that the use of newer agents along with RT-PCR monitoring of minimal residual disease can reduce or eliminate the need for standard anthracycline-based consolidation in APL while maintaining high durable remission rates.

The Australasian Leukaemia and Lymphoma Group (ALLG) treated 124 patients with newly diagnosed APL with induction consisting of: (1) ATRA on days 1-36, (2) idarubicin (12 mg/m^2) on days 2, 4, 6, and 8, (3) ATO on days 9-36, and (4) prophylactic prednisone (1 mg/kg) on days 1-10 for prevention of APL differentiation syndrome. This was followed by two courses of consolidation with ATRA and ATO and two years of maintenance with ATRA, methotrexate and mercaptopurine. With a median follow-up of 20 months, the 3-year overall survival and event-free survival rates were 93% and 87%, respectively. There were 4 early deaths and 2 relapses. This strategy maintained a high long-term remission rate with significant reduction in exposure to standard chemotherapy [57].

3.12 Induction of Telomerase-Dependent Death by ATRA/ATO. Telomerase is an enzyme that maintains the length of chromosomal ends or telomeres, which otherwise would progressively shorten after each cell division [58]. Loss-of-function mutations in the telomerase complex genes can cause bone marrow failure syndromes, presumably by limiting normal stem cell proliferation [59]. On the other hand, telomerase activity is often increased in advanced cancer cells and may be important for continuous cancer cell proliferation [60]. Indeed, shortened telomere length and elevated telomerase activity in APL patients have correlated with disease progression and relapse [61]. Early *in vitro* studies demonstrated that ATO inhibits transcription of the reverse transcriptase subunit of the human telomerase gene (*hTERT*) [62]. More recently, combination ATRA and ATO were shown *in vitro* to have a synergistic effect in triggering downregulation of telomerase

in ATRA-resistant APL cell lines [63]. Ghaffari *et al.* reported decreased telomerase activity and increased telomere length over the course of treatment with ATO in patients with APL [61]

3.13 Summary. This study seeks to minimize the morbidity and long-term complications of standard APL therapy by exploiting the antileukemic activity of ATRA and ATO. The ability to monitor the effect of treatment using RT-PCR techniques can reduce and potentially eliminate the need for standard chemotherapy. Previous studies indicate that combined ATRA and ATO produce high rates of clinical and molecular remission when used as induction [47, 48, 49, 57]. The true molecular remission rate of this combination after induction therapy, however, is unknown. The 5% molecular remission rate reported by Estey et al. is surprisingly low, possibly due to delayed initiation of ATO [48]. We hypothesize that maximally exploiting the well-described synergism of ATRA and ATO by beginning both agents on day 1 of therapy may improve the molecular remission rate after induction. In contrast to the recent study from the ALLG [57], in which idarubicin was given during induction to all patients, this study proposes to limit its use to patients with high-risk disease and those who develop leukocytosis during therapy. This population is known to be at increased risk for the APL differentiation and syndrome and relapse [13]. We expect that approximately 20-25% of patients will present with high-risk disease [22], and an additional 30-35% of patient will develop hyperleukocytosis requiring treatment with idarubicin [13, 39]. This is in contrast to other standard regimens, where up to 15 doses of anthracyclines or anthracenediones are given [20]. The use of intravenous cytarabine will be eliminated since this agent is not required for long-term remission [21, 22]. Several studies showed that ATO has activity against minimal residual disease in both patients with relapsed [38, 39] and newly diagnosed APL [48, 55]. ATRA is included during consolidation since its addition to standard anthracycline-based therapy improved disease-free survival in an earlier trial [20].

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a multicenter, phase II trial to study the efficacy of combined tretinoin and ATO in the treatment of newly diagnosed APL in an effort to reduce or eliminate the amount of standard chemotherapy required for long-term remission. Patients will be treated at Memorial Sloan Kettering Cancer Center, Northwestern University, and the Ontario Cancer Institute/Princess Margaret Hospital. The rates of molecular remission, as well as clinical CR and time to remission, will be determined after induction with combined tretinoin and ATO (along with idarubicin in patients who present with or develop leukocytosis). Minimal residual disease will be monitored by serially analyzing peripheral blood samples using quantitative RT-PCR for PML-RARα after each course of postremission therapy in local laboratories. The differentiating effects of combined tretinoin and ATO during induction will be characterized by serial flow cytometric analyses of peripheral blood in local laboratories. Effects of combined ATRA and ATO on telomerase activity, telomere length, and *TERT* expression will be assessed by analyses of serial blood and bone marrow samples in the laboratory of Dr. Neal Young at the NIH. Patients will be followed for disease-free and event-free survival, as well as toxicity, including early death, the number and length of hospitalizations, the incidence of secondary MDS/AML, and the effects of treatment on LVEF.

4.3 Intervention

Induction will consist of tretinoin 45 mg/m^2 po daily (rounded up to the nearest 10mg) in two divided doses (25 mg/m^2 in patients <20 years of age) for 35 days and ATO 0.15 mg/kg IV daily for 35 doses given 5-7 days per week. The drugs will then be discontinued, and the patient will be followed until a clinical complete remission is achieved. Idarubicin 12 mg/m^2 IV for 4 doses will be added during induction on day 2 if the presenting WBC is >10,000/µl, or if the WBC increases to 5,000/µl on day 5, 10,000/µl on day 10, or 15,000/µl on day 15, because of the increased risk of the APL differentiation syndrome and relapse in these patients. Dexamethasone 10 mg twice daily with be given on days 1-14 of induction as prophylaxis for the APL differentiation syndrome. All patients will then receive four courses of consolidation with tretinoin 45 mg/m^2 po daily (rounded up to the nearest 10mg) (25 mg/m^2 in patients <20 years of age) for 15 days and ATO 0.15 mg/kg IV for 25 doses. Patients with high-risk disease or who received Idarubicin during Induction may receive intrathecal cytarabine as CNS prophylaxis given by the treating physician during consolidation at the discretion of the site PI. High-risk patients will also receive maintenance therapy with additional courses of tretinoin and ATO every 3 months for 2 years. Each maintenance course will consist of tretinoin 45 mg/m^2 po daily (25 mg/m^2 in patients <20 years of age) for 15 days and ATO 0.15 mg/kg IV for 10 doses. Disease status will be monitored with serial analyses of peripheral blood samples using RT-PCR for PML-RARα mRNA. Patients will be followed until relapse, death, loss to follow-up, or removal from study.

Induction therapy can be given as an inpatient or outpatient. Consolidation and maintenance treatments will be given as an outpatient. Consolidation may also be given at the patient's local institution. Intrathecal cytarabine treatments will be administered as an outpatient.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Tretinoin (all-*trans* retinoic acid, ATRA, Vesanoid®). Tretinoin (Roche Pharmaceuticals, Nutley, NJ) is a retinoid that induces maturation of APL cells. It is available in a 10-mg soft gelatin-filled capsule for oral administration. Chemically, tretinoin is related to retinol (vitamin A). It is a yellow to light orange crystalline powder with a molecular weight of 300.44 g. Tretinoin should be stored at 15-30°C and protected from light.

5.2 Arsenic Trioxide (ATO, Trisenox®). ATO (Cephalon, Frazer, PA) is a trivalent inorganic arsenical. The molecular formula for the drug substance in solid state is As_2O_3, with a molecular weight, 197.8 g. The raw material is a white crystalline powder that is poorly soluble in water. The finished product, ATO, is formulated as a sterile, nonpyrogenic, clear solution in water-for-injection using sodium hydroxide and dilute hydrochloric acid to adjust to pH 8. The drug product is preservative-free. Arsenic trioxide, the active ingredient, is present at a concentration of 1.0 mg/ml. Inactive ingredients and their respective approximate concentrations are sodium hydroxide (1.2 mg/ml) and hydrochloric acid, which is used to adjust the pH to 7.0-9.0. ATO is supplied as a sterile, clear, colorless solution in 10 ml glass, single-use ampules containing 10 mg of drug. ATO should be stored at 25°C. Excursions are permitted from 15-30°C. Ampules must not be frozen.

