Protocol Title: J1240 A phase II study of 5-Azacytidine (5AC) in combination with Sargramostim (GM-CSF) as maintenance treatment, after definitive therapy with either stem cell transplant (SCT) or chemotherapy, in patients with poor-risk Acute Myeloid Leukemia (AML) or Myelodysplastic Syndrome (MDS)

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Investigation Agents:
5-Azacytidine: 5AC (Vidaza)
Sargramostim: GM-CSF: Leukine (GM-CSF)

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1. Objectives

1.1. Primary Objective

The **primary objective** of the trial is to evaluate the two-year relapse-free survival (RFS) of patients with poor-risk Acute Myeloid Leukemia (AML) or Myelodysplasia (MDS), who receive maintenance treatment with 5-Azacytidine(5AC) in combination with GM-CSF during remission, following definitive therapy with either a stem cell transplant (SCT) or consolidation chemotherapy.

1.2. Secondary Objectives

1. Describe and quantify the toxicity profile of the combination of 5AC and GM-CSF
2. Determine the impact on one-year RFS and overall survival for poor-risk myeloid disorders following maintenance therapy with 5AC and GM-CSF
3. Describe the changes in the clonogenic population during maintenance therapy, including changes in detectable cytogenetic abnormalities in blood and marrow.

2. Background

2.1. Introduction

Myeloid disorders remain a challenge for clinicians to effectively treat. Patients with AML, despite good initial responses to standard induction therapy, usually experience long term treatment failure with standard chemotherapy, particularly in patients with poor-risk disease, which includes AML arising out of MDS, treatment-related disease, AML associated with unfavorable karyotypes or FLT3/ITD mutations, and relapsed or refractory disease. Reported five-year survival rates are 15-30% with chemotherapy alone,[1] with survival rates less than 10% in poor-risk AML and only 24% in intermediate-risk disease.[2] In treatment-related AML, median survival is reported at only 10 months, with a 10% five-year overall survival.[3] Relapse is the primary reason for these dismal outcomes: 92% of patients classified as high-risk experience relapse, as do 61% of the intermediate-risk group. Allogenic hematopoietic stem cell transplant (allo SCT) is often the only curative option for poor-risk AML and MDS. Unfortunately, even allo SCT has limited efficacy, with studies demonstrating only 40% survival at four years, [4] with approximately 40% relapse rates.[5-8]

Patients who relapse after allo SCT have a dismal prognosis with a median survival of only three to four months without further therapy, and only twelve months with therapy.[9] This is in part due to the rapid rate of relapse. Studies demonstrate that up to half of all relapses occur within one year after transplant, with fully 80% occurring within 24 months.[6-10] Further contributing to poor survival are the limited treatment options at relapse, which include donor lymphocyte infusion (DLI), a second stem cell transplant, additional traditional chemotherapy, or newer biologically targeted agents (e.g., histone deacetylase inhibitors, DNA methyltransferase inhibitors,FLT3 inhibitors, and others). The only proven curative options are DLI or a second allo SCT, though few patients are candidates for these

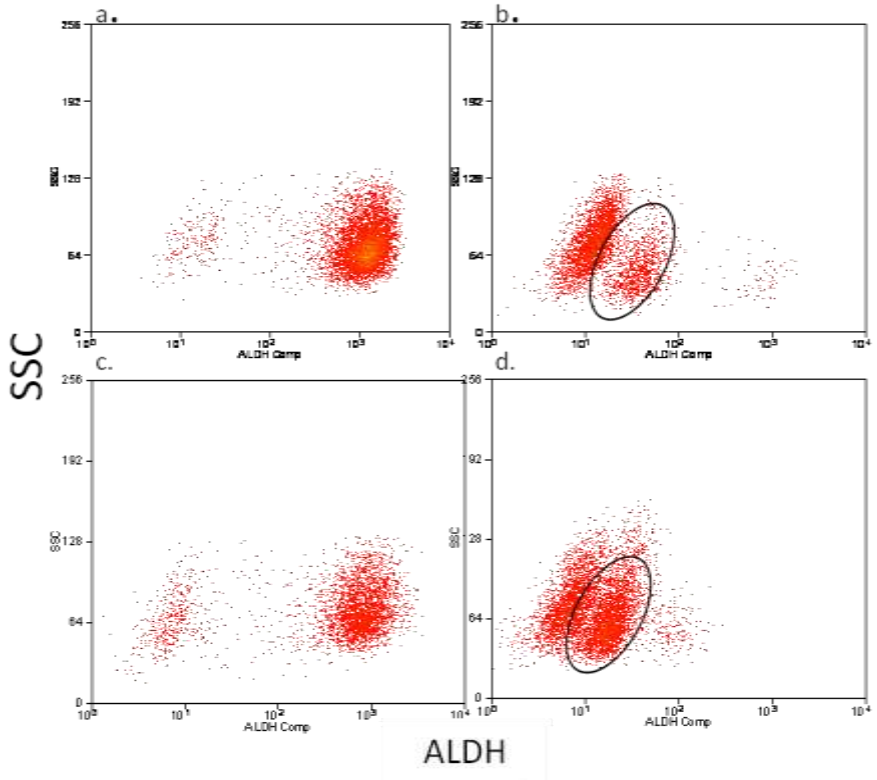
treatments and those that do undergo salvage SCTs have poor outcomes, with median overall survival under 12 months.[11;12] The success of salvage chemotherapy following allo SCT is also limited, as approximately one third of patients achieve a CR, and median disease-free survival (DFS) is just six months.[12] Due to the high relapse rate, the dismal prognosis at relapse, and the speed with which relapse occurs, novel therapies are clearly needed to maintain remission after treatment with allo SCT and traditional chemotherapy. Effective maintenance therapies would need to target and possibly eliminate the undetectable residual disease that is suspected responsible for relapse.

Pediatric patients with AML who relapse after blood or marrow transplantation (BMT) have poor outcomes.[68;69] Therapies preventing relapse would be beneficial in children as well as adults. Pediatric patients with other high-risk malignancies who are in remission after BMT have been treated safely and successfully with regimens that include GM-CSF.[70;71] In these studies, doses of GM-CSF were higher than the proposed maximum allowable dose proscribed below, 125micrograms/m^2, and were tolerated with limited toxicity.

2.2. Cancer Stem Cells

One proposed theory that may explain the high treatment failure and relapse rate following successful initial treatment of leukemia surrounds cancer stem cells. Our group has identified a population of cells that appears responsible for relapse in AML. This population of cells, not identified in normal bone marrows, is present at both diagnosis and relapse in patients with AML. When it is detected in patients in CR, it predicts relapse of their AML (Figure 2). Cells in this population share properties with both normal and leukemia stem cells (LSCs): they are phenotypically and functionally primitive, capable of both self renewal and multi-lineage differentiation; they are quiescent and relatively drug resistant, therefore difficult to eradicate by traditional cytotoxic chemotherapy and SCT regimens.[13-15] These cytotoxic therapies effectively target and eliminate the bulk leukemic blasts cells that are biologically distinct from LSCs. The population of residual LSCs appears to persist even throughout CR and, left untreated, results in relapse. It is this population of putative LSC's that is the proposed target in this study.

Figure 1



Flow analysis of aldefluor expression of $34^{+}38^{neg}$ bone marrow cells from primary patient samples. The circled population, which displays intermediate ALDH expression, is the putative LSC population. It is not identified in normal patient samples (a), but is present in AML at diagnosis (b) and relapse (d), and often disappears during complete remission (c). However when this population is present or reappears during complete remission, patients eventually relapsed.

