MSK PROTOCOL COVER SHEET

Phase I/II Study of Decitabine and All-Trans Retinoic Acid (Tretinoin) for Patients With Myelodysplastic Syndromes

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

TITLE: Phase I/II Study of Decitabine and All-Trans Retinoic Acid (Tretinoin) for Patients with Myelodysplastic Syndromes

OBJECTIVE: To determine whether the combination of decitabine with tretinoin is safe and efficacious in the treatment of Myelodysplastic Syndromes.

PATIENT POPULATION: Adults (greater than 18 years) with a confirmed diagnosis of MDS (based on the FAB MDS diagnostic criteria (1)) and an International Prognostic Scoring System (2) score of 0.5 or greater (IPSS Intermediate-1, Intermediate-2, and High-Risk categories. For IPSS schema, see Appendix 1).

STUDY DESIGN: This is a single-center, open label, phase I/II study of the combination of decitabine (DAC) and All-Trans Retinoic Acid (Tretinoin) in patients with Myelodysplastic Syndromes.

STUDY DRUG AND DOSE: Eligible patients will receive decitabine intravenously at a dose of 20 mg/m2 daily for five days for a total dose per cycle of 100 mg/m2. Decitabine will be administered as an outpatient at MSKCC. Beginning on day 10 of each cycle, patients will receive tretinoin orally for 10 days as an outpatient (See Figure 1.1 for study design schema). Three successive tretinoin dose levels will be evaluated: 45 mg/m2/day, 65 mg/m2/day, and 85 mg/m2/day given over two doses each day. A standard dose escalation scheme will be followed with cohorts of 3 to 6 patients treated at each dose level (section 14.0). A dose-limiting toxicity is defined as in section 11.5. If a DLT is seen at 45 mg/m2/day, a dose de-escalation schema will be applied (See section 9.0). If no DLT is seen amongst the three patients treated at a dose under consideration for MTD, then an additional 3 patients will be treated at this dose level to confirm this dose as the maximal tolerated dose.

The phase II study will have a 2-stage design. In the first stage of this design, twenty seven patients will be treated. Patients treated at the tretinoin MTD (6 patients) in the phase I portion of the study will be carried over and counted among the patients in stage 1 of the phase II portion of the study. If two or fewer responses (CR or PR) are seen among the first 27 patients, the study will be closed for lack of efficacy. If at least three responses are seen in the first stage, then an additional 13 patients will be accrued in the second stage.

Treatment will be recycled every 4 weeks, provided there is adequate recovery from non–hematologic toxicities. The effects of decitabine and escalating doses of tretinoin on markers of differentiation and apoptosis, and on expression of key genes will be assessed.

FIGURE 1.1: Study Design

	Treatment Period →								
	Cycle # 1 →				Cycle #2, #3, #4, etc. →				End of Study
Decitabine	X				X				
Tretinoin			X				X		
Treatment Days (D)	D1 - D5	D 6 – D 9	D10 - D19	D 20 – D 28	D 1 - D 5	D 6 – D 9	D 10 – D 19	D 20 - D 28	Day X

Patients will receive treatment for a minimum of 4 cycles, barring removal for progression of disease or excessive toxicity. Patients must achieve hematologic improvement (HI) or Bone Marrow PR/CR by the completion of cycle #6. Patients achieving HI or bone marrow PR/CR by the completion of cycle #6 may receive up to a total of 10 cycles of therapy, barring excessive toxicity. If the best response after cycle #6 is Stable Disease, these patients will be removed from study. Patients in the phase I study will continue at the study entry dose. Treatment will be interrupted for grade III or IV non-hematologic toxicity attributable to tretinoin or decitabine. Therapy may be resumed with the next cycle of treatment as long as the toxicity has decreased to < grade 2 within 8 weeks of the last treatment administration. However, these patients will receive a 25% dose reduction in tretinoin or decitabine (which ever treatment is suspected or both if the attribution is unknown) when treatment resumes. All patients will be followed for Serious Adverse Events (SAEs) for 30 days after the completion of the last cycle of therapy (each cycle is 4 weeks).

TIME TO COMPLETION: It is anticipated the study will complete accrual in 18–24 months.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary Endpoint:

Phase I: Define the hematologic and non-hematologic toxicities of combining a hypomethylating agent (Decitabine, DAC) with escalating doses of the transcriptional modifying agent tretinoin (All Trans Retinoic Acid), and define the MTD of tretinoin in combination with decitabine.

Phase II: Determine the clinical remission rate (rate of Complete Remission, Partial Remission) of the combination of decitabine and tretinoin in patients with myelodysplastic syndromes, and determine the rate of Hematologic Improvement.

Secondary Endpoints (Phase I and Phase II):

1. Evaluate the ability of decitabine and tretinoin to affect bone marrow function by monitoring frequency of transfusions, frequency of bleeding and infection, and changes in bone marrow morphology and cytogenetics.
2. Evaluate differentiation (using morphology and flow cytometry) and apoptosis (using flow cytometry) induced by treatment with decitabine and tretinoin.
3. Perform gene profiling studies to determine if gene expression changes are induced by decitabine and tretinoin.
4. Evaluate the ability of decitabine and tretinoin to cause demethylation of specific genes.
5. Correlate clinical response with gene expression, demethylation of specific genes, and flow cytometric indicators of differentiation and apoptosis.

3.0 BACKGROUND AND RATIONALE

The Myelodysplastic Syndromes:

The Myelodysplastic Syndromes (MDS) are typically characterized by refractory cytopenias in patients with a hypercellular, dysplastic bone marrow, who have a variable propensity to progress to acute myelogenous leukemia (AML). "Low risk" MDS can be distinguished from "high-risk" MDS by examining bone marrow cytogenetics, the severity and number of cytopenias, and the bone marrow blast percentage. These three parameters have been shown to correlate with survival and the likelihood of progression to AML in the International Prognostic Scoring System (2) (See Appendix 1 for the IPSS). Low risk patients, with an IPSS score of "0" have a median survival of 5.7 years, and a low risk of progression to AML. In contrast, patients with an IPSS score of 0.5 or greater have a lower survival (as low as 0.4 years) due to more rapidly progressive bone marrow failure and/or a greater risk of transformation to AML.

The standard of care for MDS is supportive care, which includes blood product transfusions and treatment of infectious complications. Growth factors are also used (rHuEPO, G-CSF), and in some patients, these agents can temporarily ameliorate the effects of some cytopenias that complicate the course of patients with MDS. In 2004, 5-azacytidine was approved for treatment of MDS, and although 5-azacytidine responders had an improved quality of life and delay in time to progression to AML, the overall response rate (CR, PR) was 23% with a CR rate of only 7% (3). Allogeneic stem cell transplantation can cure a minority of patients who are young and healthy enough to undergo such a procedure, and who have an available stem cell donor.

Aberrant differentiation and proliferation are hallmarks of MDS and AML. Agents that restore normal hematopoietic differentiation and induce appropriate apoptosis would likely improve the bone marrow function in patients with MDS.

Hypomethylating Agents in MDS: Rationale

DNA methylation patterns are frequently altered in malignancy including global demethylation and gene-specific hypermethylation of promoter CpG islands. Hypermethylation of promoter CpG islands generally result in decreased gene expression, as DNA 5-methylcytosine

formation results in a chromatin configuration that blocks the binding of transcription factors to the DNA. The genes affected by this process include a variety of tumor suppressor genes, including genes involved with cell cycle regulation(4-6).