ATO should be diluted with 100-250 ml 5% dextrose injection, USP or 0.9% sodium chloride injection, USP, using proper aseptic technique, immediately after withdrawal from the ampule. Unused portions of each ampule should be discarded properly and not be saved for later dilution and administration. ATO should not be mixed with any other medications. After dilution, ATO is chemically and physically stable for up to 24 hours

when stored at room temperature and up to 48 hours when refrigerated. ATO should be administered intravenously over 1-2 hours. The infusion duration may be extended up to 4 hours if acute vasomotor reactions are observed. A central venous catheter is not required.

5.3 Idarubicin (Idamycin®). Idarubicin (Pharmacia & Upjohn, New York, NY) is supplied as a sterile, semi-synthetic, preservative-free solution antineoplastic anthracycline for intravenous use. Chemically, idarubicin hydrochloride is 5,12-Napthacenedione, 9-acetyl-7-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)osyl]-7,8,9,10-tetrahydro-6,9,11-trihydroxyhydrochloride, (7S-*cis*). Idarubicin is a sterile, red-orange, isotonic parenteral preservative-free solution, available in 5 ml (5 mg), 10 ml (10 mg), and 20 ml (20 mg) single-use-only vials. Caution in handling the solution must be exercised as skin reactions associated with idarubicin may occur. The drug should be administered slowly over 10-15 minutes through the side arm of a freely flowing intravenous infusion of 0.9% sodium chloride injection, USP or 5% dextrose injection, USP. Unless specific compatibility data are available, idarubicin should not be mixed with other drugs. Precipitation occurs with heparin. If extravasation occurs during intravenous administration, ice packs should be placed over the area intermittently (½ hour immediately, then ½ hour 4 times daily for 3 days).

5.4 Dexamethasone (Decadron®). Dexamethasone is corticosteroid that decreases inflammation by suppression of neutrophil migration, decreases production of inflammatory mediators, and reverses increased capillary permeability. It is available for oral administration in 0.5 mg, 1 mg, 2 mg, and 4 mg, tablets and should be taken with food. It is also supplied as dexamethasone sodium phosphate for intravenous injection as 4 mg/mL (1 mL, 5 mL vials) and 10 mg/mL (10 mL vial). It is diluted in 25-50 mL D_5W or NS and administered IV piggyback.

5.5 Cytarabine (Ara-C, Cytosine Arabinoside, Cytosar-U®). Cytarabine is a pyrimidine analog supplied as powder for reconstitution in 100 mg, 500 mg, 1 gm, and 2 gm vials. It is prepared for intrathecal administration using preservative-free powder and preservative-free normal saline for injection. Five ml of preservative free NS is added to 100 mg vial to yield a 20 mg/ml solution.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

Patients with newly diagnosed APL will be eligible for this trial.

6.2 Subject Inclusion Criteria

- Previously untreated patients with a morphologic diagnosis of APL, confirmed by demonstration of t(15;17) using conventional cytogenetics OR florescence in situ hybridization (FISH), OR a positive RT-PCR assay for PML-RARα at the subject's local institution.
- Age ≥18 years.
- Karnofsky performance status of ≥ 60%.
- Adequate renal function as demonstrated by a serum creatinine ≤ 2.0 mg/dl or a creatinine clearance of > 60 ml/min.

- Adequate hepatic function as demonstrated by a bilirubin < 2.0 mg/dl (unless attributable to Gilbert's disease) and an alkaline phosphatase, AST, and ALT ≤ 2.5 times the upper limit of normal.
- Normal cardiac function as demonstrated by a left ventricular ejection fraction ≥ 50% on echocardiogram or MUGA scan.
- QTc ≤ 500 msec on baseline ECG.
- Negative serum pregnancy test in women of childbearing potential.
- Ability to swallow oral medication.
- Men and women of child-bearing potential must be willing to practice an effective method of birth control during treatment and at least 4 months after treatment is finished.
- Patients with central nervous system involvement by APL are eligible and may receive concomitant treatment with radiation therapy and/or intrathecal chemotherapy in accordance with standard medical practice.

6.3 Subject Exclusion Criteria

- Previous treatment for APL, **except tretinoin, which may be given for up to 7 days prior to study entry.**
- Active serious infections not controlled by antibiotics.
- Pregnant women or women who are breast-feeding.
- Concurrent active malignancy requiring immediate therapy.
- Clinically significant cardiac disease (NY Heart Association Class III or IV), including chronic arrhythmias, or pulmonary disease.
- Other serious or life-threatening conditions deemed unacceptable by the principal investigator.

7.0 RECRUITMENT PLAN

Potential research subjects will be identified by a member of the patient's treatment team, the protocol investigator, or research team at Memorial Sloan Kettering Cancer Center (MSKCC). Patient recruitment will occur in medical oncology clinics or the inpatient service of the Leukemia Service at MSKCC. Investigators will screen the patient's medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study.

Similar recruitment procedures will be followed at collaborating institutions. It is expected that each of the four participating sites will enroll 10-20 patients each over a 3-year period.

Participating site recruitment will be conducted as outlined within the protocol. Any participating sites that require a limited waiver must obtain it from their site IRB/PB via a separate protocol addendum or request. It is the responsibility of the MSK staff to confirm participating data collection sites have a limited waiver approved by their local IRB(s)/PBs.

As of August 19th 2016 this protocol has been closed to new patient accruals. Patients were enrolled at MSK and Northwestern University only. Treatment and/or follow up of existing patients continues.

8.1 PRETREATMENT EVALUATION

The following tests should be performed within one week of entry on study:

- Complete medical history and physical examination.
- Complete blood count with leukocyte differential.
- Comprehensive biochemistry profile (including BUN, creatinine, serum electrolytes, calcium, glucose, total bilirubin, total protein, albumin, alkaline phosphatase, AST, and ALT),
- Phosphorous
- Magnesium
- LDH
- Uric Acid
- Coagulation profile, including PT, PTT, fibrinogen, and D-dimer levels.
- Chest radiograph or Chest CT Scan.
- Electrocardiogram.
- Serum pregnancy test in women of child-bearing potential.
- Echocardiogram or MUGA scan.
- Bone marrow aspiration, including immunophenotyping, cytogenetics/FISH analysis for t(15;17), RT-PCR for PML-RARα, telomerase activity, telomere length and *TERT* expression. A biopsy may be done if clinically indicated. Alternatively, FISH and PCR studies may be sent on peripheral blood.
- Peripheral blood immunophenotyping (MSKCC patients only).
- Peripheral blood for telomerase activity, telomere length and *TERT* expression.

Immunophenotyping Studies (MSKCC patients only). Two lavender top tubes of peripheral blood and two ml of bone marrow in EDTA will be sent for immunophenotyping studies. Surface-membrane antigens will be detected by direct immunofluorescence staining with the use of fluorescein isothiocyanate (FITC)-conjugated or phycoerythrin (PE)-conjugated monoclonal antibodies against CD16, CD11b, CD33, HLA-DR, CD45, CD14, and CD117 (Beckton Dickinson, Mountain View, CA; Immunotech Immunology, Marseilles, France). Dual-color staining will be performed by incubating cells

simultaneously with two monoclonal antibodies, including CD33-PE and CD11b-FITC or CD33-PE and CD16-FITC. Apoptosis will be assayed by dual staining for Annexin V and 7-AAD. The expression of surface antigens, including CD33 and CD11b, on peripheral blood and bone marrow mononuclear cells will be plotted over time to characterize the differentiating effect of combined therapy with ATRA and ATO on APL cells during induction.