2.3. Maintenance Therapy in Leukemia

To prevent relapse, therapy must target and eradicate residual leukemia. One proposed mechanism to eradicate the LSC population is to preferentially drive those cells toward differentiation to a more mature progeny and eliminate the self-renewal capacity of the clone. Such differentiated cells are susceptible to apoptosis. Therefore, maintaining pressure towards differentiation would presumably eventually exhaust the clone and its ability to cause relapse. Our group has studied differentiation therapy and the role of combination strategies that are driven by growth factors rather than cytotoxic chemotherapy. This approach may be particularly well suited to maintenance therapy as it activates an important stem cell pathway that renders the progenitor with limited ability to self-renew.[16;17] Such long term, differentiation pressure would be expected to eradicate the malignant stem cell clone. There is evidence that this approach is effective in other leukemias.

Unfortunately, the only leukemias in which maintenance therapy has proven effective are Acute Promyelocytic Leukemia (APL) and low-risk acute lymphocytic leukemia (ALL).[18;19]

Typically, maintenance therapy in leukemia employs agents similar to those in induction therapy, but at lower doses over one to two years. Maintenance therapy for both APL and ALL typically consists of a regimen containing low-dose 6-mercaptopurine (6-MP) and (MTX) for one to two years.[20] Interestingly, the mechanism of action of these agents at these low maintenance doses is unclear. However, our group has shown that 6-MP and MTX differentiate ALL and APL cells *in vitro*.[16] Furthermore, APL maintenance therapy consists of ATRA, a known differentiating agent. This suggests that the mechanism of action of this maintenance therapy may at least in part be through induction of terminal differentiation and subsequent exhaustion of the LSC clone responsible for disease initiation and relapse.

2.4. Differentiation Therapy

We hypothesize that inducing myeloid differentiation of the LSC's over a prolonged course could exhaust the leukemic clone in AML and MDS. There are several types of differentiating agents that are active in myeloid diseases that employ different mechanisms of action, including: MS-275, a histone deacetylase inhibitor(HDACi); Bexarotene, a retinoid X receptor; Azacytidine, a DNA methyltransferase inhibitor; Bryostatin 1, a protein kinase C modulator. In general these act by restoring the capacity for cellular differentiation that the malignant clone has lost.[21] Though these differentiating agents show substantial activity *in vitro*, they are only modestly active clinically. Our group has previously shown that the ability to inhibit cell cycling appears to be a critical property of most pharmacologic differentiating agents regardless of their presumed mechanism of action; moreover, these agents require growth factors to induce terminal differentiation.[22] Most *in vitro* studies are performed with serum, which contains growth factors, which could account for the disparity in results. Our preclinical studies support this, as the addition of growth factors (GM-CSF) results in enhanced differentiation compared to these differentiating agents alone, demonstrated both by a decrease in clonogenic capacity and a change in cellular phenotype.[16;23]

2.5. Methylation Affects Differentiation in Myeloid Malignancies

The DNA methyltransferase inhibitors act via hypomethylation and epigenetic modulation. Studies have demonstrated that methylation of CpG dinucleotides in the promoter regions of cell regulatory genes is associated with gene silencing, and these changes are present in a variety of cancers, leading to loss of differentiation capacity and other critical functions.[21] Promoter methylation of this type is relatively cancer-specific, making DNA methylation an attractive target for oncology therapeutics. In malignant myeloid cells the most widely reported methylated gene is the cyclin dependent kinase inhibitor $p15^{INK4B}$, with methylation of this gene reported in up to 68% of primary AML samples.[24-30] Other genes that are methylated in myeloid malignancies include E-cadherin, p73 and RAR· , which are all implicated in hematopoietic differentiation.[31;32] Azacytidine causes hypomethylation and therefore reactivation of previously silenced genes involved in cellular differentiation, particularly P21, causing differentiation of leukemic cells.[21] Furthermore, data suggest that there is increased hypomethylation at lower doses of 5AC.(Hollenbach et al. e9001) In fact

azacytidine, a pyrimidine analog and antimetabolite, has cytotoxic effects at higher doses as opposed to differentiating effects at lower doses.

2.6. Clinical Studies of 5AC in AML/MDS

The first clinical trials of 5AC in AML met with only marginal success.[33] However, these trials used high cytotoxic doses of 5AC (150-750mg/m^2 per dose; 750–2500mg/m^2 per cycle). More recently studies have focused on the utilization of lower doses of 5AC in an attempt to promote differentiation activity.[33] Initial clinical studies of the drug at low doses were done in MDS. Phase III studies of 5AC and DAC in patients with MDS using lower doses (45-75mg/m^2 per dose; 135mg/m^2 -525mg/^{m2} per cycle) demonstrated response rates of 52-60%.[34-36] Data from these studies resulted in the FDA approval of 5AC and DAC for the treatment of CMMoL and MDS. In 2002, a CALGB phase III trial of 5AC versus supportive care, in 194 patients with high-risk MDS, reported significantly higher response rates and improved survival in the treated group. In a larger, randomized, open-label trial in patients with high-risk MDS, Fenaux demonstrated an improved overall survival in those treated with 5AC compared to the those treated with supportive care, 24.5 months versus 15 months.[37] Building upon the success in MDS, investigators now use low-dose 5AC in AML studies.

Recently several groups have reported that low dose 5AC has clinical activity in AML. These data are summarized in Table 1.[35;37-40;40-43] In a retrospective series, Sudan observed a statistically significant increase in overall survival with 5AC in patients with AML.[40] Al-Ali demonstrated activity in patients with relapsed AML with 65% response rates.[44] DAC proved active in patients with MDS and AML, all but one with refractory or relapsed disease.[39;45] Several groups advanced these agents to first line treatment in patients older than 60, who often cannot tolerate standard cytotoxic therapy. The results are promising, with response rates as high as 65 % and complete remission rates reaching 63 % in patients receiving doses of 20mg/m^2 for longer periods of time.[41-43]

Table 1

Study	Rx	Population	Dose	No pts	Response	Comments
Sudan et al. Cancer 2006	5AC	AML, retrospective evaluation	75mg/m^2 sq daily x 7 days q 28d	20	60% (12/20), 4CR, 5PR, 3HI	Survival >15mo responders vs 2.5mo non-responders ($p < 0.009$)
Al-Ali, HK et al. Blood 2006.	5AC	AML de novo, relapsed	75mg/m^2 sq daily x 5 days q 28d	17	65% (11/17), 6CR, 2PR, 2HI. Cytogenetic remission in 2 patients	Duration of response 18 wks (15-not yet reached)
Issa et al. Blood 2004	DAC	AML/MDS, relapsed, refractory	5-20mg/m^2 IV X 10 -20 days	44	27%(12/44) 7CR,5HI	9 of 12 responders treated at 15mg/m2 x 10days
Lubbert et al. ASH abstract 2007	DAC	AML, untreated	45mg/m^2 IV x 3 days x 4; 20mg/m^2 x3 days	155	54%(83/155) 23CR, 15PR, 45 ALE	Cr in 15%, ATRA in cycle for ALE or stable dz (69); 41 rec'd maintenance 20mg/m2 x3days, every 6-8 weeks
Blum et al. PNAS 2010	DAC	AML, untreated	20mg/m^2 IVx10 days	53	64%(34/53) 25CR,iCR	CR in 47%, median 3 cycles
Cashen et al. JCO 2010	DAC	AML, untreated	20mg/m^2 x 5days	55	25%(14/55) 13CR,1iCR	Median 3 cycles, up to 25 cycles

Abbreviations: CR=complete response, iCR=incomplete response, HI=hematologic improvement, PR=partial response, ALE=anti-leukemic effect

Because 5AC demonstrated activity in disease that relapsed after standard therapy, its role in post-SCT relapse was investigated. Recently there have been multiple reports of low-dose 5AC used as salvage treatment for relapse after SCT. One of the initial reports involved a patient who hade relapsed after SCT, then achieved a CR after treatment with five cycles of 5AC, subsequently received DLI, and remained in CR four months later.[46] In a series of 26 patients who received one cycle of 5AC followed immediately by DLI, two-thirds of the patients responded, a marked improvement over the historical response rate of 10-40%.[47] In different group of nine patients, five patients responded to 5AC, four of whom had overall survival greater than 12 months. [10] Although four of the nine patients did not respond to 5AC, they had all had refractory disease at time of SCT. Recently our group reported a series of 37 patients who had relapsed after transplant, 10 of whom received 5AC as salvage therapy. Six of the ten patients achieved CR, only two of whom had received DLI. Five of those patients remain disease-free compared to only five of the 27 patients who did not receive 5AC.