Inhibition of DNA methyltransferase activity has been associated with cellular differentiation in numerous hematopoietic cell systems (7-10). Differentiation has also been correlated with clinical responses in MDS patients receiving decitabine. However, if demethylation of key regulatory gene promoters is responsible for the clinical activity of demethylating agents, the exact genes involved have yet to be identified.

Transforming Growth factor-βs (TGF-β) are molecules that normally inhibit hematopoietic proliferation, initiate appropriate apoptosis, and regulate differentiation (11). Growth inhibition of leukemia cell lines by transforming growth factor β (TGFβ) is associated with activation of p15INK4b, a cell cycle regulator involved with the inhibition of progression from G1 to S phase. Inactivation of p15INK4b is associated with methylation of the 5' CpG island of the promoter region in AML cases (6, 12, 13) and in MDS patients (12, 14)(11/15 patients with MDS and blasts > 10%; Quesnel, 1998. 16/32 MDS patients, unknown blast counts; Uchida, 1997). Methylation and inactivation of the p15INK4b gene may be a mechanism for hematopoietic cell escape from the G1 regulatory effect of TGFβ, and clinical responses to decitabine in MDS patients have been associated with p15INK4b transcriptional activation (15). However, it is not clear that pharmacologic demethylation of p15INK4b was responsible for the observed *in vivo* clinical activity, since other patients responded without demethylation of p15INK4b, and all responders do not have hypermethylated p15INK4b at baseline.

Other CDKIs may affect the ability of TGFβ to induce G1 arrest, including the CDKI p27Kip1 (11). In recent *in vitro* work, decreased p27 levels or abnormal localization of p27 to the cytoplasm results in unresponsiveness of fresh AML blasts and hematopoietic cell lines, respectively, to TGFβ-mediated cell cycle arrest (16). The methylation status of the p27 promoter in MDS and AML is not well characterized. However, given the importance of TGF-β in hematopoietic differentiation and growth regulation, p27 deregulation may be important to study in MDS, and methylation is one method of dysregulation that could potentially be altered by decitabine. Another CDKI, p57 (kip2), is methylated in patients with a variety of leukemia and lymphoma including AML. We have shown the importance of p57 in the G1 growth arrest induced by TGFB in primary human hematopoietic cells (17). We have also found methylation of the p57 promoter in patients with AML and others have reported this in other hematologic malignancies. Decitabine was able to induce re-expression of p57 in B-cells. The methylation status of p57 in MDS warrants further study.

Other genes involved in the pathogenesis of MDS may be abnormally methylated and can potentially be reactivated by demethylation, including genes involved in the differentiation program of hematopoietic cells. Genes affecting apoptosis pathways may also be affected by the demethylating effect of decitabine. However, the effect of decitabine on apoptosis in MDS patients has not been well characterized to date. Since abnormal proliferation is a prominent feature of the MDS phenotype, it would be desirable to determine if apoptosis is induced in responding patients. We have recently demonstrated the effects of the histone deacetylase

inhibitor, depsipeptide, on apoptosis using flow cytometry to detect 7-AAD staining (18). This analysis can similarly be applied to patients receiving hypomethylating agents.

Hypomethylating Agents in MDS: Clinical Data

5-Azacitidine was FDA approved based on a randomized, phase III trial comparing 5-azacitidine (n=99) to supportive care (n=92); 7% of patients who received 5-azacitidine had a complete response (CR), 16% had a partial response (PR), and 37% showed evidence of hematologic improvement (HI) (3) (Response criteria used was less stringent than the IWG). Patients who received 5-azacitidine appeared to have an increased time to AML progression (22 months vs. 12 months).

In three European multi-center, phase II trials and one MSKCC phase II trial, decitabine was given to 177 elderly patients (median age 70) at a dose of 45-50 mg/m^2/day for three days every 6-8 weeks. Across those studies, the overall response rate was 49%, including a CR rate of 24%. The median response duration was 9 months (19). Decitabine was subsequently compared to supportive care in a multi-center, randomized, phase III trial in North America (20). In the phase III trial, decitabine was given as an inpatient, 15 mg/m2 IV every eight hours for 9 consecutive doses (135 mg/m2/cycle) every 6 weeks, which was the same dose and schedule given to the majority of the decitabine phase II participants. The overall response rate (CR + PR) was (15%) compared with a 0% ORR in the supportive care arm, with an 8% CR rate (IWG Response Criteria). The median time to death or AML progression (composite endpoint) overall was 491 days vs. 274 days, and survival was prolonged in responders (657 days) compared to non-responders (384 days). Patients with IPSS High-Risk disease and Intermediate-2 Risk were shown to have a statistically significant prolongation in the time to AML progression or death (12 months vs. 6.8 months, p= .03), the primary endpoint of this phase III trial. The difference was even greater when IPSS High-Risk cohort was analyzed and the treatment arm was compared to supportive care (time to AML progression or death 9.3 months vs. 2.8 months, p = .01). In another sub-group analysis, clinical response (PR + CR), a secondary endpoint, was significantly improved for the Intermediate-2 Risk group (21% vs. 0%, p = .005). The response rate for IPSS High-Risk patients was 13% (p = .234).

In a more recent three-arm, phase II study, 100 mg/ m2 decitabine was given every 4 weeks using three different outpatient schedules: 1) 20 mg/m2/day intravenously for 5 consecutive days; 2) 10 mg/m2 per day for ten days; 3) 20 mg/m2 subcutaneously for five days (21). The 5-day intravenous schedule produced a complete response rate (modified IWG criteria) of 40%, which represents a potential substantial improvement in the response rate seen for the three day schedule used previously in the bulk of the phase II studies and in the randomized phase III trial.

All-Trans Retinoic Acid (Tretinoin)

MSKCC has played a major role in demonstrating that the administration of tretinoin to patients with APL greatly improves survival. In APL, retinoic acid (RA) can usually de-repress promoters of RA target genes by blocking transcriptional inhibition caused by the PML/RARα fusion protein. Repression by PML/RARα is due to the recruitment of co-repressor

complexes, which contain histone deacetylases (HDACs), and DNA methyltransferases. With

tretinoin treatment, the block to granulocytic maturation is relieved, and granulocytic maturation proceeds normally.

Tretinoin can induce cell-cycle arrest of myeloid, non-APL leukemia cells. When 9 of 13 primary blast cultures were exposed to tretinoin >95% growth inhibition was seen (22). In this report, sensitivity to tretinoin correlated significantly with an increase in tretinoin-induced RARβ receptor expression. In other studies, tretinoin induced apoptotic cell death of non-M3 AML blasts at a concentration of 10e6 mol/l, which is clinically achievable at a dose of 45 mg/m2/day (23), over a 72 hour exposure time (24).

Anecdotal reports of non-M3 AML patients with clinical responses to tretinoin monotherapy with associated terminal differentiation suggest that some patients with MDS and AML may respond to tretinoin either alone or in combination with other agents as a result of a differentiation effect (25). There are also anecdotal reports of MDS patients responding to tretinoin monotherapy.

Decitabine in combination with Tretinoin

Decitabine was shown to activate the expression of the RARβ receptor gene in DLD-1 colon cancer cells, and more recently, to inhibit methylation at two specific RARβ promoter CpG sites in colon and breast cancer cells (26, 27). Reversal of RARβ gene promoter methylation has been studied in non-M3 AML cell lines (28). In these studies, Di Croce et. al. showed that RARβ2 promoter methylation decreased to a greater degree (75%) with the combination of decitabine and tretinoin, compared to tretinoin or decitabine alone (~ 50%) (28). The demethylation and increased RARβ2 expression was associated with morphologic evidence of differentiation.