Telomerase Activity, Telomere Length and *TERT* Expression. Telomerase activity will be determined by the florescent telomerase repeat amplification protocol (TRAPeze XL, Chemicon). Telomerase length will be determined by qPCRs performed in a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA). Each sample's telomere length (x) will be based on the telomere to single copy gene ratio (T/S ratio) and on the calculation of the ΔCt [Ct(telomeres)/Ct(single gene)]. Telomere length will be expressed as relative T/S ratio, which is normalized to the average T/S ratio of reference sample [2-(ΔCtx – ΔCtr) = 2-ΔΔCt], used for the standard curve, as reference sample, and as validation sample. In order to make comparable the results from different plate runs, the results of each plate will be approved only if the relative T/S ratio of the validation reference sample falls within 3% variation [64,65]. Expression of *TERT* will be assayed by RT-PCR (TaqMan Gene Expression Assays, Applied Biosystems) using a primer and probe premix. Two-five ml of bone marrow in EDTA will be collected at baseline and at the time of clinical CR. Two green top tubes of peripheral blood (approximately 10 ml in heparin) will be collected at baseline and weekly during induction until clinical CR is achieved. Specimens from each center will be shipped under ambient conditions overnight directly to:

Neal S. Young, MD
Hematology Branch, NHLBI
10 Center Dr., Building 10-CRC
3-5140, MSC-1202
Bethesda, MD 20892
Email: YoungNS@mail.nih.gov
Phone: 301-496-5093

Participating sites will notify the Multicenter Trial Research Staff at MSKCC when marrow and/or blood samples are shipped by using a sample requisition form. The Research Staff will contact Dr. Young's laboratory to confirm receipt of the specimens. The SKIFI system will be used to log and track samples from participating sites. The following information will be recorded: (1) MSKCC CRDB protocol participant number, (2) patient initials, (3) sample type (blood or bone marrow), (4) date specimen was obtained, (5) shipping date, and (6) date specimen was received.

Differences in telomerase activity, telomere length, and *TERT* expression pre- and post-induction will be compared using the Wilcoxon rank-sum test.

9.1 TREATMENT/INTERVENTION PLAN

At initial presentation, risk groups will be assigned as follows [22]:

- Low-risk: Presenting WBC ≤10,000/μL and platelet count >40,000/μL
- Intermediate-risk: Presenting WBC ≤10,000/μL and platelet count ≤40,000/μL
- High-risk: Presenting WBC >10,000/μL.

In an effort to minimize early death due to hemorrhagic events, treatment with tretinoin should be started at the first suspicion of the diagnosis of APL. For this reason, up to 7

days of tretinoin at any dose and schedule is allowed before entry onto study. This will not affect protocol therapy or the analysis of outcomes. The diagnosis should be confirmed as quickly as possible by cytogenetic and/or molecular testing prior to initiation of protocol therapy.

Treatment consists of induction with tretinoin and ATO (along with idarubicin in patients who present with a WBC >10,000/μL or who develop leukocytosis) followed by four cycles of consolidation with tretinoin and ATO. High-risk patients or patients who have received Idarubicin during Induction will receive CNS prophylaxis with intrathecal cytarabine during consolidation because of increased risk of extramedullary relapse [66], at the discretion of the site PI. High-risk patients will also receive maintenance therapy with additional courses of ATRA and ATO every 3 months for 8 cycles. Maintenance will not be given to patients with low- or intermediate-risk disease (Fig. 1).

9.2 Induction. All patients will begin induction therapy as an inpatient. If they are clinically stable (e.g., no evidence of coagulopathy or APL differentiation syndrome), therapy may be continued in the outpatient department at the discretion of the treating physician. Induction will consist of:

> Tretinoin 45 mg/m^2/day (25 mg/m^2/day in patients <20 years of age) orally in 2 divided doses on days 1-35 and
>
> Arsenic trioxide 0.15 mg/kg/day intravenously for a total of 35 doses.

The dose of tretinoin is reduced in patients younger than 20 years due to a higher incidence of pseudotumor cerebri [67]. Tretinoin doses will be rounded to the nearest 10-mg increment. If the total calculated dose ends in a numeral <5, the administered dose will be rounded down; if the calculated dose ends in a numeral $\geq$5, the dose will be rounded up. If the total daily dose of tretinoin is not equally divisible by 2, the larger of the two doses will be given in the morning. For example, if the total daily dose is 90 mg, the drug will be given as 50 mg every morning and 40 mg every evening. If tretinoin is given as an outpatient, patients will be given a medication log to document compliance with the drug.

ATO may be given on consecutive days, weekdays only, or some combination thereof. Generally, the drug will be administered daily while patients are hospitalized and 5-7 days per week if it is given as an outpatient. The maximum interval between doses may not exceed 2 days.

Tretinoin will be discontinued after 35 days and ATO after 35 doses. The patients will be followed until recovery of peripheral blood counts and a clinical CR (Section 12.1) is achieved.

Because of the increased risk of APL differentiation syndrome and relapse in high-risk patients, cytotoxic chemotherapy will be added on day 2 if the presenting WBC is >10,000/μL. Similarly, cytotoxic therapy will be added in patients who develop leukocytosis on treatment with a WBC >5,000/μL by day 5, > 10,000/μL by day 10, or >15,000/μL by day 15) [13]. These patients with receive:

> Idarubicin 12 mg/m^2 by IV push every other day for 4 doses.

Even if the leukocytosis resolves before completion of the entire course of idarubicin, all four doses will be administered.

9.1.1 Prevention and Management of APL Differentiation Syndrome. In order to prevent development of the APL differentiation syndrome (see Section 3.4) when the risk is greatest, all patients with receive:

Dexamethasone 10 mg twice daily (either oral or IV) on days 1-14.

Beginning on day 15, dexamethasone may be tapered over 7-10 days, at the discretion of the treating physician.

If there is suspicion of the APL differentiation syndrome during the first 14 days of therapy, and the patient is receiving dexamethasone orally, treatment should be changed to the intravenous preparation at a dose of 10 mg intravenously q 12 hours. If suspicion of the differentiation syndrome occurs later, therapy with dexamethasone should be reinstituted at a dose of 10 mg intravenously q 12 hours. Therapy should be continued for at least 3 days or longer until signs and symptoms have resolved.

9.1.2 Management of Coagulopathy. Intracerebral and pulmonary hemorrhages are relatively common life-threatening complications while the characteristic coagulopathy of APL is active. CBC's and coagulation parameters (PT/PTT, fibrinogen) should be monitored 2-4 times daily as clinically necessary. Platelets should be transfused to maintain a count of >50,000/μL. Fresh frozen plasma or cryoprecipitate should be transfused to maintain a fibrinogen level >150 mg/dL.

9.1.3 ECG Monitoring and Electrolyte Management. ATO can cause QT interval prolongation and complete atrioventricular block. QT prolongation can lead to torsade de pointes, a polymorphic ventricular arrhythmia that can be fatal. The risk of torsade de pointes may be related to the extent of QT prolongation, concomitant administration of QT-prolonging drugs, a history of torsade de pointes, preexisting QT interval prolongation, congestive heart failure, administration of potassium-wasting diuretics, or other conditions that result in hypokalemia or hypomagnesemia.

During therapy with ATO, patients may receive potassium and magnesium supplementation either orally or intravenously to keep the concentrations above 4 mEq/l and 2.0 mg/dl, respectively. ECG's will be obtained at least once per week and more frequently in patients whose routine ECG shows prolongation of the QT interval. In patients who develop an absolute QT > 500 msec, actions should be taken to correct any concomitant risk factors, and ATO will be held until the QT is < 500 msec [68]. If syncope, rapid or irregular heartbeat develops, the patient will be hospitalized for monitoring, serum electrolytes will be assessed, and ATO will be discontinued until the QTc regresses to <460 msec, electrolyte abnormalities are corrected, and the syncope and irregular heartbeat cease.