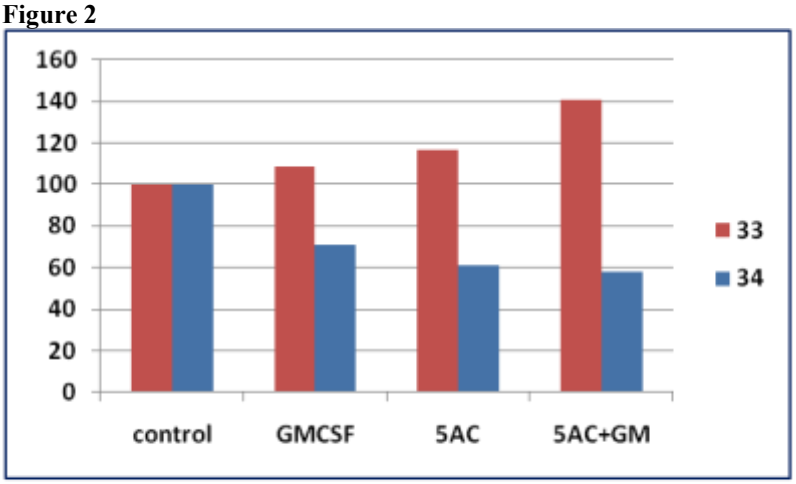
2.7. 5AC as Maintenance Therapy

Due to these favorable responses to salvage treatment after both standard treatment and SCT, together with a favorable toxicity profile, investigators began to pursue the use of low-dose azacytidine as maintenance treatment in patients with AML who were at high risk of relapse. Lubbert incorporated maintenance treatment with DAC in patients who achieved responses after initial induction therapy with the agent. With this paradigm, 57 patients received maintenance therapy, 71% of whom were in either CR or PR. They observed little toxicity and prolonged remission rates.[48] 5AC was also studied as maintenance after successful induction therapy in patients with AML or MDS; 23 patients over the age of 54 received the drug and, when compared to age-matched historical controls, experienced longer duration of CR and OS.[49] Jabbour used 5AC as maintenance treatment to prevent relapse after SCT.[10] The eight patients who received maintenance therapy had disease that was exceptionally high-risk for relapse: at time of transplant four had refractory disease, one was in CR2, and two were in CR3. Of the eight patients who received maintenance therapy, three are in complete remission after more than two years, and the only death was at 25 months. A larger dose finding study of 45 patients who received 5AC as maintenance therapy after SCT revealed a one-year event-free and overall survival of 58% and 77%, respectively.[50] These results demonstrate that 5AC could be a promising therapy not only for relapse after SCT, but perhaps have greater efficacy in a maintenance setting. Therefore, 5AC used as maintenance therapy after SCT warrants evaluation in a clinical trial.

2.8. Preclinical Studies of 5AC and GM-CSF

Our preclinical studies demonstrated that after exposure to 5AC, the AML cell line KG1 showed evidence of phenotypic differentiation, with a decrease in expression of CD34 and an increase in expression CD33. The addition of GM-CSF augmented this differentiating effect (Figure 2).

Figure 2



5AC exposure leads to differentiated cells in the AML cell line KG1, demonstrated by increased expression of CD33 and decreased expression of CD34, a phenotype characteristic of more mature myeloid cells than in the control. This differentiation is enhanced by the addition of GM-CSF.

2.9. Clinical Studies with Differentiating Agents and GM-CSF

Our group has built upon such preclinical data and has focused on studying numerous agents with differentiating properties. A phase I trial of the differentiating agent Bryostatin with GM-CSF demonstrated clinical activity in refractory myeloid malignancies but was intolerable at biologically active doses.[51] Subsequently, a Phase I study with MS-275 showed clinical activity and evidence of differentiation.[45] In a Phase II study of MS-275 and GM-CSF in patients with high-risk MDS or relapsed and refractory AML, 21% of patients achieved a response and demonstrated clinical evidence of differentiation.[52]
We propose a phase II study to determine the impact of maintenance therapy with 5-azacytidine and GM-CSF in patients with poor-risk AML or MDS, who are in remission after definitive treatment with either stem cell transplant or consolidation chemotherapy.

In order to precede relapse and to avoid lead time bias, treatment would need to commence within 185 days of definitive therapy. Furthermore, approximately 50% of relapses occur within the first year and up to 80% within two years after SCT, therefore we would limit the duration of maintenance therapy to one year, followed by two years of follow-up.

3. Patient Selection and Enrollment

3.1. Inclusion Criteria

1. Age ≥ 0.5 years
2. Initial diagnosis of poor -risk AML or MDS (defined in section 3.2), treated with either stem cell transplant or consolidation chemotherapy, within the past 60-185 days
3. ECOG performance status 0-2
4. No morphologic evidence of leukemia or active MDS as determined by JHH Hematopathologist independent review of a bone marrow aspirate and biopsy done following the completion of therapy.
5. Peripheral blood count recovery: Neutrophil count ≥ 1000 /μL, platelet count ≥ 50x 10^9 /μL without platelet transfusions, and adequate hematocrit independent of red cell transfusions .
6. No evidence of extramedullary leukemia, such as CNS or soft tissue involvement
7. Adequate end organ function as measured by the following: AST and ALT ≤ 4 x normal, total serum bilirubin < 2 x upper limit normal (unless due to hemolysis, Gilbert's syndrome, or ineffective erythropoiesis), creatinine ≤ 2 x upper limit of normal
8. Ability to give informed consent
9. In agreement to use an effective barrier method of birth control to avoid pregnancy during the study and for a minimum of 30 days after study treatment, for all male and female patients who are fertile

3.2. Inclusion Criteria Definitions

The original diagnosis of AML or MDS must have been confirmed by bone marrow aspirate and/or biopsy review by a JHH Hematopathologist. Poor-risk AML is defined as disease that is therapy-related or arises from a previous marrow disorder, or *de novo* AML that is associated with any of the following characteristics: patient age 60 years or greater, trilineage dysplasia, disease status greater than or equal to CR2, FLT3/ITD mutations, detectable disease at time of consolidation chemotherapy or SCT, or poor-risk cytogenetics, which include abnormalities of chromosome 3, 5, or 7, trisomy 8, 11q23 abnormalities, t(6;9), 20q-, and complex karyotype.

3.3. Exclusion Criteria

1. Patients with untreated or uncontrolled infections
2. Patients with untreated or uncontrolled grade 3 or 4 GVHD
3. Pregnancy and lactation
4. Concurrent use of any other investigational agents.
5. Known HIV-positive patients.
6. Known hypersensitivity to 5AC or GM-CSF

3.4. Inclusion of Women and Minorities

The proposed study is open to both men and women and to all racial/ethnic subgroups. There is no explicit mention of different treatment effects in male and female patients or in different racial/ethnic subgroups in the literature. Therefore, this study will not have separate accrual targets for these groups.

