

## **5-Azacytidine (NSC 102816): A New Drug for the Treatment of Myeloblastic Leukemia**

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The pyrimidine analog, 5-azacytidine (NSC 102816), was administered by continuous intravenous infusion in Ringer's lactate in increasing doses to sets of patients with metastatic cancer to establish a dose sufficient to produce mild toxicity. Twenty-one patients (23 trials) were treated with doses of 50–200 mg/sq m/day for 5 days every 2–4 wk. Nausea and vomiting were moderate and easily preventable. Doses of 100–200 mg/sq m for 5 days every 14 days produced granulocytopenia, usually after two courses. Less toxicity was observed when courses were given every 21–28 days. Forty-five pa-

tients with previously treated and refractory acute myeloblastic leukemia were treated. The majority received doses of 150 mg/sq m for 5 days every 2 wk. Eleven (24%) complete remissions and four partial remissions were observed. The number of courses to achieve remission averaged three and required an average of 59 days. Nine patients with blastic crisis of chronic myeloblastic leukemia and four with refractory acute lymphoblastic leukemia failed to respond. 5-Azacytidine administered by continuous infusion is well tolerated and is an active compound in acute myeloblastic leukemia.

**T**HE PYRIMIDINE ANALOG, 5-azacytidine (NSC 102816), has been shown to be an active compound in several animal and human neoplasms.<sup>1-8</sup> Its clinical usefulness has been hampered by severe nausea and vomiting and occasional diarrhea accompanying rapid intravenous injection.<sup>9</sup> It has been thought that the drug must be given by rapid intravenous infusion

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because of its instability.<sup>10</sup> However, Israili et al.<sup>11</sup> have shown, using thin-layer chromatography and nuclear magnetic resonance studies, that the  $T_{1/2}$  in buffered solutions is 60–100 hr at 25°C. Karon et al.<sup>7</sup> have found that gastrointestinal toxicity is less without reduction of antitumor activity if drug infusion is extended over 10–15 min or if the dose is divided. Moertel et al.<sup>5</sup> also have found that dividing the dose is associated with less nausea and vomiting. In an attempt to circumvent the severe gastrointestinal toxicity, the Southeastern Cancer Study Group initiated a study of continuous infusions of 5-azacytidine in patients with metastatic cancer and leukemia.

## MATERIALS AND METHODS

### *Criteria for Patient Selection*

Patients with advanced metastatic cancer who had recovered from the toxicity of any prior chemotherapy and who had a life expectancy of at least 8 wk and previously treated patients with acute leukemia or blastic transformation of chronic myelocytic leukemia giving informed consent, were eligible for study. Acute leukemia included acute lymphoblastic leukemia and acute myeloblastic leukemia (myeloblastic, myelomonocytic, monocytic). Studies were conducted at six institutions in the Southeastern Cancer Study Group (Emory University, Duke University, University of Alabama, University of Puerto Rico, Washington University, and Temple University).

### *Pretreatment Studies*

Pretreatment studies included history, physical examination, documentation of measurable neoplastic lesions, bone marrow examination, hemogram, alkaline phosphatase, SGOT, serum proteins, BUN or creatinine clearance, plasma fibrinogen and prothrombin times.

### *Studies During Treatment*

Blood counts were obtained at least twice weekly for the 6 wk of study and for 2 wk thereafter. Renal and liver function tests were repeated every 2 wk. Plasma fibrinogen and prothrombin times were repeated about every 4 wk. In the leukemic patients marrow examinations were done prior to subsequent courses of chemotherapy if blasts were absent from the peripheral blood.

### *Drug Administration*

5-Azacytidine was administered by continuous intravenous infusion over a 5-day period. The drug was dissolved in 50–100 ml of Ringer's lactate and infused over a 3–12-hr period. Fresh solution was prepared every 3–12 hr.

### *Treatment Plan*

In patients with metastatic cancers it was planned to treat three patients with 50 mg/sq m per day for 5 days followed by a 9-day rest period. If hematologic toxicity occurred, therapy was delayed. If no toxicity supervened after 6 wk of therapy (three courses), subsequent sets of three patients were to be treated with increments of 50 mg/sq m until toxicity occurred. Once toxicity occurred, six patients were to be treated at that dose before further escalation. It was planned to observe all patients for a period of 6 wk at a constant dose followed by an additional 2 wk of follow-up. Any patients showing improvement were to be continued on treatment.