9.1.4 Toxicity and Dose Modifications for Tretinoin.

Management of Headache. One of the most common adverse effects of tretinoin is headache of mild-to-moderate severity. Pediatric patients appear to be more sensitive to this particular effect, and pseudotumor cerebri has been reported. During induction therapy, intracranial hemorrhage should also be considered as a possible cause. Acetaminophen or oxycodone may be administered if headache develops. If grade 3 headache persists, the dose of tretinoin will be reduced by 50%. Once symptoms resolve, the dose may be escalated to 45 mg/m^2/day as tolerated.

Interruption of Therapy and Dose Attenuation. Treatment should be interrupted in the case of significant hepatotoxicity (defined as an increase in serum bilirubin, AST, ALT, or

alkaline phosphatase to > 5 times baseline values) for up to two weeks. Treatment may resume at 50% of the previous dose after these values recover to ≤ 4 times baseline values. If values ≤ 4 times baseline persist for more than two weeks, tretinoin will be discontinued, but patients may remain on study and continue to receive ATO.

9.1.5 Toxicity and Dose Modifications for ATO.

Management of Vasomotor Reactions. Patients who experience acute vasomotor reactions, such as hypotension (≥ 25% decrease in systolic blood pressure from baseline) or dizziness, that are temporally related to infusion of the drug will receive additional intravenous fluids as appropriate until these signs and symptoms resolve. Both the immediate and future drug infusion rates may be extended to 4 hours. Generally, however, the infusion time should be reduced as tolerated to a minimum time of 1 hour. Treatment will be discontinued in patients with persistent or recurrent episodes of hypotension that are unresponsive to these maneuvers, and they will be removed from study.

Interruption of Therapy and Dose Attenuation. Treatment should be interrupted in the case of significant hepatotoxicity (defined as an increase in serum bilirubin, AST, ALT, or alkaline phosphatase to > 5 times baseline values), nephrotoxicity (defined as serum creatinine > 3.5 times the upper limit of normal), neurological impairment (defined as somnolence, seizures, or impaired mentation), ≥ grade 3 peripheral neuropathy, or any non-hematologic grade 4 adverse event (NCI CTCAE, Version 4.0). Patients who experience such reactions that are considered drug-related should resume treatment only after resolution of the toxic event or after recovery to baseline status of the abnormality that prompted the interruption. In such cases, treatment should resume at 50% of the preceding daily dose. If the toxic event does not recur within 3 days of restarting treatment at the reduce dose, the daily dose can be escalated to 100% of the original dose. Patients who experience a recurrence of toxicity or in whom toxicity does not resolve to baseline will be removed from study. Patients with therapy interrupted for conditions that were not considered probably related to ATO could resume treatment at the full dose at the discretion of the investigator.

Management of Overdose. If symptoms suggestive of serious acute arsenic toxicity (*e.g.*, convulsions, muscle weakness, and confusion that cannot be attributed to other causes) appear, ATO should be discontinued immediately, and the patient removed from study. Chelation therapy should be considered. A conventional protocol for acute arsenic intoxication includes dimercaprol administered at a dose of 3 mg/kg intramuscularly every 4 hours until immediate life-threatening toxicity has subsided. Thereafter, penicillamine at a dose of 250 mg orally, up to a maximum frequency of four times daily, may be given for up to 3 months.

9.2 Consolidation Therapy. All patients will receive four consolidation courses. In general, this treatment will be given as an outpatient, or at the patient's local institution; however, administration of this therapy as an inpatient is allowed at the discretion of the treating physician. Consolidation 1 will begin 2-6 weeks after documentation of clinical CR. Consolidations 2, 3, and 4 will begin 2-4 weeks after completion of the prior consolidation course. Each consolidation course will be given over approximately 5 weeks and will consist of:

Tretinoin 45 mg/m^2/day (25 mg/m^2/day in patients <20 years of age) orally in 2 divided doses for 15 days and

Arsenic trioxide 0.15 mg/kg/day intravenously for a total of 25 doses.

ATO may be given on consecutive days, weekdays only, or some combination thereof. Generally, the drug will be administered 5 days per week for 5 weeks. Although the schedule may omit holidays, the maximum interval between doses in a course of therapy may not exceed 4 days. Since tretinoin will be administered as an outpatient, patients will be given a medication log to document compliance with the drug.

The APL differentiation syndrome has not been described in patients receiving tretinoin or ATO while in clinical remission. ECG's will be monitored and electrolytes will be managed as described in Section 9.1.3. Toxicities and dose modifications for tretinoin and ATO are described in Sections 9.1.4 and 9.1.5, respectively.

9.2.1 Consolidation Therapy at Local Institution

Patient's may receive consolidation therapy at their local institutions. Treatment decisions based on labs received at local institutions will be made by the treating physician at the local institution. Treating physicians at the local institution will monitor these labs according to parameters listed in Appendix A. The MSK or participating site treating physician will review these lab values weekly to ensure protocol adherence and patient management.

9.3 Central Nervous System Prophylaxis. Patients presenting with WBC > 10,000/μL have been noted to be at increased risk for the development of extramedullary relapse, particularly CNS disease [66]. Patients, who are high-risk or have received idarubicin during induction, will receive prophylactic intrathecal chemotherapy, at the discretion of the site PI. Treatment will consist of:

Cytarabine 70 mg intrathecally for a total of 6 doses.

The first dose of intrathecal therapy will be given within 2 weeks of the documentation of clinical complete remission upon recovery from induction. Subsequent doses will each be given approximately 2-4 weeks apart during consolidation. These treatments will be given in the outpatient clinic or in the Neuroradiology Department under fluoroscopic guidance, if necessary.

9.4 Maintenance Therapy (High-Risk Patients Only). Because of the increase risk of relapse in patients presenting with WBC > 10,000/μL, maintenance therapy will be administered to these patients. Maintenance therapy should begin 4-6 weeks after the completion of Consolidation 4. Each course of maintenance therapy will be given over 15 days and will consist of:

Tretinoin 45 mg/m^2/day (25 mg/m^2/day in patients <20 years of age) orally in 2 divided doses for 15 days and

Arsenic trioxide 0.15 mg/kg/day intravenously for a total of 10 doses.

Treatment will be repeated every 3 months for 2 years, for a total of 8 cycles. ATO may be given on consecutive days, weekdays only, or some combination thereof. Generally, the drug will be administered 5 days per week for 2 weeks. Although the schedule may omit holidays, the maximum interval between doses in a course of therapy may not exceed 4 days. Since tretinoin will be administered as an outpatient, patients will be given a medication log to document compliance with the drug. ECG's will be monitored and electrolytes will be managed as described in Section 9.1.3. Toxicities and dose modifications for tretinoin and ATO are described in Sections 9.1.4 and 9.1.5, respectively.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Listed below are the schedules for follow-up evaluations during Induction (Table I), Consolidations 1 – 4 (Table II), Maintenance (Table III) and Long-term Follow-up (Table IV).

Table I. Evaluation on Study: Induction

Week:	**Pre-treatment (within 7 days of study entry)**	**1**	**2**	**3**	**4**	**5**	**6-10**
Day:		**1-7**	**8-14**	**15-21**	**22-28**	**29-35**	**36-70**
Tretinoin (ATRA)		Daily	Daily	Daily	Daily	Daily	
Arsenic Trioxide (ATO)		Daily	Daily	Daily	Daily	Daily	
Idarubicin (for patients with leukocytosis only)[1]		Days 2, 4, 6, and 8					
Physical exam	X	Daily	Daily	Twice[2]	Twice[2]	Twice[2]	Weekly until count recovery[3]
CBC	X	Daily[4]	Daily[4]	Daily	Daily	Daily	Weekly until count recovery[3]
Electrolytes, BUN, creatinine, Mg		4 times[5]	4 times[5]	4 times[5]	4 times[5]	4 times[5]	
Comprehensive biochemistry profile,[6] PO_4, Mg, LDH, uric acid	X	3 times[7]	3 times[7]	3 times[7]	3 times[7]	3 times[7]	
PT, PTT, fibrinogen, D-dimer	X	Daily[4]	Daily[4]				
Chest radiograph or Chest CT Scan	X						
ECG	X	Once	Once[8]	Once[8]	Once[8]	Once[8]	
Seum pregnancy test (in women of child-bearing potential)	X						
Echocardiogram or MUGA scan	X						
Bone marrow aspiration[9]	X						At count recovery[3]
Peripheral blood immunophenotyping (MSKCC patients only)[10]	X		Once[8]	Once[8]	Once[8]	Once[8]	At count recovery[3]
Peripheral blood RT-PCR for PML/RARα							At count recovery[3]
Peripheral blood for telomerase activity, telomere length, and *TERT* expression[10]	X		Once[8]	Once[8]	Once[8]	Once[8]	At count recovery[3]

[1]Only if presenting WBC is >10,000/μL. Alternatively, if WBC is 5,000/μL by day 5, 10,000/μL by day 10, or 15,000/μL by day 15, therapy with idauricin will be initiated on the same dose and schedule.