3.5. Off-study Criteria

Patients may be removed from the study for any of the following:

1. Death
2. Completion of follow-up as per section 5.4
3. Elective withdrawal of consent by patient

3.6. Informed Consent

All patients eligible for the study must be evaluated by one of the study investigators. Informed consent must be obtained and the consent form signed. A Johns Hopkins On-Study Form will be completed following fulfillment of the on-study requirements for laboratory work and eligibility criteria. For enrollment of non-English-speaking candidates, the policies and procedures mandated by the JH IRB will be followed as listed at the following website: https://irb.jhmi.edu/Guidelines/nonenglishconsent.html

4. Pretreatment Plan

A complete history and physical examination and list of medications will be documented for each patient within two weeks of enrollment. A bone marrow aspirate or biopsy confirming the suspected diagnosis will be documented in the electronic medical records. Bone marrow aspirate / biopsy documenting no evidence of leukemia will be performed after completion of therapy. Evaluations within 14 days of beginning therapy will include:

1. A history and physical exam, including a list of active medications
2. Hematologic studies: complete blood count with platelet and differential cell counts.
3. Chemistry panel (including electrolytes, creatinine, uric acid, albumin, total protein, calcium and phosphate)
4. Hepatic panel: serum SGOT, SGPT, alkaline phosphatase, total bilirubin, PT/PTT.
5. Reticulocyte count
6. An EKG

5. Treatment Plan

5.1. Treatment Schedule

The planned initial dose of 5AC is 37.5mg/m^2 (Level 0) and this will be administered subcutaneously or intravenously for days 1through 5 of a 28 day cycle. Patients who tolerate the combination with no SAEs during two consecutive cycles will be eligible for a dose escalation to 50mg/ m^2/day for 5 days of every 28 day cycle (Level +1). Patients who tolerate Dose Level +1 with no SAEs during two consecutive cycles will be eligible for a dose escalation to 75mg/ m^2/day for 5 days of every 28 day cycle (Level +2). Patients will

continue at Dose Level +2 for the study's duration provided there are no toxicities requiring dosing delays or dose reduction.

Dose reductions are permitted as per the standard treatment approach with 5AC and are listed below in Table 2. Patients who experience toxicity or dosing delays, but return to pre-therapy baseline within 56 days of the first day of their most recent cycle, will be eligible to continue at the next lower Dose Level (Table 2). Patients unable to tolerate the lowest dose of 12.5mg/m^2 /day for 5 days (Dose Level -2) due to toxicities or cytopenias, will be removed from the study. Patients requiring a dose reduction are not eligible for re-escalation. Azacitidine dosing is the same regardless of subject age.

Table 2

Dose Level	5AC
-2	12.5mg/m^2
-1	25mg/m^2
0	**37.5mg/m^2 (starting dose level)**
+1	50mg/m^2
+2	75mg/ m^2

GM-CSF will be self-administered subcutaneously for days 1-10 of each 28 day cycle. For subjects unable to self-administer, GM-CSF may be administered by a caregiver or on site at Johns Hopkins or a local provider's office. Dosing will be based on weight and rounded to a standard dosing size: patients who weigh less than or equal to 70kg will receive 125 micrograms/day and those who weigh over 70kg will receive 250 micrograms/day. Pediatric subjects will receive 125 micrograms/day regardless of weight. Subjects will be given prescriptions for pre-dosed syringes of GM-CSF. If the starting dose is not tolerated due to traditional side effects of GM-CSF, such as muscle aches and pains, bone or joint discomfort, or elevated WBC > 25,000, the drug will be held through resolution of these side effects. Patients who return to their pre-dosing baseline will re-start the GM-CSF at the next cycle start date at the next lower Dose Level (Table 3). Patients unable to tolerate the minimum dose (Dose Level -3) for 7 days due to either persistently elevated WBC or other side effects will be removed from the study.

Table 3

Dose Level	GM-CSF patients >18 years	Patients $\leq$ 18 years
-3	50micrograms x 7 D	50micrograms/ m^2 x 7D
-2	50 micrograms x10 D	50micrograms/ m^2 x 10D
-1	62.5 micrograms x 10D	62.5micrograms/ m^2 x10D
0	**125 micrograms (starting dose weight <70 kg) x 10D**	**125micrograms/** m^2 **x10D[1]**
+1	**250 micrograms (starting dose weight >70 kg) x 10D**	

5.2. Dosing Delays and Modifications for Toxicity

5.2.1. Dosing Delays for Hematologic Toxicity

If a patient develops critical drug induced neutropenia or thrombocytopenia (as defined in sections 5.22), 5AC and GM-CSF will be held. If neutrophil count rises to 100-500/mm^3, or platelet count rises to 10,000-50,000/mm^3, the patient may resume the next cycle at the next lower dose level. If the neutrophil count is greater than 500/mm^3 or the platelet count greater than 50,000/mm^3, the patient may resume the next cycle at the same dose level. If there is inadequate peripheral count recovery within 56 days from the first day of the most recent cycle the patient will be removed from the study and a bone marrow biopsy and aspirate will be performed.

5.2.2. Definitions of Hematologic Toxicity

Critical neutropenia or thrombocytopenia is defined by a neutrophil count <100/mm^3 or a platelet count < 10,000/mm^3. Persistent critical cytopenias are those that last for greater than 3 weeks.

5.2.3. GVHD

If a patient develops clinical grade 3 or 4 acute GVHD[53], study therapy will be held until GVHD has stabilized or resolved. Patients who develop clinical Grade 2 GVHD may continue on therapy with close observation (typically clinic visits 2-3 times / week), but will have study therapy held for advancing GVHD. Study therapy will be held should a patient develop Severe chronic GVHD until stabilization or resolution. If a patient develops mild or moderate chronic GVHD[54], the patient may continue on therapy with close observation (typically clinic visits 2-3 times per week), but study therapy must be held for advancing or uncontrolled GVHD.

5.2.4. Non-hematologic Adverse Events

Patients with grade 3 or 4 non-hematological adverse event, deemed by the investigator to be possibly, probably or definitely related to the study drugs, will hold both 5AC and GM-CSF through the start of the next planned cycle. If the adverse events resolve to pre-treatment baseline, the study drugs will be resumed starting with the following scheduled cycle at the next lowest Dose Level. To allow for resolution of adverse events, patients may have study drugs held for up to 58 days from the start of the most recent cycle prior to being removed from the study.

5.3. Duration of Therapy

Treatment is planned for a total of 12 cycles of therapy and will continue until one of the following occurs:

1. Disease relapse
2. Serious illness that prevents further administration of treatment (i.e., progressive infection not responding to appropriate antibacterial and/or anti-fungal therapies)
3. Unacceptable adverse event(s)

4. Elective withdrawal of consent by the patient
5. Determination by the physician that it is no longer in the patient's best interest to continue on trial
6. Delay of the study drug dose for more than 56 days from the first day of the most recent cycle due to unresolved toxicities
7. Inability to tolerate either study drug at the lowest allowable dose levels
8. Initiation of breast feeding by the patient.
9. Patient pregnancy

All subsequent cycles may be initiated no sooner than 28 days and no later than 56 days from Day 1 of the previous cycle.

5.4. Duration of Follow-up

Patients will be followed for relapse and death for three years from the first day of the first cycle of treatment. Patients are routinely followed and monitored by JH physicians after SCT or chemotherapy, and patients who complete the study and continue to receive their care at JH will have survival captured annually at least.

5.5. Supportive Care Guidelines

Supportive care, including infection management and transfusion support, will follow good medical practice and institutional standard guidelines.

5.6. Study Parameters

The studies planned during the patient evaluation and therapy are listed in Table 4. This represents the basic follow-up plan and this will change for a change in patient condition. Patients will be requested to maintain a diary documenting administration of drug and any symptomatology. All attempts will be made to collect all the correlative studies, however, due to laboratory constraints (weekends, holidays, laboratory emergencies or staffing difficulties) and patient constraints (related to intercurrent illnesses, transportation, or inadequate sample), some samples will not be collected or will not be suitable for evaluation. A patient will be considered suitable for evaluation independent of the availability of correlative study material. A variation, of +/- 3 days from any weekly scheduled testing and +/- 7 days for all other study visits, is permitted.

Table 4

Parameter	Pre-treatment[1]	Weekly during first cycle	Day 1 of each cycle	End of Treatment
History/ Physical	X		X	X
Weight	X		X	X
Performance Status	X		X	X
CBC with Differential	X	X	X	X
PT, PTT	X			
Reticulocyte Count	X			X
Comprehensive Metabolic Panel	X	X	X	X
Phosphate	X		X	X
Magnesium	X		X	X
Toxicity Notation	X	X	X	X
EKG	X			
Review diary	X		X	X
Serum HCG for applicable women only	X			

[1] Within 14 days prior to enrollment.