In patients with acute leukemia, it was planned to start at 50 mg/sq m in the first set of three patients, but if after a 9-day rest period, there was no change or the white count had increased, the dose was to be escalated by 50 mg/sq m increments in the second and subsequent courses until a hematologic effect was noted. If no effect on the white count occurred at the 50 mg/sq m starting dose, the initial dose was to be escalated in subsequent sets of three patients by 50 mg/sq m increments.

### Evaluation of Toxicity

Hematologic, hepatic, and renal toxicity were graded as previously reported.<sup>9</sup> Gastrointestinal toxicity was graded as follows: (1) nausea without vomiting while on therapy (mild); (2) nausea and vomiting while on therapy controlled by antiemetics (moderate); (3) nausea and vomiting not controlled with antiemetics (severe).

### Criteria of Response

In metastatic cancer patients' objective responses were rated as previously reported,<sup>9</sup> requiring at least a 20% reduction in measurable tumors at 12 wk.

In patients with acute leukemia responses were judged according to the criteria as previously reported.<sup>12</sup> Those demonstrating a clearing of leukemic cells from the blood, changes in marrow status from an M-3 to 2 or less and a reduction in hepatomegaly and splenomegaly when present, but not achieving remission status, were considered to have had an antileukemic effect.

Patients with metastatic cancer were judged evaluable for toxicity if they received at least one 5-day course of treatment and had adequate follow-up information to determine the effect of 5-azacytidine on hemogram, liver, and renal function. Patients were judged evaluable for a therapeutic response if they received at least three courses of therapy.

In leukemia patients all studies were judged evaluable if they received one 5-day course of 5-azacytidine according to protocol.

## RESULTS

### Metastatic Cancer

Table 1 summarizes the toxicity observed in 23 trials in 21 patients entered on study with metastatic cancers.

The major hematologic toxicity was granulocytopenia. In most instances patients received two courses of therapy before toxic levels were reached. The mean nadir day was day 32 (range 22-45) with recovery (above 1500/cu mm) by day 39 (range 25-56). All six patients given infusions of 150 mg/sq m every 14 days had granulocytopenia. When the infusions were given every 21 days or later, two of four experienced granulocytopenia.

No patient had hepatic or renal toxicity which was thought to be drug related. No changes in fibrinogen or prothrombin time were noted although only a few tests were done serially.

Nausea and/or vomiting occurred in 20 patients, and was considered grade 2 in 6. Symptoms were worse the first day or two of treatment. Nausea was usually well controlled with antiemetics. Only one patient refused subsequent courses because of nausea and vomiting. An occasional patient experienced mild diarrhea.

**Table 1. Metastatic Cancer Toxicity**

Dose mg/sq m/day	Frequency of Course (days)	No. of Patients	Hematologic					Gastrointestinal		
			Granulocytes		Platelets		Hemoglobin >3 Drop	1	2	>2
			<1500	<750	<100,000	<50,000				
50	14	4	1	0	0	0	0	2	0	0
100	14	4	0	3	0	0	2	3	1	0
100	21	1	0	0	0	0	0	1	0	0
150	14	8	1	5	0	0	0	5	3	0
150	21-28	4	1	1	1	0	1	3	1	0
175	—	1	0	0	0	0	0	0	1	0
200	—	1	0	1	1	0	0	1	0	0

**Table 2. 5-Azacytidine in Leukemia (Evaluable Patients)**

Leukemia*	Dose (mg/sq m/day)	No. of Patients	Dose Escalated	GI Toxicity			Response†		
				1	2	>2	CR	PR	ALE
AML	50	1	1	1	0	0	0	0	1
	100	7	5	3	2	0	3	0	3
	150	32	7	16	8	0	7	4	7
	200	5	0	3	2	0	1	0	0
	Total	45	13	23	12	0	11	4	11
Blast phase of CML	100	2	1	1	0	0	0	0	1
	150	4	0	0	0	0	0	0	3
	200	3	0	3	0	0	0	0	3
	Total	9	1	4	0	0	0	0	7
ALL	150	3	1	1	0	0	0	0	0
	200	1	0	0	0	0	0	0	0
	Total	4	1	1	0	0	0	0	0

\*AML, acute myeloblastic leukemia; CML, chronic myeloblastic leukemia; ALL, acute lymphoblastic leukemia.