[2]3-5 days apart, e.g., Monday-Thursday or Tuesday-Friday.
[3]ANC ≥1,500/μL, platelet count ≥100,000/μL (see Section 12.1).
[4]2-4 times daily as clinically indicated until coagulopaty resolves.
[5]On days when comprehensive biochemical panel is not obtained.
[6]Includes BUN, creatinine, serum electrolytes, calcium, glucose, total bilirubin, total protein, albumin, alkaline phosphatase, AST, and ALT.
[7]2-3 days apart, e.g., Monday, Wednesday, and Friday.
[8]5-8 days apart.
[9]Including morphology, cytogenetics OR FISH for t(15;17) OR RT-PCR for PML-RARα, immunophenotyping, telomerase activity[10], telomere length[10], *TERT* expression[10],and biopsy (if clinically indicated).
[10]**Research non-billable test** (RNB): Blood and bone marrow specimens for telomerase activity, telomere length and *TERT e*xpression will be collected, stored and shipped according to procedures given in Section 8.0.

Table II. Evaluation on Study: Consolidations 1 – 4

Week:	**Pretreatment**[1]	**1**	**2**	**3**	**4**	**5**	**6**
Day:		**1-7**	**8-14**	**15-21**	**22-28**	**29-35**	**36-42**
Tretinoin (ATRA)		Daily	Daily	Day 15			
Arsenic Trioxide (ATO)[2]		5 times	5 times	5 times	5 times	5 times	
IT Cytarabine (high-risk patients or patients that have received Idarubicin during Induction)	First dose[3]						
Physical exam	X	Once					
CBC	X	Twice[5]	Twice[5]	Twice[5]	Twice[5]	Twice[5]	Once
Electrolytes, BUN, creatinine, Mg		4 times[6]	4 times[6]	4 times[6]	4 times[6]	4 times[6]	
Comprehensive biochemistry profile,[7] PO_4, Mg, LDH, uric acid	X	Once	Once[4]	Once[4]	Once[4]	Once[4]	Once[4]
ECG		Once	Once[4]	Once[4]	Once[4]	Once[4]	
Peripheral blood RT-PCR for PML/RARα		X[8]					

[1]Within 14 days of beginning therapy of week 1 Consolidation 1.
[2]ATO may be given on consecutive days, weekdays only, or some combination thereof. Generally, the drug will be administered 5 days per week for 5 weeks (see Section 9.2).
[3]A total of 6 doses of intrathecal cytarabine will be given to high-risk patients or patients that have received Idarubicin, at the discretion of the site PI. The first dose of intrathecal therapy will be given within 2 weeks of the documentation of clinical complete remission upon recovery from induction. Subsequent doses will each be given approximately 2-4 weeks apart during consolidation (see Section 9.3).
[4]5-8 days apart.
[5]3-5 days apart, e.g., Monday-Thursday or Tuesday-Friday..
[6]On days when ATO is administered but comprehensive biochemical profile is not done.
[7]Includes BUN, creatinine, serum electrolytes, calcium, glucose, total bilirubin, total protein, albumin, alkaline phosphatase, AST, and ALT.
[8]Prior to receiving treatment on consolidations 2, 3, and 4 only.

Table III. Evaluation on Study: Maintenance (High-Risk Patients Only)

	Cycle 1						**Cycles 2-8**
Month:	**1**				**2**	**3**	
Week:	**1**	**2**	**3**	**4**			
Day:	**1-7**	**8-14**	**15-21**	**22-28**			
Tretinoin (ATRA)	Daily	Daily	Day 15				As in cycle 1
Arsenic Trioxide (ATO)[1]	5 times	5 times					
Physical exam	Once						
CBC	Once	Once[2]					
Electrolytes, BUN, creatinine, Mg	4 times[3]	4 times[3]					
Comprehensive biochemistry profile[4], Mg, LDH	Once	Once[2]					
ECG	Once	Once[2]					
Echocardiogram or MUGA scan (whichever was done during pre-treatment evaluation)[5]	Once						Once during cycle 5 only
Peripheral blood RT-PCR for PML/RARα	Once						Once per cycle[6]

[1]ATO may be given on consecutive days, weekdays only, or some combination thereof. Generally, the drug will be administered 5 days per week for 5 weeks (see Section 9.4).
[2]5-8 days apart.
[3]On days when ATO is administered but comprehensive biochemical profile is not done.
[4]Includes BUN, creatinine, serum electrolytes, calcium, glucose, total bilirubin, total protein, albumin, alkaline phosphatase, AST, and ALT.
[5]To be completed at the discretion of the treating physician.
[6]To be completed during Week 1 of each cycle.

Table IV. Long Term Follow Up

Year[7]	1	2	3	4	5
Physical exam	Monthly x 6[5], then q 3 months[6]	q 3 months[6]	q 3 months[6]	q 6 months[6]	q 6 months[6]
CBC	Monthly x 6[5], then q 3 months[6]	q 3 months[6]	q 3 months[6]	q 6 months[6]	q 6 months[6]
Echocardiogram or MUGA scan (whichever was done during pre-treatment evaluation)[1]	Once	Once[2]	Once[2]	Once[2,3]	Once[2,3]
Peripheral blood RT-PCR	q 3 months[4, 6]	q 3 months[6]	q 3 months[6]		

[1]Only in patients who have received idarubicin during induction, at the discretion of the treating investigator
[2] To be performed 11-13 months apart.
[3]Omit in high-risk patients.
[4]To be completed within 4-6 weeks of Consolidation or Maintenance (for high risk patients only)
[5]A +/- 7 day window will be allowed for completion of these assessments.
[6]A +/- 14 day window will be allowed for completion of these assessments.
[7]At least 2.5 years must be completed.

11.0 TOXICITIES/SIDE EFFECTS

11.1 Toxicities of Tretinoin. The most frequently reported adverse events associated with tretinoin are similar to those described in patients taking high doses of vitamin A and include headache (86%), fever (83%), skin/mucous membrane dryness (77%), nausea or vomiting (57%), rash (54%), mucositis (26%), pruritus (20%), visual changes (17%), and bone pain (3%). Approximately 25% of patients undergoing induction therapy with tretinoin can experience APL differentiation syndrome characterized by fever, dyspnea, weight gain, pulmonary infiltrates, and pleural or pericardial effusions. Section 9.1.1 describes the APL differentiation syndrome and its treatment in detail. Hyperleukocytosis has been frequently observed (75%), sometimes associated with the APL differentiation syndrome. Arrhythmias have been reported in 23% of patients. Central nervous system effects include dizziness (20%) and paresthesia (17%). Children are more sensitive to the CNS effects of tretinoin, and there have been reports of pseudotumor cerebri in this population. Hypercholesterolemia and/or hypertriglyceridemia have been seen in up to 60% of patients. Elevated liver function tests occur in 50-60% of patients while taking tretinoin. Guidelines for management of toxicity are given in Sections 9.1.1 and 9.1.4.