6. Treatment Evaluation

6.1. Response Criteria: Measurement of Effect

The NCI criteria for relapse in AML and MDS will be used. Relapse following complete remission is defined as:

1. Peripheral Blood Counts: it is expected that cycles of treatment will be associated with cytopenias, however recurrent cytopenias or the appearance of blasts in the peripheral blood will warrant a bone marrow aspirate and biopsy evaluation.
2. Bone Marrow Aspirate and Biopsy: Presence of > 5% blasts not attributable to another cause (e.g., bone marrow regeneration), dysplasia in greater than 10% of any lineage with cytopenias in one or more lineages, or the presence of a cytogenetic abnormality consistent with MDS or AML.
3. Development of extramedullary disease.

7. Pharmaceutical Information

7.1. Toxicity Concerns

7.1.1. GVHD

We will monitor GVHD as there are reports that 5AC is associated with immunomodulatory effects that both enhance and inhibit GVHD.[55;56] 5AC may aid antigen presentation which may be expected to both enhance GVHD[57] and concurrently improve any graft versus leukemic effects.(Pinto, Coral) 5AC may also enhance any anti-leukemic effects by inducing cytotoxic T lymphocytes against AML cells.[57] In contrast, there is also evidence that 5AC may inhibit T-cell proliferation and activation, resulting in inhibition of GVHD and graft versus leukemia effects.[58] Whatever the over-riding immune mechanisms of 5AC, no exacerbations of GVHD were observed in two reports of 5AC used after SCT.[10;59] We will monitor GVHD according to institutional standards for all post-transplant patients.

7.1.2. Drug Combination

As to the safety of the combination, GM-CSF is often used as standard supportive care in patients receiving 5AC and tolerated well. Therefore, we do not expect increased toxicity from the combination**.** In fact, GM-CSF may mitigate the myelosuppressive effects of 5AC.

7.2. Dosing

7.2.1. 5-Azacytidine

There are no standard doses for 5AC as maintenance therapy. As primary therapy for MDS, 75mg/m^2 for 7 days is the approved dose. This dosing has also been studied in patients with active AML.[40] Lower doses are also being investigated. One study of MDS treatment compared three dosing schedules: 75mg/m^2 for 5 days, 50mg/ m^2 for 10 days and 75mg/ m^2 for 7 days. All three schedules produced responses, with the first schedule experiencing the least serious adverse events and the greatest response rates for both hematologic response and transfusion independence.[60] A regimen of 50mg/m^2 for 10 days, every 28 days is currently in use in a CALGB trial. A comparison of 10mg/m^2 IV daily for 10days and 20mg/m^2 IV and SC for 5 days reported responses in both groups.[61] As maintenance therapy, doses varying between 16-75mg/m^2 daily have been studied.[10;49;62;63] 75mg/m^2 for seven days

was not well tolerated due to neutropenia, particularly in the older population. However a portion of patients did tolerate 75mg/m^2 without incident. In addition, many of these studies included patients with active or partially treated disease. Most studies suggest that cytopenias remain of concern in patients with active marrow disease. Our study proposes to treat patients who are currently in remission and we plan to use low doses of growth factors which would support the marrow and minimize this potential toxicity. Furthermore, data suggest that there is increased hypomethylation at lower doses of 5AC,[40] which may lead to increased reactivation of genes involved in differentiation. Therefore, a relatively low does that still maintains differentiation activity would be optimal. Dose escalation and de-escalation are outlined in section 5.1 to help guide physicians and support patient safety.

7.2.2. GM-CSF

GM-CSF will be initiated at two flat doses based on weight and rounded to a standard dosing size, as done commonly in practice. Patients who weigh less than or equal to 70kg will receive 125 micrograms/day and those who weigh over 70kg will receive 250 micrograms/day. This dosing is based in part on our previous experiences in myeloid disorders using GM-CSF in combination with standard therapy in both the non-transplant[51;64;65] and the post-transplant setting.[66;67] Each of these studies supported the biologic activity of GM-CSF and determined that it was well tolerated. Patients will be given needle disposal boxes and instructions on how to properly dispose of the used syringes and needles. The needle box will be returned to the study research nurse for disposal through the pharmacy.

7.3. Timing of Initiation and Duration of Treatment

After SCT the majority of acute GVHD occurs within the first 60 days. To ensure patients are stable prior to initiating maintenance therapy, the study will start no sooner than after the day 60 post-transplant bone marrow biopsy results are available. As many relapses occur within the first several months,[9] subjects must be enrolled within 185 days of transplant or chemotherapy. Treatment with 5AC and GM-CSF must then begin within 14 days of enrollment. Approximately half of relapses occur within the first year after SCT, with 80% occurring within the first two years.[9] Accordingly, we would limit the duration of maintenance therapy to one year and follow patients for an additional two years.

7.4. 5-Azacytidine (NSC 102816)

1. Chemical Name: 4-Amino-1-ß-D-ribofuranosyl-1,3,5-triazin-2(1H)-one
2. Other Names: 5-Azacytidine, 5-AZA, Mylosar7, Ladakamycin, Vidaza
3. Supply: 5AC is supplied as 100 mg of white, lyophilized powder with 100 mg of mannitol, USP in 30 ml flint vials. The contents of each vial should be dissolved in 4mL of sterile water or 0.9% sodium chloride to provide a 25 mg/ml slurry. 5AC does not go into solution but forms loose slurry when reconstituted in this fashion. *Do not inject the slurry intravenously.* Single injections should not exceed 2ml. Doses requiring larger volumes may be split into multiple injection

sites if volume to be administered is too large. Injection sites should be rotated on a daily basis.

4. Storage and Stability: Store un-reconstituted vials at 25 degrees C (77 degrees F); excursions permitted to 15-30 degrees C (59-86 degrees F) (See USP Controlled Room Temperature). 5AC reconstituted for subcutaneous administration may be stored for up to 1 hour at 25 degrees C (77 degrees F) or for up to 8 hours between 2-8 degrees C (36 and 46 degrees F). The constituted solutions hydrolyze at room temperature and should be used within 30 minutes for delivery of maximum potency.
5. Route of Administration: Route of administration is via subcutaneous or intravenous injections. Injections are to be given by trained nursing staff at each institution. Reconstituted solutions of 5AC are unstable. Upon reconstitution, the material should be injected within 30 minutes. To provide a homogeneous suspension, the contents of the syringe must be re-suspended by inverting the syringe 2-3 times and vigorously rolling the syringe between the palms for 30 seconds immediately prior to administration until a uniform, cloudy suspension is achieved.
6. Toxicities: Hematologic toxicities include leukopenia, neutropenia, thrombocytopenia, and anemia. GI toxicities include nausea and vomiting, diarrhea, stomatitis and mucositis. Increased liver function tests and renal tubular acidosis can be seen. Rarely, pulmonary edema, arrhythmia, pericarditis, hypotension, hepatic coma, CNS and neuromuscular toxicity have been seen. Rash, allergic reactions, fever, conjunctivitis and alopecia have also been noted. Local injection reactions are common and include burning, pain, violaceous (purple) discoloration lasting up to ten days, and persistent brown discoloration. In clinical studies, the most commonly occurring adverse reactions were nausea (71%), anemia (70%), thrombocytopenia (66%), vomiting (54%), pyrexia (52%), leucopenia (48%), diarrhea (36%), fatigue (36%), injection site erythema (35%), constipation (34%), neutropenia (32%) and ecchymosis (31%). Other adverse reactions included dizziness (19%), chest pain (16%), febrile neutropenia (16%), myalgia (16%), injection site reaction (14%) and malaise (11%). Complete and updated adverse events are available in the Investigational Drug Brochure and the IND safety letters.