This effect was also demonstrated in non-M3 AML by Trus, et al. (29). Tretinoin alone induced little or no evidence of differentiation or RARβ upregulation, but when OCI/AML-2 cells and non-M3 primary cultures were incubated with decitabine before exposure to tretinoin, differentiation was seen (upregulation of CD11b and Cd14), as was upregulation of RARβ. Similarly, Niitsu et. al. studied the response of three non-M3 AML cell lines to tretinoin alone, or tretinoin combined with decitabine. All three non-M3 AML cell lines studied were effectively induced to differentiate by the combination.[30] The combination of tretinoin and decitabine may therefore induce additive or synergistic differentiation activity.

Tretinoin has also been shown to induce apoptosis in *in vitro* systems (see Section 3.0, "Tretinoin" sub-section) and demethylation of tretinoin-responsive (30) after tretinoin, then any apoptosis induced by tretinoin may abrogate the demethylating and cytotoxic effects of decitabine, which require S-phase and, at least for the demethylation effect, a minimum of 3-5 days (31-33). With the exception of Oki, et al., the "kinetics" of decitabine-induced demethylation has primarily been studied in *in vitro* systems, as cited above. Interestingly, the data presented by Oki et al. indicate that the maximal demethylating activity in patient samples after decitabine administration may be closer to 12 days, since demethylation was tested in patient samples at 5, 12, and 26 days after decitabine monotherapy was started, and the relative decrease in demethylation was greatest at day #12. These data appear to support the need for decitabine treatment to precede the start of tretinoin by possibly up to 12 days.

Although these effects have largely been explored in AML cell lines and AML primary cultures, it is possible that in MDS, upregulation of the RARβ receptor by demethylation may enhance the sensitivity of blast cells to the growth inhibitory or differentiation effects of tretinoin. In this way, decitabine may induce a more "permissive" environment for the effects of tretinoin by activating genes required for differentiating or apoptosis "programs", and allowing access of transcriptional regulatory complexes to DNA.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a phase I/II study designed to evaluate the safety, biologic effects, and preliminary clinical efficacy of the combination of decitabine and tretinoin. Approximately 45 eligible patients with a myelodysplastic syndrome will receive the combination of decitabine and tretinoin. Decitabine will be administered at a dose of 20 mg/m2/day for five days every 4 weeks. Tretinoin will be administered from days 10-19 of each cycle in escalating doses to determine the MTD of Tretinoin in combination with decitabine.

The initial dose level will be 45 mg/m2/day, which is 30% of the MTD for Tretinoin in MDS patients (MTD = 150 mg/m^2)(34), and is a dose that has been shown to produce apoptosis in non-M3 AML(23), and differentiation in APL. This is also the standard dose of tretinoin that has been administered safely to patients with acute promyelocytic leukemia in combination with cytotoxic chemotherapy. Three dose levels will be tested, with the maximum dose being 85 mg/m2/day. The rationale for this design is that tretinoin given at doses of 90 mg/m2/day or greater is more likely to produce side effects (primarily skin and mucosal toxicity) that we wish to avoid in this generally elderly patient population. Also, decitabine is expected to induce myelosuppression, and infection risk may be increased by compromised mucosal integrity. If 2 patients experience DLT at 45 mg/m2/day, a dose de-escalation scheme will be employed to determine the MTD below 45 mg/m2/day. Once the MTD is established, additional patients (to equal 6 patients) will be treated to confirm the MTD. If dose escalation proceeds to 85 mg/m2/day, and no DLT is seen in three patients, an additional 3 patients will be treated at this dose level to confirm this dose as the maximal tolerated dose. Once the tretinoin MTD is determined, the phase II portion of the study will proceed with tretinoin at the MTD in combination with decitabine.

The phase II study will have a 2-stage design. In the first stage of this design, twenty seven patients will be treated with decitabine (20 mg/m2/day x 5 days) and tretinoin, using the tretinoin MTD established during the phase I portion of the study. Patients treated at the tretinoin MTD (6 patients) in the phase I portion of the study will be carried over and counted among the patients in stage 1. If two or fewer responses (CR or PR) are seen among the first 27 patients, the study will be closed for lack of efficacy. If at least three responses are seen in the first stage, then an additional 13 patients will be accrued in the second stage.

Prior to treatment, blood and bone marrow samples will be obtained for baseline research studies. At defined time points after the start of the treatment, blood and bone marrow samples will be obtained for research studies as well. Research studies (at all time points) will include flow cytometry to analyze for evidence of differentiation and apoptosis. In addition, gene promoter methylation studies and Affymetrix gene profiling will also be performed. Patients will be monitored for clinical response. Clinical responses will be correlated with the laboratory studies.

4.3 Intervention

Decitabine will be administered by intravenous infusion, at a dose of 20 mg/m2 per day for 5 days as an outpatient. Beginning on day 10 of each cycle, tretinoin will be self-administered daily for 10 days. Tretinoin will be given using a dose escalation scheme to determine the MTD of tretinoin in combination with decitabine during the phase I portion of the study. Tretinoin and decitabine are commercially available and the cost of this medication will be charged to the patient and/or their insurance company.

The rationale for this intervention is that the addition of tretinoin, an established differentiating agent, may enhance the differentiation effects seen with decitabine alone, and possibly increase the response rate over decitabine alone and/or decrease the time to response. Tretinoin may also induce apoptosis based on *in vitro* studies, and therefore, will be started on day 10. It is anticipated that any apoptotic effect of tretinoin (if this effect is seen) will be induced after maximal demethylation has been achieved. Demethylation and/or re-expression of specific genes, and/or flow cytometric evidence of differentiation and apoptosis may be surrogate markers for clinical response and these parameters will be studied after exposure to decitabine and again after treatment with tretinoin. Correlation of these analyses with clinical responses will assist in gaining a better understanding of the mechanism of action of decitabine and tretinoin in MDS patients.

5.1 THERAPEUTIC/DIAGNOSTIC AGENTS

5.2 Decitabine (5-aza-2'-deoxycytidine)

Description: 5-aza-2'-deoxycytidine is an analog of 2'-deoxycytidine with a nitrogen atom substituted for carbon at the 5-position of the ring.

Other names: Dacogen (™)
DAC
Deoxyazacitidine

Drug supply and distribution: Decitabine is formulated and commercially supplied by MGI Pharma.

How supplied: Lypholized powder for injection, 50 mg in 20 ml vials.

Storage: Unopened vials are to be stored at controlled room temperature per USP and are stable for 3 years. Reconstituted decitabine should be stored for no longer than 7 hours at 2-8 °C.

Solution Preparation: Each vial is reconstituted with 10 ml of Sterile Water for Injection, USP. One ml of reconstituted solution will contain 5 mg of decitabine at a pH of 6.8-7.0. This reconstituted solution can be further diluted to the desired concentration using infusion fluids such as 0.9% Sodium Chloride, 5% Dextrose Injection, or Lactated Ringers to a final drug concentration of 0.1-1.0 mg/ml. Unless used within 15 minutes upon reconstitution, the diluted solution must be prepared using cold infusion fluids and stored at 2-8°C (36-47°F) for a maximum of 7 hours prior to administration.