11.2 Toxicities of ATO. The most common (≥ 5% of patients) severe and life-threatening (grade 3 and 4) adverse events attributed in ATO in previous clinical studies included APL differentiation syndrome, febrile neutropenia, leukocytosis, prolonged QT interval, torsade de pointe, hyperglycemia, peripheral neuropathy, atrial fibrillation, complete atrioventricular block, spontaneous abortion, hemorrhage, infections, pain, diarrhea, and nausea. Prolonged QT as determined by ECG analyses occurred in 70% of patients. Guidelines for ECG monitoring and electrolyte management are given in Section 9.1.2. Leukocytosis occurred in 50% of patients with APL. The most common adverse events (occurring in ≥ 25% of patients) related to treatment with ATO were hyperglycemia, hypokalemia, headache, nausea, and fatigue. Signs of arsenic overdose include convulsions, muscle weakness, and confusion. Guidelines for management of toxicity are given in Sections 9.1.1, 9.1.3, and 9.1.5.

11.3 Toxicities of Idarubicin. Severe myelosuppression is the most frequent adverse effect associated with idarubicin. This can lead to infection (95%) and hemorrhagic complications (63%). Cardiotoxicities include decreased left ventricular function, congestive heart failure, arrhythmias, and myocardial infarction. Gastrointestinal side effects include nausea/vomiting (82%), stomatitis (50%), abdominal cramps and diarrhea (73%). Elevations in creatinine have been reported in 1% of patients receiving idarubicin. Elevation of liver function tests have been described in < 5% of patients. Alopecia is common (77%). The drug is a vesicant; guidelines for management of extravasation are given in Section 5.3.

11.4 Toxicities of Dexamethasone. The most common toxicities of dexamethasone are: weight gain and fluid retention, gastritis, development of peptic ulcers, insomnia, and mood changes. Less likely toxicities include: glucose intolerance, diabetes mellitus, hypertension, osteoporosis, alopecia, decreased muscle mass, and myopathy. Rare side effects include: development of cataracts, skin changes, aseptic necrosis of the hip, and impaired immunity.

11.5 Toxicities of Intrathecal Cytarabine. Administration of cytarabine intrathecal has commonly been associated with fever, headache, nausea, and vomiting. Rare but serious side effects include accessory nerve paralysis, necrotizing leukoencephalopathy (particularly when given concurrently with cranial irradiation and other intrathecal chemotherapy), and paraplegia.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

12.1 Clinical Complete Remission. All three criteria defined by Cheson *et al.* [69] must be met for clinical complete remission:

- *Peripheral Blood Counts.* The peripheral blood neutrophil count should be ≥1,500/μl (sustained without growth factor support), and the platelet count should be ≥100,000/μl (without transfusion). No circulating blasts or abnormal promyelocytes should be detected.

- *Bone Marrow Aspirate.* The cellularity of the bone marrow should approximate normal. There must be evidence of maturation of all cell lines. The bone marrow aspirate should contain <5% blasts and no abnormal promyelocytes. Auer rods should not be detected.

- *Extramedullary Leukemia.* Extramedullary leukemia, such as CNS or soft tissue involvement, must not be present.

12.2 Molecular Remission. A molecular remission is defined as a clinical CR with a negative RT-PCR assay for PML-RARα performed on bone marrow.

12.3 Clinical Relapse. Clinical relapse is defined as the reappearance of blasts or abnormal promyelocytes in the peripheral blood or the finding of more than 5% blasts and/or abnormal promyelocytes in the bone marrow aspirate. These findings should not be attributable to another cause (*e.g.*, myeloid growth factor administration, bone marrow regeneration).

12.4 Molecular Relapse. Molecular relapse is defined as the conversion of RT-PCR for PML-RARα from negative to positive on two consecutive tests performed on bone marrow at least one week apart, while still meeting criteria for clinical CR.

13.1 CRITERIA FOR REMOVAL FROM STUDY

- If the patient fails to achieve a clinical complete remission after Induction, he/she will be taken off study.

- If the patient fails to achieve a molecular remission following completion of Consolidation 4 or has a clinical or molecular relapse at any time, he/she will be taken off study and referred for alternative therapy.

- If at any time the patient develops unacceptable toxicity as defined in Sections 9.1.3, 9.1.4, and 9.1.5, he/she will be removed from study.

- If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (*i.e.*, a change in diagnosis), the patient will be removed from study.

- If the patient fails to comply with the defined treatment plan and follow-up evaluations, the patient will be removed from the study.

- If the patient withdraws consent for continued participation, he/she will be removed from study.

14.1 BIOSTATISTICS

The major objective of this phase II trial is to determine the molecular remission rate after induction therapy combined with ATRA and ATO. (Patients with high-risk disease at presentation or who develop leukocytosis will also receive idarubicin.) The standard of care for patients with APL is induction with ATRA plus chemotherapy [13, 15, 20]. Based on previous studies the molecular remission rate after induction with ATRA and idarubicin is approximately 60% [15]. In this trial, we will utilize a Simon Minimax two-stage design in which a 50% molecular remission rate is considered not promising, a 70% molecular remission rate is considered promising, and the probabilities of a type I error (falsely accepting a non-promising therapy) and type II error (falsely rejecting a promising therapy) are set at 0.10 and 0.10, respectively. In this scenario, the maximum trial size will be 39 patients. In the first stage of this design, 23 patients will be accrued. If at least 12 patients achieve molecular remission after induction among these 23 patients, then an additional 16 patients will be accrued to the second stage. If 11 or less molecular remissions are seen after induction, the study will be terminated and declared negative. This design yields at least a 0.90 probability of a positive result if the true molecular response rate is at least 70% and yields a 0.90 probability of a negative result if the true response rate is 50%.

Any patient receiving any part of therapy on study will be considered evaluable, even if removed from study for toxicity. Patients who attain a clinical CR but not a molecular remission after induction will remain on study and be followed for secondary endpoints as described below. Patients who have not achieved at least a clinical CR after induction and a molecular remission after consolidation 4 will be considered treatment failures and removed from study.

A number of secondary endpoints will be studied. The clinical CR rate will be estimated after induction. The proportion of RT-PCR-negative patients and the 95% confidence interval will be estimated at: (1) study entry, (2) after induction therapy, (3) after each course of consolidation with ATRA and ATO, and (4) every 3 months thereafter for 3 years. Proportions will be calculated at the conclusion of the trial by taking the number of RT-PCR-negative patients at a particular time point divided by the total number of patients who received any part of induction therapy. The median time to clinical and molecular remissions will be estimated by standard life-table methods.

Disease-free and event-free survival will be estimated using actuarial methods. Disease-free survival analysis will include only those patients who achieve at least a clinical CR after induction and will be calculated from the time of clinical CR (or better) to relapse (molecular or clinical) or last follow-up. Event-free survival will be calculated from the start of induction therapy to relapse (molecular or clinical), death, or last follow-up. Event-free survival analyses will include all patients who receive any part of induction therapy.

Frequencies of toxicities based on the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0 will be tabulated. The incidence of APL differentiation syndrome and death within 30 days of beginning induction will be calculated. The number of hospitalizations and number of hospital days will be tabulated for each patient. The incidence of secondary MDS/AML will be calculated. Immunophenotyping of peripheral blood will be performed weekly during induction, and the expression of surface antigens, including CD33 and CD11b, will be plotted over time to characterize the differentiating effect of combined therapy with ATRA and ATO on APL cells during induction. Expected accrual at all sites is approximately 10-15 patients per year. This trial will take approximately 3-4 years to complete. Bone marrow samples will be analyzed at baseline and at the time of clinical CR for telomerase activity, telomere length and *TERT* expression. Peripheral blood samples will be analyzed at baseline and weekly during induction until clinical CR is achieved.

Differences in telomerase activity, telomere length, and *TERT* expression pre- and post-induction will be compared using the Wilcoxon rank-sum test.

