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Hematologic and Cytogenetic Remission of Blastic Transformation in Chronic Granulocytic Leukemia

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Thirty patients in the blastic phase of chronic granulocytic leukemia were treated with a combination of vincristine, 2.0 mg/sq m weekly, and prednisone, 60 mg/sq m orally each day. Remission was achieved in nine patients (30%), six of whom had a complete remission, and three had a good partial remission. The survival of responding patients was significantly improved over the non-responders. Cytogenetic studies were performed on all patients in the chronic phase of the disease, and in 28 during the blastic phase. All were Philadelphia chromosome-positive throughout their course. Aneuploidy developed in 68% of the patients entering the blastic phase. Complete hematologic remission was accompanied by disappearance of aneuploid blast cell lines in the five patients in which they were detected, with return of the chromosomal constitution to that which characterized the chronic phase of their disease. Hypodiploidy in blastic transformation of CGL appeared to predict for a favorable response to vincristine and prednisone. Subsequent relapse of the disease in previously remitted patients was associated with further degrees of aneuploidy, suggesting clonal evolution of a resistant cell line.

THE TERMINAL PHASE of chronic granulocytic leukemia (CGL) is characterized by an increasing proportion of blast cells in the bone marrow and peripheral blood, eventuating in a state resembling acute granulocytic leukemia. The treatment of this latter phase of the disease with cytotoxic drugs has met with only limited success in attaining hematologic and clinical remissions.[1] Because the overall remission rate has consistently remained below 10%, it has been regarded as one of the most refractory forms of leukemia.[2]

The present report documents the use of a combination of drugs, vincristine

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and prednisone, in the treatment of the blastic phase of CGL. They were selected in the hope that they might spare the platelet and granulocyte reserve and still have a cytotoxic effect on blast cell proliferation.

Thirty consecutive patients in the blastic phase of CGL were treated. The chemotherapeutic effects of this regimen were correlated with cytogenetic findings at the onset of the blastic phase and during the course of treatment. The results of the program appear superior to previously reported experience and suggest that responses might be predictable on the basis of chromosomal findings.

Materials and Methods

Thirty patients with Philadelphia chromosome (Ph') positive chronic granulocytic leukemia entering the blastic phase of their disease between January 1968, and July 1970, were treated on the protocol. All but one patient had been previously treated with busulfan or dibromomannitol for the chronic phase of their disease.

The minimal diagnostic criteria for transition of CGL to its blastic phase are illustrated in Table 1. The most consistent finding suggesting transition into the blastic phase of CGL is an increasing percentage of blasts in the bone marrow. This can occur rapidly without detectable splenomegaly. In addition, a leukocytosis with increasing numbers of early myeloid cells and blasts, progressive anemia, and thrombocytopenia, which are no longer responsive to previously effective chronic phase therapy, are important criteria. Three patients in this series presented with pancytopenia, insignificant splenomegaly, and extensive blast infiltration of the marrow within 4 wks of their last followup, at which time their counts had been within normal limits. The recognition of arbitrary percentages of blast cells in the marrow is not entirely adequate in the evaluation of this disease, since progressive clinical and hematologic deterioration can ensue before a certain percentage of blast cells are seen in a given marrow specimen. The age and sex distribution of the patients treated are tabulated according to the responsiveness to this regimen (Table 2).

Bone marrow aspirations and biopsy specimens were obtained from all patients prior to study. Cytogenetic studies were performed according to the method of Tjio and Whang.[3]

Drug Protocol

All patients had been off other forms of chemotherapy for at least 1 wk prior to entry into the protocol. Vincristine sulfate, 2.0 mg/sq m body surface area, was administered as a single intravenous dose on a weekly schedule. Prednisone, 60 mg/sq m/orally per day, was added and maintained for at least 2 wk, at which time a gradual tapering of the dose was begun. All patients received at least three weekly doses of vincristine. At that point, the

Table 1.—Diagnosis of Blastic Transformation of CGL

Principal features:
Increasing percentage of blasts in the marrow (20–30%) with or without splenomegaly
Progressive leukocytosis with decrease in mature granulocytes, decreasing platelet count, anemia unresponsive to chronic phase therapy
Other:
Progressive splenomegaly
Cytogenetic evidence of aneuploidy
Extramedullary tumors
Rising leukocyte alkaline phosphatase
Bone pain
Fever, chills

Table 2.—Age, Sex Distribution

	No. of Patients	Age (Mean, Range)	Male	Female
Complete, partial response	9	39 (13–59)	6	3
Peripheral effect	13	41 (23–70)	5	8
No response	8	45 (19–62)	6	2
Totals	30	41 (13–70)	17	13

patient's status was reevaluated. Progression of the disease or failure to achieve an improvement in marrow morphology and peripheral counts led to cessation of therapy. Treatment was continued in patients in whom progressive hematologic improvement occurred.