†CR, complete remission; PR, partial remission; ALE, antileukemic effect.

No significant therapeutic benefit was observed in this group of 21 patients, which included 6 with lung carcinoma, 5 with melanoma, 3 with breast carcinoma, 2 with hypernephroma, 2 with leiomyosarcoma, 1 with mesothelioma, 1 with colon carcinoma, and 1 with fallopian tube carcinoma. One patient with melanoma had stabilization of disease but with less than 20% regression in the size of measurable lesions.

### Results in Leukemia

**Acute myeloblastic leukemia.** Forty-nine patients with acute myeloblastic leukemia were entered on study and 45 were evaluable. Of the four inevaluable patients, two were ineligible for the study, in one the protocol was not followed and one was lost to follow-up. A summary of these cases is given in Table 2. The initial dose of 50 mg/sq m was administered to only one patient and no hematologic effect was noted. The dose was escalated in subsequent courses. A reduction in leukocyte count was noted, but no remission occurred despite 13 courses over a year's time.

Seven patients began at 100 mg/sq m; in five the dose was escalated to 150 mg/sq m. One of these five and two others obtained a complete remission and three had an antileukemic effect. Five experienced mild nausea and vomiting.

Thirty-two patients started at 150 mg/sq m. In seven, the dose was escalated in subsequent courses and in one, it was reduced. The maximum doses given were 300 mg/sq m/day. There were seven complete remissions, four partial remissions, and seven patients who had an antileukemic effect. Mild nausea and vomiting occurred in 24 patients.

Granulocytopenia occurred in ten patients and was usually prolonged. One patient developed pleuritic chest pain with the last three of her six courses of chemotherapy, and this was thought to be drug related. Hyperglycemia occurred in two patients, and an inappropriate ADH syndrome was observed in one patient.

Five patients were started at 200 mg/sq m, and the doses were not escalated. One obtained a complete remission. Two experienced moderate nausea and vomiting.

Courses of 5-azacytidine were repeated every 2 wk except in the event of prolonged cytopenia, when treatment was delayed until evidence of recovery occurred. Bone marrows were monitored at frequent intervals and therapy was reinstated whenever the percentage of blasts increased even though the peripheral blood count remained low. Many patients could tolerate two courses with a 9-day interval, but subsequent courses were delayed.

Thus, of 45 evaluable patients with acute myeloblastic leukemia, complete remissions occurred in 11 (24%) and partial remissions in four patients. An antileukemic effect was observed in 11 patients. Reduction of white count was achieved in almost all patients. Dose escalation in two of the patients with partial remissions failed to induce a complete remission. No gastrointestinal toxicity greater than two was observed.

Table 3 summarizes our experience in those 11 patients who achieved complete remissions. The number of courses required to achieve remission varied from one to seven with an average of three and seemed to be unrelated to dose. The beginning of repeated courses varied from 13 to 29 days with an average of 15 days. Four patients achieved remission without marrow hypoplasia, and the remainder demonstrated prolonged marrow hypoplasia after two or more courses. The time to achieve remission varied from 27 to 92 days with an average of 59 days.

Patients received varying maintenance programs, and assessment of remission duration is difficult. The duration of remission ranged from 26 to 600 days with a median of 88 days.

*Blast crisis of chronic myelocytic leukemia.* There were nine patients with blastic transformation treated with 5-azacytidine in doses ranging from 100 to 200 mg/sq m (Table 2). Seven patients had an antileukemic effect after one to three courses, but no remissions were obtained. Three patients had prolonged granulocytopenia.

*Acute lymphoblastic leukemia.* Four patients with acute lymphoblastic

**Table 3. Acute Myeloblastic Leukemia—Complete Remissions**

Starting Dose (mg/sq m/day)	Pt. No.	No. of Courses	Interval (days)	Aplasia (days)	Days to Remission
100	1	2	15	21	54
	2	2	20	31	59
	3	7	14	0	92
150	4	4	19,29,19	0	67
	5	2	13	0	27
	6	3	14	21	61
	7	1	—	0	38
	8	2	14	29	64
	9	3	14	22	75
	10	2	12	41	50
200	11	2	16	26	58

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