Stability: The concentration of decitabine decreases over time once the lyophilized preparation is reconstituted. Therefore, the sterile water used to reconstitute the lyophilized preparation and the 0.9% Sodium Chloride (or 5% Dextrose or Lactated Ringer's) diluent should be between 2-8 °C to minimize decomposition. Reconstituted decitabine should be stored at refrigerator temperatures for no longer than 7 hours prior to administration.

Decitabine will be administered intravenously at a dose of 20 mg/m^2 daily for 5 days. The infusion period is over one hour (no greater than 3 hours for solution that has been kept at 2-8 °C for 7 hours or less).

Precautions: Skin contact with the solution should be avoided and protective gloves should be worn. Spilled drug can be inactivated by a 2 M sodium hydroxide solution. Exposed skin can be treated with a borax buffer solution pH 10, to be followed by a thorough washing with soap and water.

Mechanism of Action: Decitabine is a cytosine nucleoside analog that induces inhibition of DNA methylation. This effect was first demonstrated by Jones and Taylor in 1980 (7). More recent investigations have determined that this effect appears to be brought about by the trapping of DNA methyltransferases (DNMT) (35). After the DNMTs are trapped, they are degraded. DNA hypomethylation has been linked to activation of gene transcription in many human and animal cell systems. Moreover, inhibition of DNA methylation has been associated with cellular differentiation in studies of hematopoietic cell systems (7-9).

The MTD for 5-aza-2'deoxycytidine was established in phase I studies to be 1,500 – 2,250 mg/m^2 per cycle. At these higher doses, there was significant myelosuppression and infectious complications, which proved to be dose-limiting. In addition to the apparent cytotoxicity that causes myelosuppression, a cytotoxic effect was also noted in various *in vitro* systems as well (36), where, at minimal cytotoxic concentrations, greater cell kill was seen with sustained

exposure to 5-aza-2'deoxycytidine (37, 38). Therefore, a second mechanism of action may be cytotoxicity, even at the lower doses proposed for this study. The cytotoxic effect follows the incorporation of the drug into newly synthesized DNA, and therefore, appears to be S-phase specific. Interestingly, 5-aza-2'deoxycytidine does not block the progression of cells from G1 into S-phase (in contrast to cytosine arabinoside and 5-azacytidine), suggesting that its cytotoxic activity is not self-limiting (39).

5.3 All-Trans Retinoic Acid (Tretinoin)

Description: Tretinoin is a retinoid derived from naturally occurring all-trans-retinol (Vitamin A1). The compound is formed *in vivo* from retinol and can be detected at nanogram levels in normal and human plasma (40). Tretinoin binds to serum albumin, is not stored in the liver, and probably enters cells by diffusion. In the cytoplasm, Tretinoin binds to cellular retinoic acid binding proteins (CRABPs) and is transported to the nucleus (41), where it is released and binds to nuclear RARs. The signaling pathway after ligand-RAR binding is poorly characterized. Isomers of tretinoin including 9-cis RA and 13-cis RA, as well as vitamin A, β-carotene and various synthetic retinoids, have been clinically studied for anticancer and /or cancer chemoprevention activity.

Other names: Vesanoid ®

Drug supply and distribution: Tretinoin is manufactured and distributed by Hoffman LaRoche.

How supplied: 10 mg capsules

Storage: Store at 15 to 30 degrees Celsius (59 to 86 degrees F) and protect from light.

Precautions: Tretinoin is teratogenic and fetotoxic in rats at doses 1000 and 500 times the topical human dose, respectively. Tretinoin is contraindicated in persons sensitive to parabens, vitamin A, and other retinoids.

Mechanism of Action: Numerous reports have documented tretinoin induced differentiation of APL cell in vitro. Retinoids are known to induce granulocytic differentiation and inhibition of clonal growth in the HL-60 cell line (42). Both tretinoin and 13-cis-RA isomers are active in HL-60 at approximately 10-6 M. Response to retinoids in other leukemia cell lines has been less consistent. KG-1 cells showed growth inhibition at 10-9 M but exhibited no morphologic, histochemical or functional differentiation (43). K-562 cells treated with 10-6 M tretinoin displayed a normal growth pattern. U937 cells underwent monocytoid differentiation at 10-6 M and THP-1 cells showed functional (but not morphologic) differentiation at 10-9 M (43). Tretinoin has produced dramatic clinical benefit in patients with APL, and as a single agent has significantly survival in this disease (44). The drug is commercially approved for this indication.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

- Diagnosis of a myelodysplastic syndrome according to the FAB classification system, which has been confirmed at MSKCC
- Age greater than or equal to 18 years
- IPSS score of greater than or equal to 0.5.
- Performance Status of $\geq$ 60 % (Karnofsky)
- Serum total bilirubin $\leq$ 2.5
- SGOT and SGPT should be $\leq$ 2 times the upper limit of normal.
- Serum creatinine should be $\leq$ 1.5 x ULN, or 24 hour creatinine clearance must be $\geq$ 60 cc/min.
- Negative pregnancy test for women with child-bearing potential.
- Female patients with child-bearing potential or male patients who are sexually active must agree to use an accepted form of contraception.
- Signed informed consent
- Transplant ineligible prior to therapy
- Prior 5-azacytindine recipients are eligible regardless of prior response to 5-azacytidine.
- Prior decitabine recipients must have had a response (HI or better per IWG Response Criteria), followed by POD (POD after decitabine was discontinued).
- Patients will be eligible for entry if they have IPSS Intermediate-1, Intermediate-2 or High Risk disease.

6.3 Subject Exclusion Criteria

- Pregnant or lactating women. Patients with childbearing potential will be required to practice an accepted mode of birth control.
- Cytotoxic chemotherapy or radiotherapy within 4 weeks of starting treatment
- Experimental therapy within 4 weeks of starting treatment
- Medical condition which, in the opinion of the treating physician, would put the patient at excessive risk of toxicity from the treatment
- Inability to adhere to treatment or monitoring schedule
- Unwillingness to provide research specimens as outlined in the protocol
- Presence of another malignancy which will likely require systemic chemotherapy within 4 months of starting decitabine and tretinoin.
- Decitabine refractory MDS
- Allergy to parabens, vitamin A, or retinoids

7.0 RECRUITMENT PLAN

The study will be conducted at MSKCC. The study will accrue approximately 45 evaluable patients. Approximately 200 new patients with a diagnosis of MDS are seen annually by physicians on the Hematology, Leukemia and Allogeneic Bone Marrow Transplant Services. It is estimated that 2.0 years will be required to accrue ~ fifty eligible patients. Efforts will be made to enroll women and minorities onto this trial. It is expected that the sex and racial breakdown of patients entered into this trial will reflect the overall referral patterns seen at MSKCC. However, because of the small number of patients entered into this trial, an absolutely representative cohort may not be accrued.

8.1 PRETREATMENT EVALUATION

8.2 Pre-study Evaluation (Eligibility Assessment)

Eligibility evaluations to be performed within 30 days of starting therapy with decitabine and tretinoin will include the following:

- Bone marrow aspirate for histology, cytogenetics, flow cytometry, and a bone marrow biopsy.
- Baseline bone marrow aspirate will also be obtained for research studies. Therefore patients will be consented prior to their baseline bone marrow procedure.
- Physical exam with review of systems, medical history, Karnofsky performance status, , FAB classification, history of prior medications, prior MDS treatments, history of transfusions requirements for two months prior to registration.
- Determination of elements of IPSS (bone marrow aspirate and complete blood count), which produces IPSS score of 0.5 or greater.
- Electrocardiogram (EKG)
- Comprehensive metabolic panel, CBC with differential and smear review, reticulocyte count, pregnancy test for women with child-bearing potential.
- Assessment of ability of the patient to comply with the requirements of the protocol.