7.5. GM-CSF (NCI 613795)

1. Other names: Leukine®, GM-CSF
2. Classification: Recombinant human GM-CSF produced in yeast formulation.
3. Supply: GM-CSF is supplied as either 250 mcg liquid vials or as 500 mcg lyophilized powder, multi dose vials.
4. Storage: Intact vials should be stored refrigerated at 2 – 8 C.
5. Stability: After reconstitution with 1 mL Sterile Water for Injection (SWFI), GM-CSF should be used immediately within 6 hours. After reconstitution with 1 mL Bacteriostatic Water for Injection (BWFI), GM-CSF is stable for up to 20 days in

vials and 14 days in syringes refrigerated at 20 – 80 C.

6. Route of Administration: Subcutaneous. Do not filter during preparation or administration.
7. Method of Preparation: GM-CSF solution can be prepared in prefilled BD syringes in the clinic for outpatients' treatment. Refrigerate prepared prefilled BD syringes at (2 - 8C) for up to 20 days.
8. Drug Ordering and Drug Accountability:
9. Patient Care : Patients will be provided a prescription for pre-filled, pre-dosed syringes for self-administration as per routine instructions (or administration on site or by provider as noted earlier). In addition, patients will be provided a needle and syringe disposal box and instructions on the proper disposal of the used needle and syringe. The needle box will be returned to the study research nurse for final disposal through the pharmacy.
10. Toxicities: GM-CSF may cause lethargy, myalgia, bone pain, anorexia, weight change, generalized skin eruptions, and flushing. These side effects are generally tolerated by patients.

8. Laboratory Correlative Studies

The bone marrow biopsy and aspirate will be performed as standard of care pre-treatment and as needed per the subject's primary oncologist. 10 ml in a sodium heparin green top of bone marrow aspirate may be obtained during each of these procedures for biologic correlative laboratory studies described below.

In order to describe changes of the clonogenic population, the putative LSC population, including changes in detectable cytogenetic abnormalities and antigen expression pattern, we will study freshly isolate cells from patient bone marrow aspirate. The 34^+ cells will be isolated by Ficoll density centrifugation separation followed by selection using anti-CD34 magnetic beads. Then analysis and sorting of cells based on expression of CD34, CD38, and Aldeflour will be performed. Sorted cells will be used for *in vivo* and *in vitro* studies of antigen expression and differentiation and for FISH analysis of cytogenetic abnormalities.

9. Statistical Methods

This is a phase II study of 5AC in combination with GM-CSF as maintenance therapy after definitive therapy with either stem cell transplant or consolidation chemotherapy in adults with poor risk Acute Myeloid Leukemia or MDS. We hypothesize that prolonged pressure on the leukemia stem cell to differentiate would eventually exhaust the leukemic clone and therefore reduce relapse rates.

9.1. Objectives

9.1.1. Primary Objective

The **primary objective** of the trial is to evaluate the two year relapse-free survival (RFS) of maintenance treatment with 5AC in combination with GM-CSF in patients with poor-risk Acute Myeloid Leukemia (AML) and Myelodysplasia (MDS) in

complete remission after definitive therapy with either a stem cell transplant or consolidation therapy with chemotherapy. RFS will be defined as time to relapse or death. The start time of RFS will be the date of first remission marrow following induction treatment. Patients will be required to have no morphologic evidence of leukemia or active MDS before entry onto the protocol. This study will monitor three separate cohorts:

1. Patients in complete remission (CR) following a full myeloablative stem cell transplant
2. Patients in CR following a non-myeloablative stem cell transplant
3. Patients in CR following standard consolidation therapy

9.1.2. Secondary Objectives

1. Describe and quantify the toxicity profile of the combination of 5AC + GM-CSF
2. Determine the impact on one year RFS and overall survival for poor risk myeloid disorders following maintenance therapy with 5AC + GM-CSF
3. Describe the changes in the clonogenic population, including changes in detectable cytogenetic abnormalities in blood and marrow, during maintenance therapy.

9.2. Study Design

This will be a three-arm phase II study with historical references for each arm. The historical two-year RFS and corresponding event rates in these arms are assumed to be: myeloablative, 40% (0.46 per person year); non-myeloablative, 34% (0.54 per person year); and standard consolidation , 20% (0.80 per person year). The non-parametric Kaplan-Meier estimate will be used to monitor the relapse-free survival function in each cohort.

9.3. Futility Analysis

After 10 patients have been enrolled in an arm, the design will include interim analyses for futility that could halt that arm of the trial if it seems likely that the combination of 5AC and GM-CSF does not have sufficient clinical activity. The study is designed to stop any arm if it is 70% certain that the two-year RFS is below the historical two-year RFS: 40% in the myeloablative arm, 34% in the non-myeloablative arm, and 20% in the standard consolidation group.

9.4. Early Stopping Guideline for Futility and Simulations for Each Cohort

The simulation studies demonstrate the power for a sample size of 25 in each cohort. The study will be monitored for futility with a primary endpoint of two-year RFS. If the study meets the accrual goal of 10 patients per year per arm, 20 patients would be accrued by the time the first patient enrolled on study reaches the two-year efficacy endpoint. We will therefore monitor the study based on one-year RFS, knowing that if the one-year RFS is convincingly less than our targeted RFS at two years, the study would not meet its efficacy

objective and the trial should be halted. The non-parametric Kaplan-Meier estimate will be used to estimate the RFS function at one year.

The sample size of 25 and the study design operating characteristics assume a 2.5 year accrual period with an additional follow-up of one year. The null two-year RFS is 40% in the myeloablative group, 34% in the non-myeloablative group and 20% in the non-transplant cohort. We have designed each arm to stop early only if the posterior probability is 70% or higher that one-year RFS is less than that arm's null two-year RFS. Simulations were carried out with exponential survival and staggered patient entry. We assumed on average 10 patients will enter each arm of the study per year. Initial interim analyses occurred after 10 patients enrolled and was repeated every 5^{th} patient to the maximum of 25. The interim analysis estimates of one-year RFS are based on analyses with an underlying Dirichlet process prior. We approximate the posterior distribution, which is actually a mixture of Beta distributions, the mixture depending on the amount of censoring, with a single Beta distribution. The parameters of this posterior Beta distribution are based on the number of failures and the effective sample size at one year, combined with the parameters of the prior.

The following tables summarize the operating characteristics under various scenarios for the underlying exponential RFS, based on 1000 simulations. The uncertainty of the null two-year RFS estimates are characterized with three priors: beta(2 ,3) in the myeloablative group (Table 5) and beta(2,4) in the non-myeloablative group (Table 6) and beta(1,4) in the non-transplant group (Table 7). This implies that our prior guess at the two year RFS for these three cohorts are respectively 40, 34 and 20%.

The highlighted row gives the power of the study under the scenario where the median RFS is increased from 1.5 to 3.1 years in myeloablative patients, 1.3 to 3.1 years in non-myeloablative patients and 10.4 months to 18 months in the non-transplant patients. The 95% posterior intervals are the quantiles of the simulated one-year RFS corresponding to probabilities of .025 and .957. The interpretation is that the posterior probability that the estimate of one-year RFS lies in this interval is 95%.

These futility stopping rules are not excessively stringent: for instance the risk of not stopping the arm if the treatment is equal to the null hypothesis (the historical RFS rates), and therefore not effective, is approximately 50% in all three arms. However, we do not want to accept the null hypothesis and stop the trial if there is a reasonable probability that the experimental treatment may be effective. The treatment is relatively non-toxic, we are monitoring toxicities and adjusting doses accordingly, the risk of relapse is very high, and upon relapse the mortality rate is very high. Therefore, we are willing to accept the characteristics of these stopping rules.