Results

Cytogenetic Findings

All 30 patients had cytogenetic studies performed during the chronic phase of their disease, and 28 of the patients were studied at the onset of blastic transformation. The Philadelphia chromosome was present in all patients throughout the course of their disease. Nineteen of these 28 patients (68%) demonstrated a major aneuploid cell line, which was not present during the chronic phase of their disease. The distribution varied from 44 chromosomes to 51 (Table 3). Six of the 19 demonstrated duplication of the Ph′ chromosome, in addition to the other abnormalities. In one patient the same aneuploid cell line was detected in a myeloblast-infiltrated lymph node prior to its subsequent infiltration of the bone marrow.

Response to Therapy

Nine patients of the 30 treated (30%) achieved a complete or partial remission. Six of the nine had a complete hematologic remission, with a return of the marrow morphology to normocellularity or the granulocytic hyperplasia, characteristic of CGL, with less than 5% blast forms. In five of these patients, the aneuploid cell line that characterized their blastic transformation completely disappeared with a return to the chromosomal constitution of the chronic phase of the disease (46 chromosomes, Ph′ positive). The hematologic and cytogenetic characteristics prior to therapy and in remission are shown in Table 4. In all patients peripheral hematologic values returned to normal, except the hemoglobin in patient J. R. It is of note that splenomegaly was not present in

Table 3.—Cytogenetic Studies During Blastic Transformation of Chronic Granulocytic Leukemia (Vincristine–Prednisone Study)

	Principal Cytogenetic Cell Line (All Ph′-positive)								
Total patients studied—28									
Number of patients	44	45	46	46*	47	48	49	50	51
	1	4	9	5	3	2	1	1	2

* Pseudodiploid.

Table 4.—Remission of Blastic Transformation of Chronic Granulocytic Leukemia: Hematologic and Cytogenetic Characteristics

			Pretreatment						Posttreatment				
Patient	Age and Sex		Duration of CGL (mo)	Hgb. (g/100 ml)	WBC/% Blasts	Platelets	Spleen Size	Cytogenetic Data	Hgb. (g/100 ml)	WBC	Platelets	Spleen Size	Cytogenetics
J.H.	45	M	42	11.0	2000/10	18,000	0	44 Ph′/46 Ph′	15.0	6,000	300,000	0	46 Ph′
W.P.	50	M	4	10.2	95,000/20	86,000	0	45 Ph′/46 Ph′	13.0	10,000	280,000	0	46 Ph′
L.M.	59	M	108	11.1	40,300/85	70,000	0	46* Ph′/46 Ph′	12.0	8,000	500,000	0	46 Ph′
J.R.	25	M	7	10.3	65,000/20	325,000	Splenectomy	48 2 Ph′/46 Ph′	9.6	5,200	306,000	Splenectomy	46 Ph′
V.S.	47	F	36	11.5	44,800/26	134,000	10 cm	46 Ph′	12.6	6,000	181,000	0	46 Ph′
W.U.	20	M	2	12.6	13,300/14	40,000	12 cm	45 Ph′/46 Ph′	12.0	7,000	140,000	0	46 Ph′

* Pseudodiploid

three patients in whom there was an abrupt transition to the blastic phase. One patient had had a previous splenectomy. The other three patients achieved a significant partial remission, but were excluded from the complete remission group because of persistent splenomegly, despite marked reduction in size, or the continuous demonstration of aneuploidy in the marrow. Eight of the remaining patients achieved a greater than 50% reduction in the peripheral blast count only, but otherwise were without significant hematological improvement, and 13 patients demonstrated no antileukemic effect on this regimen. The presence of reticulin-positive myelofibrosis was noted in 11 patients, but did not influence the likelihood of response. A mean of 3.5 wk of therapy (range 3–6 wk) was required before remission was achieved.

Once a remission was obtained, maintenance was attempted with either oral hydroxyurea or biweekly methotrexate. The mean duration of the first remission in the six patients who achieved complete remission was 5 mo (range 1–9 mo). Reinduction with vincristine alone was attempted in four of these patients during subsequent relapse and was successful in three cases. Two patients achieved a third remission. The interval of remission duration became progressively shorter after the second and third inductions.