8.3 Baseline evaluations

Baseline evaluations to be performed within 7 days of starting therapy with decitabine and tretinoin will include the following:

- Interim medical history including review of systems, height, weight, vital signs, and calculation of body surface area, medications, transfusion requirements, Karnofsky performance status.

- Comprehensive metabolic panel, CBC with differential and smear review, reticulocyte count, PT, PTT, pregnancy test for women with child-bearing potential.
- Patients must meet all eligibility criteria at the time of the baseline evaluation.

9.0 TREATMENT/INTERVENTION PLAN

Eligible patients will receive Decitabine at a dose of 20 mg/m2 daily for 5 days as an outpatient and will receive a total dose per cycle of 100mg/m2. Beginning on day 10 of each cycle, patients will self-administer tretinoin for 10 days (See Figure 1.1). Treatment will be recycled every 28 days. The tretinoin dose will be escalated within the range of 45 mg/m2/day to 85 mg/m2/day to determine the MTD within that range. Although unlikely, if DLT is seen in two patients at 45 mg/m2/day, a dose de-escalation scheme will be employed to explore the safety of 30 mg/m2/day and 15 mg/m2/day.
The tretinoin dose escalation will proceed as follows: Three patients will receive 45 mg/m2/day given over two doses each day (standard dose used for acute promyelocytic leukemia alone or in combination with cytotoxic chemotherapy). If no dose-limiting toxicity is observed (see section 11.5 for definition of DLT) after completion of one cycle of therapy, three additional patients will receive 65 mg/m2/day given over two doses each day. If no dose-limiting toxicity is observed after one cycle of therapy, three additional patients will receive 85 mg/m2/day given over two doses each day. Doses above 85 mg/m2/day will not be tested in this trial. Based on prior studies with tretinoin monotherapy, toxicity that is unacceptable for the planned patient population is expected at or beyond a daily dose of 90 mg/m2, and biological effects of tretinoin can be expected as doses lower than 85 mg/m2/day. A DLT in one patient at any of the three dose levels tested will require the addition of three patients at that dose level. If DLT recurs in a second patient (2/6), the next lower dose level will be considered the MTD. If no DLT is seen amongst the three patients treated at the highest tretinoin dose tested (85 mg/m2), an additional 3 patients will be treated at this dose level to confirm this dose as the maximal tolerated dose.
If required, the tretinoin dose de-escalation schema will proceed as follows: If two patients experience DLT at 45 mg/m2/day during the first cycle of therapy, and the toxicities are likely attributable to tretinoin, patients will be enrolled to receive tretinoin at a dose of 30 mg/m2/day, and 15 mg/m2/day if 30 mg/m2/day is not tolerated (if 2/6 patients experience DLT). If 15/mg/m2 is not tolerated (2/6 patients experience DLT), the study will be terminated due to excessive toxicity of the regimen.

Once the MTD is established, patients will be accrued to the phase II portion of the study which will evaluate the efficacy of decitabine in combination with the MTD of tretinoin. The phase II study will have a 2-stage design. In the first stage of this design, twenty seven patients will be treated. Patients treated at the tretinoin MTD (6 patients) in the phase I portion of the study will be carried over and counted among the patients in stage 1. If two or fewer responses (CR or PR) are seen among the first 27 patients, the study will be closed for lack of efficacy. If at least three responses (CR or PR) are seen in the first stage, then an additional 13 patients will be accrued in the second stage. To qualify as a non-responder, patients must undergo disease re-evaluation studies (bone marrow and complete blood counts) after completing four cycles of therapy.

Treatment will be recycled every 28 days, provided there is adequate recovery from non-hematologic toxicities. The start of cycle #1, or subsequent cycles may be delayed for up to 2 weeks in the event of 1) uncontrolled infection, or 2) other abnormalities that would preclude patient eligibility (see sections 6.1 and 6.2).

Patients will receive treatment for a minimum of 4 cycles, barring removal for progression of disease or excessive toxicity. Patients must achieve hematologic improvement (HI) or Bone Marrow PR/CR by the completion of cycle #6. Patients achieving HI or bone marrow PR/CR by the completion of cycle #6 may receive up to a total of 10 cycles of therapy, barring excessive toxicity. If the best response after cycle #6 is Stable Disease, these patients will be removed from study. Patients in the phase I study will continue at the study entry dose. Treatment will be interrupted for grade III or IV non-hematologic toxicity attributable to tretinoin or decitabine. Therapy may be resumed with the next cycle of treatment as long as the toxicity has decreased to < grade 2 within 8 weeks of the last treatment administration. However, these patients will receive a 25% dose reduction in tretinoin or decitabine (which ever treatment is suspected or both if the attribution is unknown) when treatment resumes. All patients will be followed for Serious Adverse Events (SAEs) for 30 days after completion of the last cycle of therapy (each cycle is 28 days).

Effects of decitabine and tretinoin on markers of differentiation and apoptosis, and on the methylation and expression of various genes will also be assessed.

Myeloid growth factors will only be allowed in the setting of febrile neutropenia according to established guidelines for use (45).

10.1 EVALUATION DURING TREATMENT

10.2 Safety Monitoring:

Weekly while on study: Complete blood count (including differential)

Within 7 days of starting each cycle: CBC, Comprehensive metabolic panel, pregnancy test for patients with child-bearing potential, physical exam, assessment of performance status, medications and adverse events. Patients will be asked to maintain a symptom diary each cycle, which will be reviewed prior to starting each subsequent cycle. Patients will keep a tretinoin administration log, which will be reviewed with the patient. A pill count will be performed and correlated with the patient's tretinoin administration log to assess compliance with tretinoin administration. Physical exam and toxicity assessments may be required more frequently if clinically indicated.

10.3 Tests for Disease Response:

Complete blood count with differential and smear review prior to start of all treatment cycles.

Bone marrow aspirate and biopsy after even-numbered cycles (eg., 2, 4, 6, etc). Bone marrow aspirate studies for disease assessments will include flow cytometry, cytogenetics, and histochemistry.

10.4 Research Samples:

Research samples will be obtained on patients treated in the phase I and the phase II portion of this study at the following time points:

(1) Prior to treatment (during the screening period when screening baseline bone marrow and bloodwork is obtained), blood (30 cc in heparin) and bone marrow aspirate (10 cc in heparin) will be obtained.

(2) During cycle #1 and cycle # 2, on days 10 and 19, blood (30 cc in heparin) will be obtained for research purposes only (blood to be drawn with weekly routine blood work).

(3) During cycle #1 only, on days 10 and 19, bone marrow aspirate (10 cc in heparin) will be taken for research purposes only.

(4) At the time of scheduled disease assessments done after cycles 2 and 4, blood (30 cc in heparin) and bone marrow aspirate (10 cc in heparin) will be obtained.

10.5 Research Tests:

The following research studies will be performed at the time points indicated in 10.3 when feasible:

(1) Bone marrow aspirate and peripheral blood mononuclear cells will be assessed for the extent of DNA methylation using array-based whole genome methylation analysis(NimbleGen) (46). The expression and methylation status of specific genes will be analyzed, focusing on, but not necessarily restricted to the following genes involved in the TGFB signaling pathway: p57, p 21, p73, p15INK4b, TGFβRII, RARβ, and JunB.

(2) Bone marrow aspirate and peripheral blood mononuclear cells will be analyzed using gene profiling (Affymetrix) which will be performed by the Core Facility Laboratory.