Table 5

1year RFS	Prob Stop for Futility	Avg N	Estimated 1 yr RFS	lo 95% Post Int'l	hi 95% Post Int'l	Prob H0 Rejected (power)
0.20	0.95	11.22	0.185	0.0	0.36	0.0
0.30	0.77	14.26	0.264	0.0	0.48	0.01
0.40	0.46	18.51	0.360	0.1	0.60	0.09
0.50	0.16	22.68	0.487	0.2	0.72	0.36
0.60	0.06	24.07	0.588	0.3	0.80	0.69

Myeloablative: prior is beta(2,3) : Mean is 0.40

Table 6

1year RFS	Prob Stop for Futility	Avg N	Estimated 1 yr RFS	lo 95% Post Int'l	hi 95% Post Int'l	Prob H0 Rejected (power)
0.20	0.86	13.22	0.178	0.0	0.36	0.01
0.34	0.45	19.2	0.307	0.1	0.52	0.13
0.40	0.30	21.16	0.364	0.1	0.56	0.24
0.50	0.09	23.86	0.485	0.2	0.68	0.58
0.60	0.02	24.73	0.59	0.36	0.80	0.88

Non-myeloablative: prior is beta(2,4) : Mean is 0.34

Table 7

1year RFS	Prob Stop for Futility	Avg N	Estimated 1 yr RFS	lo 95% Post Int'l	hi 95% Post Int'l	Prob H0 Rejected (power)
0.10	0.92	12.35	0.081	0.0	0.20	0.0
0.20	0.52	18.17	0.168	0.0	0.36	0.08
0.30	0.20	22.25	0.277	0.0	0.48	0.36
0.40	0.06	24.2	0.390	0.10	0.60	0.74
0.50	0.01	24.87	0.502	0.32	0.68	0.95

Non-transplant : prior is beta(1,4) : Mean is 0.20

9.5. Early Stopping Rules for Safety

This study will monitor acute grade III/IV GVHD in the two transplant arms. If it becomes evident that the proportion of acute grade III/IV GVHD convincingly exceeds 10%, the study will be halted for a safety consultation. The stopping rule will be applied to both transplant arms and will hold enrollment if the posterior probability of toxicity more than

0.10 is 75% or higher. The prior for this monitoring rule is beta(1,9). This means that our prior guess at the proportion of acute grade III/IV GVHD is 10%, and there is 90% probability that this proportion is between 0.57% and 28.3%. The operating characteristics of the stopping rule are given in the Table 8 and are based on 5000 simulations:

Toxicity stopping rule:

- Serious AE in 2 out of 2 patients. Pr(Risk>0.1| Data) = 0.91
- Serious AE in 2 out of 3 patients. Pr(Risk>0.1| Data) = 0.889
- Serious AE in 2 out of 4 patients. Pr(Risk>0.1| Data) = 0.866
- Serious AE in 2 out of 5 patients. Pr(Risk>0.1| Data) = 0.842
- Serious AE in 2 out of 6 patients. Pr(Risk>0.1| Data) = 0.816
- Serious AE in 2 out of 7 patients. Pr(Risk>0.1| Data) = 0.789
- Serious AE in 2 out of 8 patients. Pr(Risk>0.1| Data) = 0.762
- Serious AE in 3 out of 9 patients. Pr(Risk>0.1| Data) = 0.902
- Serious AE in 3 out of 10 patients. Pr(Risk>0.1| Data) = 0.885
- Serious AE in 3 out of 11 patients. Pr(Risk>0.1| Data) = 0.867
- Serious AE in 3 out of 12 patients. Pr(Risk>0.1| Data) = 0.848
- Serious AE in 3 out of 13 patients. Pr(Risk>0.1| Data) = 0.828
- Serious AE in 3 out of 14 patients. Pr(Risk>0.1| Data) = 0.807
- Serious AE in 3 out of 15 patients. Pr(Risk>0.1| Data) = 0.786
- Serious AE in 3 out of 16 patients. Pr(Risk>0.1| Data) = 0.764
- Serious AE in 4 out of 17 patients. Pr(Risk>0.1| Data) = 0.888
- Serious AE in 4 out of 18 patients. Pr(Risk>0.1| Data) = 0.873
- Serious AE in 4 out of 19 patients. Pr(Risk>0.1| Data) = 0.858
- Serious AE in 4 out of 20 patients. Pr(Risk>0.1| Data) = 0.842
- Serious AE in 4 out of 21 patients. Pr(Risk>0.1| Data) = 0.825
- Serious AE in 4 out of 22 patients. Pr(Risk>0.1| Data) = 0.807
- Serious AE in 4 out of 23 patients. Pr(Risk>0.1| Data) = 0.789
- Serious AE in 4 out of 24 patients. Pr(Risk>0.1| Data) = 0.77
- Serious AE in 4 out of 25 patients. Pr(Risk>0.1| Data) = 0.75

Table 8

True Tolerability Risk	Prob. Declare Treatment Not Tolerable	Avg. Sample Size
0.02	1.5%	24.8
0.05	8.5%	23.7
0.10	34.8%	20.2
0.15	62.8%	16.0
0.20	81.8%	12.6
0.25	92.8%	9.7
0.30	97.8%	7.6

Operating characteristics of stopping rule based on 5000 simulations.

10. Data Safety and Monitoring Plan

The study PI will be responsible for the conduction of the study, including the monitoring of the study's safety and oversight of the data collection. The PI will follow the Data Safety and Monitoring plan outlined in the Sidney Kimmel Comprehensive Cancer Center's policy. This trial is designated as a category 1 protocol based on the risk and monitoring needs.

10.1. Data Reporting

This is a DSMP Level I High-Medium Risk study under the SKCCC Data Safety Monitoring Plan (9/22/2011). The Clinical Research Office QA Group will perform an audit after the first subject has been treated and then periodically depending on the rate of accrual and prior audit results. All trial monitoring and reporting will be reviewed annually by the SKCCC Safety Monitoring Committee. The PI is responsible for monitoring the study. Data must be reviewed to assure the validity of data, as well as, the safety of the subjects. The PI will also monitor the progress of the trial, review safety reports, and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study. The PI will be responsible for maintaining the clinical protocol, reporting adverse events, assuring that consent is obtained and documented, reporting of unexpected outcomes, and reporting the status of the trial in the continuing renewal report submitted to the IRB and to the trial monitoring review group. Content of the continuing renewal report at a minimum should include year-to-date and full trial data on: accrual and eligibility, protocol compliance, treatment administration, toxicity and ADR reports, response, survival, regulatory compliance, compliance with prearranged statistical goals. The report should be submitted in a timely manner according to the schedule defined by Johns Hopkins Medicine Institutional Review Board."

10.2. Adverse Event Definition

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, regardless of whether the occurrence is considered to have a causal relationship with treatment. An AE can therefore

be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. AEs are to be coded using an internationally recognized dictionary.

AEs will be recorded and reported beginning with the first dose of study drug. All events occurring between informed consent and the first dose will be recorded as medical history.

This study will utilize the Common Toxicity Criteria (CTC) version 4.0 for toxicity where applicable for adverse event reporting. In cases where CTC cannot be applied to the toxic event, the investigator will quantify the toxicity based on intensity as defined:

1. Mild: The subject is aware of signs or symptoms but they are easily tolerated; usually does not require additional therapy or discontinuation of study drugs.
2. Moderate: The signs and symptoms are sufficient to restrict but do not prevent usual activity; possible requires additional therapy but usually does not require discontinuation of study drugs.
3. Severe: The Subject is unable to perform usual activities and usually requires discontinuation of study drugs.
4. Life-threatening consequences; urgent intervention indicated.

Patients are to be followed for adverse events for 30 days after the last does of study drug and any adverse event occurring in a patient up to 30 days after stopping the study drug must be reported. The surveillance period after study drug discontinuation may be extended if there is a strong suspicion that the drug has not yet been eliminated or if the nature of a particular event may suggest long-term effects by the investigational drug, as assessed by the investigator.

10.3. Drug Relationship

The investigator will classify the study product relationship of an adverse event to the investigational product according to the following definitions:

1. None: The time course between the administration of study product and the occurrence or worsening of the adverse event rules out a causal relationship and or another cause is confirmed and no indication of involvement of the study product in the occurrence/worsening of the adverse event exists.
2. Unlikely: the time course between administration of the study product and occurrence or worsening of AE makes a causal relationship unlikely; the known effects of the study product or of the substance class provide no indication of involvement in AE and another cause adequately explains the AE; regarding the AE, a plausible causal chain may be deduced from the known effects of the study product or the substance class but another cause is much more probably; or another cause is confirmed and involvement of the study product in the AE is unlikely.