14.2 Stopping Rule for Treatment-Related Mortality. Treatment-related mortality during induction in excess of 15% would be unacceptably high. If at any time, five patients die from treatment-related causes, the trial will be terminated. The probability of stopping the trial is 72% if the true rate of mortality is 15%, 35% if the true mortality rate is 10%, and 4% if the true mortality rate is 5%.

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Central registration for this study will take place at MSK.

To complete registration and enroll a participant from another institution, the participating site must contact the MSK study coordinator to notify him/her of the participant registration.

The following documents must be sent to the MSK study coordinator for each enrollment **within 24 hours** of the informed consent form being signed:

- The completed or partially completed MSK eligibility checklist
- The signed informed consent and HIPAA Authorization form
- Supporting source documentation for eligibility questions (e.g. laboratory results, pathology report, radiology reports, MD notes, physical exam sheets, medical history, prior treatment records, and EKG report).

Upon receipt, the MSK study coordinator will conduct an interim review of all documents. If the eligibility checklist is not complete or source documentation is missing, the participant will be registered PENDING and the participating site will be responsible for sending the completed registration documents within 30 days of the consent.

If the external registration submission is complete, the participating site IRB has granted approval for the protocol, and the participating site is in good standing, the MSK study coordinator will send the completed registration documents to the MSK Protocol Participant Registration Office for participant enrollment as stated in the protocol.

Once the participant is registered, the participant will be assigned a protocol participant number in the MSK Clinical Research Database (CRDB). This number will be relayed back to study staff at the registering participating site via e-mail and will serve as the enrollment confirmation. The number is unique to the participant and must be written on all data and correspondence for the participant.

15.3 Randomization

There is no randomization in this trial.

16.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into a secure database, the MSKCC Clinical Research Database (CRDB). Source documentation will be available to support the computerized patient record.

16.1.1 Data and Source Documentation for Participating Sites

Data: Standardized Case Report Forms (CRFs), directions for use and sign off requirements have been generated for this study. Blank case report forms will be sent to the study staff at each participating site for use. The participating Site PI is responsible for ensuring these forms are completed accurately, legibly and in a timely manner.

Source Documentation: Source documentation refers to original records of observations, clinical findings and evaluations that are subsequently recorded as data. Source documentation should be consistent with data entered into CRFs. Relevant source documentation to be submitted throughout the study includes:

- Baseline measures to assess pre-protocol disease status (ex. CT results, bone marrow)
- Treatment records
- Grade 3-5 toxicities/adverse events not previously submitted with SAE Reports
- Response designation
- Any other forms of source documentation requried per protocol

Source documentation should include a minimum of two identifiers to allow for data verification. MSK will maintain the confidentiality of any subject-identifiable information it may encounter.

16.1.2 Data and Source Documentation Submission for Participating Sites

Participating sites should e-mail CRFs and source documentation to MSKCC research staff. Submissions should include a cover page listing all CRFs enclosed per participant.

16.1.3 Data and Source Documentation Submission Timelines for Participating Sites

Data and source documentation to support data should be transmitted to MSKCC according to Table V below:

Table V. Data and Source Submission Requirements and Timelines

	Baseline	***Induction***	***Consolidations 1, 2, 3, & 4***	***Maintenance***	***Long-term Follow-up***	***SAE***	***Off Study***
SUBMISSION SCHEDULE							
Source Documentation	Within 24 hours (see Section 15.1.1)	Within 14 days of the end of each week	Within 14 days of the end of each cycle	Within 14 days of the end of each cycle	Within 14 days after each visit	Within 3 days of event (see section 17.2.1); updates to be submitted as available	Within 14 days of visit
CRFs	Within 7 days of initial evaluation						
Required Forms							
Demographics Form	X						
Medical History Form	X						
Concomitant Medications Form	X	X	X	X	X		X
Physical Exam Form	X	X	X	X	X		X
Treatment Form		X	X	X			X
Laboratory Form	X	X	X	X	X		X
Disease Status Form		X	X	X	X		X
Adverse Event Form		X	X	X	X	X	X
Serious Adverse Event Form						X	
Off Study Form							X

16.1.4 Data Review and Queries for Participating Site Data

Research staff at MSKCC will review data and source documentation as it is submitted. Data will be monitored against source documentation and discrepancies will be sent as queries to the participating sites. Queries will be sent by MSKCC Research staff twice a month.

Participating sites should respond to data queries within 14 days of receipt.

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action. Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated. Additionally, monthly teleconferences including principal investigators and/or their designees from all sites will be held to expedite the review of toxicity and efficacy data.

16.2.1 Quality Assurance for Participating Sites

Quality Assurance will be conducted according to MSK guidelines and Multicenter SOPs.

Research staff at MSK will conduct periodic reviews of regulatory documentation, protocol compliance and data, and issue queries as appropriate. The level and frequency of monitoring or auditing may be adjusted based on ongoing site performance.

16.2.2 Response Review

Since therapeutic efficacy is a stated primary objective, all sites participant's responses are subject to review by MSKCC's Therapeutic Response Review Committee (TRRC). Radiology, additional lab reports and possibly bone marrow biopsies and/or aspirates will need to be obtained from the participating sites for MSKCC TRRC review and confirmation of response assessment. These materials must be sent to MSKCC promptly upon request.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: *http://www.cancer.gov/clinicaltrials/conducting/dsm-guidelines/page1*. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: http://inside2/clinresearch/Documents/MSKCC%20Data%20and%20Safety%20Monitoring%20Plans.pdf

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (*e.g.*, protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control. Additionally, two institutional committees are responsible for monitoring the activities of our clinical trials programs. The committees, *Data and Safety Monitoring Committee (DSMC)* for

Phase I and II clinical trials and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board. Patients treated collaborating institutions will also be included in these reviews.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (*e.g.*, NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed, and the monitoring procedures will be established at the time of protocol activation.

16.3 Regulatory Documentation

Site Activation

Prior to implementing this protocol at MSK, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MSK Institutional Review Board/Privacy Board (IRB/PB). There will be one protocol document and each participating site will utilize that document.

The following documents must be provided to MSK before the participating site can be initiated and begin enrolling participants:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved informed consent form and HIPAA authorization
- Participating Site IRB's Federal Wide Assurance number and OHRP Registration number
- Curriculum vitae and medical licenses for each investigator and consenting professional
- Documentation of Human Subject Research Certification training for investigators, consenting professionals and key study personnel at the participating site
- Documentation of Good Clinical Practice training for the participating site PI and co-PI
- Participating site laboratory certifications and normals

Upon receipt of the required documents, MSK will formally contact each participating site and grant permission to proceed with enrollment.

16.3.1 Amendments

Each change to the protocol document must be organized and documented by MSK and approved first by the MSK IRB/PB. Protocol amendments that affect MSK only (e.g. change in MSK Co-Investigator, MSK translation, etc.) do not require IRB review at the participating site(s). All other protocol amendments will be immediately distributed to each participating site upon receipt of MSK IRB/PB approval.

Each participating site must obtain IRB approval for all amendments within 90 calendar days of MSK IRB/PB approval. If the amendment is the result of a safety issue or makes eligibility criteria more restrictive, participating sites will not be permitted to continuing enrolling new participants until site IRB approval of the revised protocol documents is granted and submitted to MSK.

16.3.2.1 Additional IRB Correspondence

Continuing Review Approval
The Continuing Review Approval letter from each participating site's IRB and the most current approved version of the informed consent form must be submitted to MSK within 7 days of expiration. Failure to submit the re-approval in the stated timeline will result in suspension of new participant enrollment.

Deviations
A protocol deviation on this study is defined as any incident involving non-adherence to an IRB approved protocol. Deviations typically do not have a significant effect on the rights, safety, or welfare of research participants or on the integrity of the resultant data. Deviations that represent unanticipated problems involving risks to participants or others, or serious adverse events should be reported according to sections 17.3 and 17.5.