(3) Bone marrow aspirates and peripheral blood mononuclear cells will be analyzed by flow cytometry for evidence of differentiation using fluorescein isothiocyanate (FITC) - conjugated or phycoerythrin (PE)-conjugated monoclonal antibodies: CD16, CD11b, CD33, CD13, HLA-DR, CD34, CD45 and CD14. Combinations to be examined to assess for differentiation include CD33-PE and CD34-FITC, CD33-PE and CD11b-FITC, CD13-PE and CD34-FITC or CD11b-PE and CD34-FITC. Apoptosis will be assayed using flow cytometry and dual staining with Annexin V-PE and 7-AAD.

11.1 TOXICITIES/SIDE EFFECTS

11.2 Expected Side Effects of Decitabine

Common – Myelosuppression, nausea, vomiting.
Uncommon – Diarrhea, fever, anorexia, abdominal pain, mucositis, alopecia.
Rare – Death.

11.3 Expected Side Effects of Tretinoin

Common – Headache; dryness, scaling or flaking of skin; dry mouth and mucous membranes; cracking of lips; hyperlipidemia.
Uncommon – Increase in hepatic transaminases; hypercalcemia; nausea and vomiting;
Rare – Pancreatitis

11.4 Adverse Event Reporting

Adverse events will be followed after the patient has received their first dose of therapy on this trial, and will continue until 30 days after the completion of the last cycle of therapy. Toxicity will be graded and recorded using the NCI Common Toxicity Criteria version 2.0, to allow the use of the "leukemia" scale for grading hematologic toxicity.

Clinically significant adverse events will be noted and compared to the baseline condition. Adverse events that will be noted will include expected side effects, and any other clinically significant symptom or laboratory value. An assessment will be made concerning the severity of the event, and if the event appears to be related to the study drug(s). Adverse events, toxicity grading, and attributions will be recorded in the CRDB by the RSA after review of the data by the Principal Investigator. Hematologic toxicity will be graded using the "leukemia" grading scale in the NCI CTC version 2.0.

11.5 Serious Adverse Event (SAE) Reporting

An SAE is defined as any adverse effect occurring in a patient receiving the study drug(s) that results in the following:

- Death
- A life-threatening illness or abnormality
- Hospital admission or prolongation of pre-existing hospitalization
- Persistent or debilitating disability
- Birth defect

Please refer to Section 17.2 and 17.2 for further SAE reporting guidelines.

11. 5 Establishment of Maximum Tolerated Dose (MTD)

In the phase I portion of this study, the MTD of tretinoin will be determined (within the range of 15 mg/m2 - 85 mg/m2) when given in combination with decitabine. At least three patients will be treated at each dose level, starting with 45 mg/m2/day, which is 30% of the MTD (in a phase I MTD of tretinoin monotherapy in MDS patients, the MTD was 150 mg/m2/day when given daily for 6 weeks (34)). Additional dose levels will be tested as follows: 65 mg/m2/day and 85 mg/m2/day.

A dose-limiting toxicity is defined as a grade III or IV non-hematologic toxicity (NCI CTC, ver. 2.0). If at any dose level one patient experiences grade III or IV non-hematologic toxicity (according to the NCI CTC version 2.0) attributable to tretinoin, three additional patients will be added to that dose level. If a second patient experiences grade III or IV non-hematologic toxicity, the dose level below will be considered the MTD, which is defined as the dose level below that which produces Grade III/IV non-hematologic toxicity in $\geq$ 33% of patients.

If a DLT is experienced at 45 mg/m2/day and is attributable to tretinoin, a dose de-escalation schema will be employed (see section 9.0) to determine the MTD below 45 mg/m2/day.

If no DLT is seen after the 85 mg/m2/day dose level is complete, the phase II portion will proceed using tretinoin at a dose of 85 mg/m2/day. However, once the MTD is established, a total of 6 patients will be treated at that level to confirm the MTD before proceeding to the phase II portion of the study.

12.1 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Response will be determined by the MDS International Working Group criteria (47).

12.2 <u>Complete Remission (CR)</u>

Bone marrow evaluation: Repeat bone marrow showing 5% or less myeloblasts with

normal maturation of all cell lines. Persistent dysplasia will be noted. (*)

Peripheral Blood: Absolute values must last at least 1 months. In some circumstances, protocol therapy may require the initiation of further treatment before the 1 month period. Such patients can be included in the response criteria into which they fit at the time therapy is started. Transient cytopenias during repeated chemotherapy courses should not be considered as interrupting durability of response, as long as they recover to the improved counts of the pervious course.

Hgb $\geq$ 11 g/dL (untransfused, patient not on erythropoietin)
Neutrophils $\geq$ 1500/mm3 or more (not on myeloid growth factor)
Platelets $\geq$ 100,000/mm3 or more (not on thrombopoietic factor)
Blasts 0%

(*) Dysplastic changes should consider the normal range of dysplastic changes (modification).

12.3 Partial Remission (PR)

Absolute values must last at least 1 months unless interrupted by therapy as described above.

All the CR criteria (if abnormal before treatment) except:

Bone marrow evaluation: Blasts decreased by 50% or more over pretreatment, but still more than 5 %.. Cellularity and morphology are not relevant

12.3 Marrow CR

Bone Marrow: 5% myeloblasts or less and decrease by 50 % or greater over pretreatment.

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

12.4 Stable Disease

Failure to achieve at least a PR, but with no evidence of progression for at least 2 months

12.5 Failure

Death during treatment or disease progression characterized by worsening of cytopenias, increase in the percentage of bone marrow blasts, or progression to an MDS FAB subtype more advanced than pretreatment. Progression to AML (30% or more bone marrow blasts)

12.6 Relapse after CR or PR

One or more of the following:

a) Return to pretreatment bone marrow blast percentage
b) Decrement of 50% or greater from maximum remission / response levels in granulocytes or platelets
c) Reduction in hemoglobin concentration by at least1.5 g/dL or new/recurrent transfusion dependence (in the absence of another explanation such as treatment effect, acute infection, gastrointestinal bleeding, hemolysis and so on)

12.7 Disease Progression

a) For patient with less than 5% blasts: a 50% or more increase in blasts to more than 5% blasts
b) For patients with 5% to 10% blasts: a 50% or more increase to more than 10% blasts.
c) For patients with 10% to 20% blasts: a 50% or more increase to more than 20% blasts
d) For patients with 20% to 30% blasts: a 50% or more increase to more than 30% blasts.
e) One or more of the following: 50% or greater decrement from maximum remission/response levels in granulocytes or platelets, reduction in hemoglobin concentration by at least 2 g/dL, or transfusion dependence (in absence of another explanation such as acute infection, G.I. bleeding, hemolysis etc.)

12.8 Hematologic Improvement (HI)

Improvements must last at least 2 months in the absence of ongoing cytotoxic therapy. When protocol therapy requires the initiation of further treatment before the 2 month period, patients can be included in the response criteria into which they fit at the time therapy is started.

Hematologic improvement should be described by the number of individual, positively affected cell lines (eg, HI-E; HI-E + HI-N; HI-E + HI-P + HI-N).

1. Erythroid response (HI-E)
For patients with pretreatment hemoglobin less than 11g/dL: greater than 1.5 g/dL increase in hemoglobin, relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC/8wk compared with the pretreatment transfusion number in the pervious 8 wk. (*)

(*) Only RBC transfusions given for Hgb > 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation.