3. Possible: Regarding the AE, a plausible causal chain may be deduced from the pharmacological properties of the study product or the substance class, but another cause just as likely to be involved is also known; although the pharmacological properties of the study product or the substance class provide no indication of involvement in the AE, no other cause gives adequate explanation.
4. Probable: the pharmacological properties of the study product or of the substance class and/or the course of the AE suggest involvement of the study product in the AE, although another cause cannot be ruled out.
5. Definite: the pharmacological properties of the study product or of the substance class and the course of the AE indicate involvement of the study product in the AE and no indication of other causes exists.
6. Unclassifiable: only used for SAE: the available information is not sufficient for causality assessment.

10.4. Outcome

The investigator will record the outcome of the AE choosing one of the following categories:

1. Recovered/resolved
2. Recovering/resolving
3. Not recovered/ not resolved
4. Recovered/resolved with residual effects as specified
5. Fatal
6. Unknown

10.5. Serious Adverse Event (SAE) Definition

As defined by the FDA CFR 312, a serious adverse event is one, occurring at any dose (including overdose), that results in any of the following:

1. Death
2. Life-threatening illness
3. Inpatient hospitalization or prolongation of existing hospitalization
4. Persistent or significant disability or incapacity
5. A congenital anomaly or birth defect
6. An important medical event
7. Pregnancy

10.6. Adverse Drug Reaction and Toxicity Monitoring

The study team (research nurse, study coordinator or attending) will assign toxicity scores using the NCI common Toxicity Criteria version 4.03, weekly through the first cycle of therapy and at least every cycle thereafter. A copy of the CTC version 4.0 is available at the CTEP home page (http://ctep.info,nih.gov). If an unexpected and serious toxicity occurs that would result in patients being subjected to unacceptable risk, the trial will be placed on hold while this toxicity is investigated.

10.7. Toxicity Reporting

The Principal Investigator is responsible for the ongoing safety evaluation of the study. Unanticipated events will be reported to IRB as per JHMIRB policy 103.6b, *Organization Policy on reports of Unanticipated Problems Involving Risks to Participants or Others.* Expectedness of events will be determined by the product inserts for 5AC and GM-CSF. All other events will be logged on a Master AE Log as per SKCCC policy.

10.8. Pregnancies

Pregnancies occurring while the subject is on 5AC or within 4 weeks after the subject's last dose are considered unanticipated reportable events. If the subject is on 5AC, it is to be discontinued immediately. The Investigator will follow the subject until completion of the pregnancy, and will provide any new information as a follow-up to the initial SAE. The subject should be referred to an obstetrician/gynecologist experienced in reproductive toxicity for further evaluation and counseling. All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as unanticipated SAEs. In addition, any infant death after 30 days that the Investigator suspects is related to the *in utero* exposure to 5AC should also be reported.

10.9. Data Handling and Record Keeping

10.9.1. Case Report Forms

The PI and study coordinator will document in the patient files. Data required according to this protocol will be recorded on the electronic CRFs developed by the PI.

Any documents related to the study must be archived at the study site or in a central archive. This includes the careful listing of the identities of the subjects involved in the study. This list and the signed informed consent statements are key documents in the files to be stored by the PI. Subject files will be archived according to local regulations. All documents related to the study must be retained until at least 15 years after the end of the study.

10.9.2. Subject Registry

The PI should maintain a registry of all subjects entered into the study in the event that a safety issue arises after study completion.

11. Ethics

11.1. Institutional Review Board

The study protocol and any amendment that is not solely of an administrative nature must be approved by an Institutional Review Board (IRB).

11.2. Ethical Conduct of the Study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki.

11.3. Evaluation of Benefits and Risks/Discomforts

11.3.1. Potential Benefits

Patients will receive evaluation and treatment of their malignancy as a result of participating in this trial. The trial will provide information on how the combination of 5AC and GM-CSF should be administered to patients, but may or may not help a specific patient personally. This treatment may offer temporary control of the disease, but is not expected to be curative by this protocol. Alternative approaches to entering this trial, including supportive care only, will also be discussed before the verbal and written consent is obtained regarding the risks, benefits, and the treatment requirements of this trial.

11.3.2. Measures for Minimizing Risk

Administering 5AC and GM-CSF to patients may involve risks that are currently unforeseeable. Side effects can be unpredictable in nature and severity, although all care will be taken to minimize them. If patients suffer any physical injury as a result of participating in this study, immediate medical treatment is available at the treatment center. Frequent blood work will be taken to monitor side effects. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations. Malignancies with no further standard treatment options generally have a poor prognosis. Therefore, patients may experience significant treatment-related morbidity, and/or complications from progression of their disease.

11.3.3. Risks/Benefits Analysis

Data gathered from both clinical and laboratory evaluations in this trial will be analyzed frequently to ensure safety of patients. Any new or significant finding(s) found during the course of the research will be shared and explained to each participant since that may affect a patient's willingness to participate further. Patient's anonymity will be protected to the maximum extent in all publications and presentations that result from this research.

11.3.4. Patient Information and Consent

The investigator or consent designee will explain the nature of the study, its purpose and associated procedures, the expected duration, and the potential benefits and risks of participation to each patient prior to his/her entry into the study (i.e., before examinations and procedures associated with selection for the study are performed). Each patient will have ample opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision. Following this informative discussion, a patient will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the patient voluntarily agrees to sign the informed consent statement and has done so, may he/she enter the study. The patient will receive a copy of the signed and dated informed consent form. The signed informed consent statement is to remain in the investigator's files. The informed consent form and any other written information provided to patients will be revised whenever important new information becomes available that may be relevant to the patient's consent, or there is an amendment to the protocol which necessitates a change to the content

of the written informed consent form. The investigator will inform the patient of changes in a timely manner and will ask the patient to confirm continuation of his/her participation in the study by his/her signature on the revised informed consent form. Any revised written informed consent form must receive the IRB's approval/favorable opinion in advance of use.

11.4. Financial Disclosure

Each investigator (including the principal investigator and any sub-investigators) who is directly involved in the treatment or evaluation of research subjects must disclose certain financial arrangements. A financial disclosure statement must be provided to the sponsor for each investigator (including each sub-investigator in IND studies identified on FDA Form 1572) at a study site before the study can commence. Financial disclosure statements must also be provided at the time the study is closed and at the 1-year anniversary of study closure.

The following arrangements with, and interests of, investigators (including the spouse and dependent children) should be disclosed to the FDA:

1. Compensation made to the investigator in which the value of compensation could be affected by study outcome (e.g., higher compensation for a favorable outcome than for an unfavorable outcome, or a royalty interest related to product sales)
2. A proprietary interest by the investigator in the tested product, including, but not limited to, a patent, trademark, copyright or licensing agreement
3. Any equity interest in the sponsor of this study, i.e., any ownership interest, stock options, or other financial interest whose value cannot be readily determined through reference to public prices, or any equity interest in a publicly held company that exceeds $ 50,000 in value held during the time the investigator is carrying out the study and for 1 year following completion of the study
4. Significant payments of other sorts, i.e., payments that have a cumulative monetary value of $25,000 or more, made by the sponsor of a covered study to the investigator or the investigator's institution to support activities of the investigator exclusive of the costs of conducting the clinical study or other clinical studies, (e.g., a grant to fund ongoing research, compensation in the form of equipment or retainers for ongoing consultation or honoraria) during the time the investigator is carrying out the study and for 1 year following completion of the study
5. In this context "investigator" is defined as all individuals listed on FDA form 1572 - or for non-IND studies performed outside the U.S. listed in the signature list.
6. Page 44 directly involved in the treatment or evaluation of research subjects. The term also includes the spouse and each dependent child of the investigator.

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