Deviations that do not adversely affect the rights and/or welfare of the participant or the scientific validity of the study and are related to protocol scheduling changes outside of the allowed window due to a holiday (e.g., New Year's, Thanksgiving, etc.) and/or inclement weather or other natural event do not require reporting to the MSK IRB/PB. However, they must be clearly documented in the patient's medical record.

Prospective Deviations
Deviations to the research protocol that involve patient eligibility, an informed consent procedure change, and/or treatment/pharmacy alterations that are not allowed by the protocol require prospective approval from the MSK IRB/PB prior to the change being carried out. Participating sites should contact the MSK PI who will in turn seek approval from the MSK IRB/PB.

Retrospective Deviations
Deviations that include a change or departure from the research protocol without prior approval from the MSK IRB/PB are considered retrospective deviations. Retrospective deviations should be reported to the MSK PI as soon as possible, who will in turn report the deviation to the MSK IRB/PB as per MSK guidelines.

Participating Site IRB Reporting
Participating sites should report all deviations to their institution's IRB per local guidelines. Approvals/acknowledgments from the participating site IRB for protocol deviations should be submitted to MSK upon receipt.

Other Correspondence
Participating sites should submit all other correspondence to their institution's IRB according to local guidelines, and submit copies of official site IRB correspondence, including approvals and acknowledgements, to MSK.

16.3.3 Document Maintenance

The MSK PI and participating site PI will maintain adequate and accurate records to fully document protocol implementation and allow data to be subsequently verified.

The participating sites will ensure that all regulatory documents and participating site IRB correspondence are maintained in an on-site regulatory binder and sent to MSK. The on-site regulatory binder will be reviewed by the designated study monitor at monitoring visits. A

regulatory binder for each participating site will also be maintained at MSK within the institution's Protocol Information Management System (PIMS).

After study closure, the participating sites must maintain all source documents, study related documents and CRFs for 3 years.

16.4 Noncompliance

If a participating site is noncompliant with the protocol document, accrual privileges may be suspended and/or contract payments may be withheld, if applicable, until the outstanding issues have been resolved.

17.1 PROTECTION OF HUMAN SUBJECTS

17.2 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.3 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org containing the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

17.4 Serious Adverse Event (SAE) Reporting for Participating Sites

Responsibilities of Participating Sites

- Participating sites are responsible for reporting all SAEs to their site IRB per local guidelines. Site IRB approvals/acknowledgments must be sent to MSK upon receipt.
- Participating sites are responsible for submitting the SAE Report form to MSK within 3 calendar days of learning of the event.
- When a life threatening event or death is unforeseen and indicates participants or others are at increased risk of harm, participating sites should notify the MSK PI as soon as possible but within 24 hours of the time the site becomes aware of the event.

Responsibilities of MSK

- MSK is responsible for submitting all SAEs to the MSK IRB/PB and funding entities (if applicable) as described in the protocol.
- MSK is responsible for informing all participating sites about all unexpected SAEs that are either possibly, probably, or definitely related to the study intervention within 15 days of receiving the stamped SAE report from the MSK IRB/PB.
- MSK is responsible for informing all participating sites within 24 hours or on the next business day about a life threatening event or death that is unforeseen and indicates participants or others are at increased risk of harm.

17.5 Safety Reports

MSK must submit outside safety reports to the MSK IRB/PB according to institutional guidelines. All outside safety reports will be made available to the participating sites. Outside safety reports that are reportable to the MSK IRB/PB will be distributed to the participating sites immediately upon receiving a stamped copy from the MSK IRB/PB. Participating sites will receive a special alert for any outside safety reports that warrant a significant change to the conduct of the study. Outside safety reports that are not reportable to the MSK IRB/PB, will be sent to the participating sites monthly.

Participating sites are responsible for submitting safety reports to their site IRB per their local guidelines. All site IRB approvals/acknowledgments of safety reports must be sent to MSK upon receipt.

17.6 Unanticipated Problems

Unanticipated problems involving risks to participants or others (UPs) are defined as any incident, experience or outcome that meets all of the following criteria:

- Unanticipated (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; **and**
- Related or possibly related to participating in the research (possibly related means there is a reasonable probability that the incident, experience or outcome may have been caused by procedures involved in the research); **and**
- Suggests that the research place participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Participating sites are responsible for reporting all UPs to MSK as soon as possible but within 3 calendar days of learning of the event. UPs that are SAEs should be reported to MSK via SAE Report form. All other UPs should be reported to MSK in a memo signed by the site PI.

MSK is responsible for submitting UPs to the MSK IRB/PB according to institutional guidelines. In addition, MSK is responsible for notifying participating sites of all non-SAE UPs that may affect the sites.

17.7 Potential Benefits.

While current therapeutic approaches for APL produce long-term remissions in 75-90% of patients, short-term complications include prolonged hospitalizations and morbidity due to multiple courses of intensive anthracycline-based chemotherapy. This therapy can produce late cardiotoxicity and secondary MDS/AML in up to 6.5% of patients. A treatment regimen that combines highly active agents for APL in a strategy that minimizes exposure to anthracycline-based chemotherapy using molecular monitoring of response may reduce hospitalizations, morbidity, and delayed complications of therapy while still maintaining high long-term remission rates.

17.8 Risks in Relation to Anticipated Benefits.

This is a phase II trial to study the efficacy of combined ATRA and ATO in the treatment of newly diagnosed APL and the use of molecular monitoring of minimal residual disease to reduce or eliminate the amount of standard chemotherapy required for long-term remission. The risks of this investigational approach may include increased risk of relapse in exchange for the benefit of decreased courses of chemotherapy with less exposure to the toxic side effects of such treatment. If results from the study show an equivalent probability of long-term remission to those found in previous trials (80% ± 7% at three years) with less short-term morbidity and late complications, a randomized study would be needed to verify these findings and determine the risk-benefit ratio.

17.9 Procedures for Protecting Against and Minimizing Risks.

Guidelines for the management of APL syndrome are given in Section 9.1.1. ECG monitoring and electrolyte management guidelines in order to avoid complications caused by a prolonged QT interval are described in Section 9.1.3. Management of toxicities and dose modifications for ATRA are given in Section 9.1.4. Management of toxicities and dose modifications for ATO are given in Section 9.1.5. Left ventricular function will be assessed with serial echocardiograms or MUGA scans, at the discretion of the treating physician, to detect changes from baseline cardiac function. Pack red blood cell transfusions will be given as clinically indicated. Disseminated intravascular coagulation will be treated with transfusions of platelets and fresh frozen plasma or cryoprecipitate according to guidelines given in Section 9.1.2. Infections will be treated with intravenous antibiotics as clinically indicated. Treatment-related mortality during induction in excess of 15% would be unacceptably high. If at any time, four patients die from treatment-related causes, the trial will be terminated.

17.10 Alternative Treatments

Alternative therapy would include a standard regimen consisting of induction with ATRA and an anthracycline with or without cytarabine followed by 2-3 courses of anthracycline-based consolidation with or without maintenance therapy, consisting of ATRA ± oral mercaptopurine and methotrexate.

17.11 Incentives/Costs

No incentives will be offered to patients/subjects for participation in the study. Participation is voluntary. The patient/subject will be responsible for the costs of standard medical care, including ATRA, ATO, idarubicin, all other standard medications, blood and platelet transfusions, radiographic studies, laboratory tests, and all hospitalizations, even for complications of treatment. Weekly peripheral blood immunophenotyping during induction as well as peripheral blood and bone marrow analyses for telomerase activity, telomere length, and *TERT* expression are research non-billable studies.

18.1 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

18.2 For Participating Sites

The investigators listed on the Consenting Professionals Lists at each participating site may obtain informed consent and care for the participants according to Good Clinical Practice and protocol guidelines.

A note will be placed in the participant's medical record documenting that informed consent was obtained for this study, and that the participant acknowledges the risk of participation.

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20.0 APPENDICES

Appendix A: Local Institution Guidelines *(Local Institution Consolidation Therapy Guidelines*

Appendix B: Consolidation/Maintenance Pill Diary