2. Platelet response (HI-P)

For patients with a pretreatment platelet count less than 100 000/mm³: an absolute increase of 30 000/mm³ or more for patients starting with more than 20 000/mm^3 platelets, an increase from less than 20 000/mm^3 to greater than 20 000/mm^3, and by at least 100%.

3. Neutrophil response (HI-N)
For patients with pretreatment neutrophil count less than 1 000/mm3: at least a 100% increase and an absolute increase of more than 500mm³.

4. Progression/relapse after HI
One or more of the following: A 50% or greater decrement from maximum response levels in granulocytes or platelets, a reduction in hemoglobin concentration by at least 1.5 g/dL, or transfusion dependence (in the absence of other explanation, such as hemolysis, hemorrhage, etc).

13.0 CRITERIA FOR REMOVAL FROM STUDY

13.1 Progressive disease and transformation to acute leukemia (Response criteria are defined in section 12.0)
13.2 Failure to achieve at least a PR after 6 cycles of treatment
13.3 Unacceptable toxicity defined as grade III or IV non-hematologic toxicity requiring greater than 8 weeks to return to < grade 2 (NCI CTC version 2.0).
13.4 Patient's withdrawal of consent for any reason
13.5 Patient non-compliance with the treatment and monitoring plan
13.6 Patient death

14.1 BIOSTATISTICS

Phase I Component: The following design will be used to determine the maximum tolerated dose (MTD) of tretinoin in combination with decitabine. Three dose levels of tretinoin will be investigated (45 mg/m2/day, 65 mg/m2/day, and 85 mg/m2/day). Patients will be treated in cohorts of size three to six and the dosage will be escalated if the clinical toxicity is acceptable. Dose-limiting toxicity (DLT) is defined as a grade 3-4 non-hematologic toxicity observed within the first cycle of treatment. The design is constructed to reduce the chance of escalating the dose when the probability of DLT is high, and increase the chance of escalating the dose when the probability of DLT is low. The maximum tolerated dose is defined as the highest dose level with an observed incidence of DLT in no more than one out of six patients treated at a particular dose level. The dose escalation scheme is as follows:

1) If none of initial three patients at a given dose level experience DLT, the next dose level is studied in another cohort of three patients.

2) If one of the initial three patients at a given dose level experience DLT, up to three additional patients will be treated at that same dose level. Escalation will continue if one of the six patients experience DLT.

3) If two or three patients experience DLT in the first three patients, or two or more patients experience DLT in six patients at a given dose level, the MTD will be determined as the preceding dose level.

4) If three or fewer patients are treated at a dose under consideration as the MTD, additional patients to total six will be treated at that level to confirm MTD

The dose escalation scheme provides the following probabilities of escalation based on the true chances of DLT at a specific dose level. One can see that the probability of escalation is high if the toxicity risks are low.

True Probability of Toxicity:	.10	.20	.30	.40	.50	.60
Probability of Escalation:	.91	.71	.49	.31	.17	.08

If 2 patients experience DLT at the first dose level (45 mg/m2/day), a dose de-escalation scheme will be employed, testing doses of 30 mg/m2/day and 15 mg/m2/day. Cohorts of three to six patients will be treated at each dose level, if necessary.

A minimum of two and a maximum of 18 patients could be treated in this portion of the trial. At the end of this study, the phase II component of the trial will begin at the MTD determined. The six patients treated at the MTD will be included as part of the phase II component of the trial.

Phase II Component: The primary objective of the phase II component of the trial is to determine the response rate (defined as Complete Remission or Partial Remission) to the combination of decitabine and tretinoin (with tretinoin administered at the MTD from the phase I component above) in patients with myelodysplastic syndromes. A Simon minimax two-stage design will be utilized, in which a 10% response rate is considered not promising, a 25% response 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 the first stage of this design, 27 patients will be accrued. If at least 3 patients achieve a complete remission or partial remission among these 27 patients, then an additional 13 patients will be accrued to the second stage. If two or fewer responses are seen, the study will be terminated and declared negative. At the end of the trial, the combination will be considered

worthy of further study if 7 or more responses are observed out of 40 patients. This design yields at least a 0.90 probability of a positive result if the true response rate is at least 25% and yields a 0.90 probability of a negative result if the true response rate is 10%.

Additionally, secondary objectives will be examined in patients enrolled into phase I and phase II components of the trial. The proportion of Hematologic Improvements will be recorded. The frequency of transfusions, bleeding and infections will be summarized. Peripheral blood and bone marrow aspirate mononuclear cells will be assessed for the extent of DNA methylation using PCR based technologies. The expression and methylation status of specific genes will be analyzed, focusing on, but not necessarily restricted to the following genes: p57, p 21, p73, p15INK4b, TGFβRII, RARβ, and JunB. In this study we will examine whether methylation of these genes (yes/no) as well as flow cytometric indicators of differentiation and apoptosis, correlate with response to treatment. Associations will be examined using a Fishers exact test. These analyses are exploratory and hypothesis-generating.

In addition, bone marrow and blood mononuclear cells will be analyzed using gene profiling (Affymetrix). In order to attempt to identify genes that are most affected by treatment, a paired sample t-test will be used to rank the genes. Samples pre and post chemotherapy will be paired together and a fold change will be computed for each pair. The log of the fold change will then be used and genes will be scored by how large and consistent the fold change is among the patients. To deal with issues of non-normality of the signal levels p-values will be computed using a permutation algorithm which shuffles the sample labels and recomputes the t-score. To correct for multiple testing the False Discovery Rate (FDR) method will be used to compute rate of false positives. Due to the two-stage design, and uncertainty regarding the rate of disease response, the exact number of samples that will be included in the microarray analysis is unknown.

Frequencies of toxicities based on the NCI Common Toxicity Criteria version 2.0 will be tabulated.

A minimum of 27 patients (including 6 patients from the phase I component) and a maximum of 40 patients will be accrued to the phase II component of this trial. Approximately 3-4 patients will be accrued per month, and hence, the trial will be completed in approximately 2 years.

15.1 PATIENT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Patient Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.
All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations can be phoned in or faxed. The completed signature page of the written consent/verbal script and a completed Eligibility Checklist must be faxed to PPR.

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 Clinical Research Database (CRDB). Source documentation will be available to support the computerized patient record.

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 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.

16.3 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://cancertrials.nci.nih.gov/researchers/dsm/index.html. 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://mskweb2.mskcc.org/irb/index.htm

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, plus there are two institutional committees that 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.

During the protocol development and review process, each protocol will be assessed for it's 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.

17.1 PROTECTION OF HUMAN SUBJECTS

Potential Risks: Based on prior clinical trials (for decitabine, tretinoin) and post-marketing surveillance (tretinoin), the following adverse events can be expected:

Decitabine:
Common – Myelosuppression, nausea, vomiting.
Uncommon – Diarrhea, mucositis, abdominal pain, anorexia, seizures, alopecia, fever.
Rare – Death.

Tretinoin:
Common – Headache; dryness, scaling or flaking of skin; dry mouth and mucous membranes; cracking of lips; hyperlipidemia.
Uncommon – Increase in hepatic transaminases; hypercalcemia; nausea and vomiting;
Rare – Pancreatitis

Benefits: Decitabine has been administered to patients with myelodysplastic syndromes (MDS) in phase II studies where the overall response rate was ~ 50%. A phase III study was performed that compared decitabine on a three day schedule compared to best supportive care. That trial showed a 15% response rate (compared to a 0% response rate for the supportive care arm) with an 8% complete response rate. Responders had a prolonged survival and reduced risk of AML transformation, and this was significant in the IPSS high-risk cohort. A recent small phase II study showed that a 5-day regimen of 20 mg/m2/day every 28 days produced 40% complete responses, which is the schedule that will be used in this study. The rationale for the current study is that the addition of tretinoin to decitabine may improve the response rate and increase the CR rate in patients with MDS over treatment with decitabine alone.

Alternatives: The standard treatment for patients with MDS who are not candidates for an allogeneic stem cell transplant is Supportive Care or 5-azacytidine. Other alternatives include treatment with other agents in other clinical trials.

Incentives: Participation in this trial is voluntary. No incentive, financial or otherwise, will be offered in exchange for participation in this study.

Financial Considerations: All standard tests, physician visits, and hospitalization for treatment and management of treatment/disease complications will be billed to the patient and their insurance company. Both tretinoin and decitabine will be billed to the patient and their insurance company. .

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.

17.3 Serious Adverse Event (SAE) Reporting

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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 the CRDB:

- Subject's name
- Medical record number
- Disease/histology (if applicable)
- Protocol number

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 information:
  - 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.

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.

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20.1 APPENDICES

Appendix I: The International Prognostic Scoring System (2)

BM Blast %	<5%	0.0
	5-10%	0.5
	11-20%	1.5
	21-30%	2.0
Cytogenetics	Good Risk [-Y, del(5q),del(20q), normal]	0.0
	Intermediate Risk [8+, other]	0.5
	Poor Risk [Ch 7 abn., ≥ 3 abnormalities]	1.0
Cytopenias	0-1 cytopenia	0.0
	2-3 cytopenias	0.5

Cytopenias: ANC < 1.5, HGB < 10.0, PLT < 100,000

Calculating the IPSS Score and determining the IPSS risk category:

Using the table above, total the scores obtained for the three individual categories (i.e., cytopenias, cytogenetics and bone marrow blast count).

Depending on the total score, the patient is determined to have IPSS Low, Intermediate-1, Intermediate-2, or High risk MDS.

Total IPSS score of 0 = Low Risk
Total IPSS score of 0.5-1.0 = Intermediate-1 Risk
Total IPSS score of 1.5-2.0 = Intermediate-2 Risk
Total IPSS score of ≥2.5 = High Risk

Appendix II: French-American-British (FAB) Criteria for MDS

Five subtypes of MDS are recognized in the FAB classification system. These subtypes are distinguished by the percentage of myeloblasts, presence or absence of ringed sideroblasts, or a monocytosis as summarized in the following table:

FAB Category	Myeloblast %		% Ringed Sideroblasts	Monocytes>1x 10^9/L
	Bone Marrow	Peripheral Blood	Bone Marrow	Peripheral Blood
Refractory Anemia (RA)	**<5**	**<1**	**<15**	**-**
RA with ringed sideroblasts (RARS)	**<5**	**<1**	**≥15**	**-**
RA with excess blasts (RAEB)	**5-20**	**<5**	**Variable**	**-**

Chronic Myelomonocytic leukemia (CMML)	**≤20**	**<5**	**Variable**	**-**
RAEB in transformation (RAEB-t)	**21-30 or Auer rods present**	**Auer rods present**	**Variable**	+/-

Appendix III: Tretinoin Med Log

TRETINOIN MEDLOG			# OF PILLS	TIME TAKEN	# OF PILLS	TIME TAKEN	INT
1	**MON**						
2	**TUES**						
3	**WED**						
4	**THUR**						
5	**FRI**						
6	**SAT**						
7	**SUN**						
8	**MON**						
9	**TUES**						
10	WED			AM PM		AM PM	
11	THUR			AM PM		AM PM	
12	FRI			AM PM		AM PM	
13	SAT			AM PM		AM PM	
14	SUN			AM PM		AM PM	
15	MON			AM PM		AM PM	
16	TUES			AM PM		AM PM	
17	WED			AM PM		AM PM	
18	THUR			AM PM		AM PM	
19	FRI			AM PM		AM PM	
20	**SAT**						
21	**SUN**						
22	**MON**						
23	**TUES**						
24	**WED**						

25	**THUR**						
26	**FRI**						
27	**SAT**						
28	**SUN**						

Please complete this diary on a daily basis. Write in the number of capsules of Tretinoin you took and the time you took them in the appropriate date box. On the days that you do not take any capsules, please write "0." If you forget to take your daily dose, please write "0", but remember to take your prescribed dose at the next regularly scheduled time

Tretinoin Dose Level: ____ mg (__10mg pills + __5mg pill)

PROTOCOL 06-054: CYCLE 1

HEALTH PROBLEMS/MEDICAL COMPLAINTS:

Please record ALL Health Problems/Medical Complaints you experience below

Please describe what you experienced	Start Date	Stop Date
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		

OTHER MEDICATIONS:

Please record ALL medication other than tretinoin
Including: prescription, over-the-counter, herbal, and vitamins

Medication	Why did you take this medication?	Start Date	Stop Date
1.			
2.			
3.			
4.			

5.			
6.			
7.			
8.			
9.			

*** If you have any questions, please call Dr. Rampal's office at 646-608-3746.**

Appendix IV: Schema

	Pre-treatment		Cycle 1 (Subsequent Odd Cycles)				Cycle 2 (Subsequent Even Cycles)			
	Within 30days	Within 7days	Week1 Day 1-7	Week2 Day 8-14	Week3 Day15-21	Week4 Day22-28	Week1 Day 1-7	Week2 Day 8-14	Week3 Day15-21	Week4 Day21-28
Decitabine			Day1-5				Day1-5			
Tretinoin				Day 10 – Day 19				Day 10 – Day 19		
Informed Consent	X									
Inclusion/Exclusion Criteria	X									
Physical Exam		X				X				X
Medical History		X								
KPS		X				X				X
IPSS	X[3]									
Fab Classification	X									
EKG	X									
Comp		X				X				X
CBC - differential & smear		X	X	X	X	X	X	X	X	X
Reticulocyte Count		X								
B HCG (if applicable)		X				X				X
Basic Metabolic Panel				X				X		
Height/Weight/BSA		X								
Vital Signs		X								
Prior Medications		X				X				X
Transfusion Requirements		X								
PT/PTT		X								
DNA Methylation/Array/Flow on Blood (30 cc in heparin)	X			Day10[1]	Day19[1]			Day10[1]	Day19[1]	X
DNA Methylation/Array/Flow on BM (10 cc in heparin)	X			Day10[2]	Day19[2]					X
Routine Disease Assessment: BMbx, BMA, Cytogenetics,	X									X
Pill Count						X				X
Diary Card Check						X				X
Adverse Events						X				X

[1] Day 10 & Day 19 Research Blood Sample collected only in Cycle 1 & Cycle 2.
- Day 10 Blood Sample: after completing decitabine, but prior to first dose of tretinoin
- Day 19 Blood Sample: after completion of tretinoin.

[2] Day 10 & Day 19 Research Bone Marrow Aspirate Sample collected only in Cycle 1
- Day 10 Bone Marrow Aspirate Sample: after completing decitabine, but prior to first dose of tretinoin
- Day 19 Bone Marrow Aspirate Sample: after completion of tretinoin

[3] Baseline IPSS score needs to be determined to be $\geq$0.5 within 30 days of the initiation of decitabine and tretinoin. Bone marrow cytogenetic results do not need to be resulted at the time of registration if the IPSS score is $\geq$0.5 by other criteria.