Drug Toxicity

The mean dose of vincristine administered to the responding group before achieving a remission was 8.2 mg/sq m body surface. The mean and range of total dose in the various response groups is illustrated in Table 5. It can be seen that somewhat less vincristine was administered to the nonresponders, because of the tendency to stop treatment after 3 wk if no improvement was noted, or if frank progression of the disease occurred despite therapy.

The administration of vincristine was associated with neurotoxicity; including numbness, jaw pain, constipation, and foot drop. It was severe enough to limit drug dosage in only one patient. Another patient received a total of 7.5 mg/sq m in 3 wk without any toxicity.

The predisposition to some forms of serious infectious diseases associated with the administration of corticosteroids was suggested by three patients, who died of documented *Pneumocystis carinii* pneumonia. Two of these were in remission at the time of death.

Cytogenetic Correlations

The type of chromosomal abnormality present at the time that treatment was initiated is tabulated according to the response to this drug combination in Table 6. Patients in blastic transformation with evidence of hypodiploidy appear more likely to respond than any other group, since all five hypodiploid

Table 5.—Total Dose of Vincristine

	No. of Patients	Vincristine Dose (mg) Mean, Range
Complete and partial remission	9	12.2 (5.7–22.5)
Peripheral effect only	8	9.5 (5–15)
No response	13	8.4 (5.2–16)

Table 6.—Principal Cytogenetic Findings in Blastic Phase of CGL: Effect of Vincristine, Prednisone (Chromosomal Number, ALL Ph′ POS)

	44	45	46	46°	47	48	49	50	51
Complete and partial remission	1	3	3	1		1			
Peripheral effect only		1	2	1			1	1	1
No response			4	3	3	1			1

° Pseudodiploid.

patients either had a remission or demonstrated a marked peripheral effect, whereas the majority of hyperdiploid patients were resistant. The differences between these groups are significant ($p<0.023$), chi-square analysis.

Cytogenetic studies were repeated during the subsequent course of the blastic phase in 22 patients. During leukemic relapse in those six patients who had previously achieved complete remission, five developed additional cell lines with further degrees of aneuploidy associated with progressive resistance to vincristine. Only one patient relapsed with the original hypodiploid cell line.

One of the three partial responders and only two of the 13 nonresponders, subsequently studied, developed additional lines. The latter two patients had a marked peripheral antileukemic effect without remission.

Hyperdiploidy occurred in six of the eight cases who developed an additional aneuploid cell line, by means of the acquisition of a second Ph′ (two cases), or other additional chromosomes.

Survival

Those patients in whom a remission could be obtained had a significantly longer survival ($p< 0.01$) than the nonremitting group (Fig. 1). Remission was associated with a median survival of 10.5 mo, whereas in the nonresponders the median was 2.5 mo. By comparison, the mean survival of a group of additional patients previously treated at this institution with a variety of other drug programs was 2.5 mo, with only 7.5% of the patients attaining a remission.[4]

Discussion

The natural history of chronic granulocytic leukemia terminates, in the

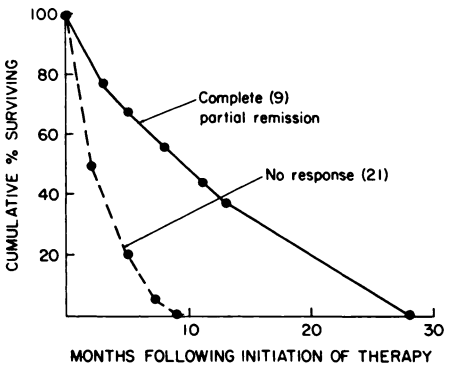

Fig. 1.—Survival in blastic transformation of chronic granulocytic leukemia according to response to vincristine and prednisone. Numbers in parentheses indicate number of patients.

Table 7.—Cytogenetic Evolution During Blastic Phase: Complete Remission Patients

Patient	Duration (mo)	Pretreatment	Relapse
L.M.	8	46 Pseudodiploid Ph′	48 Ph′
J.H.	13	45 Ph′	45 2 Ph′
J.R.	4	48 2 Ph′	49 2 Ph′
W.P.	28	45 Ph′	44 Ph′
W.U.	10	44 Ph′	44 Ph′
V.S.	12	46 Ph′	47 Ph′

majority of patients, in an accelarated or blastic phase, which in some instances is indistinguishable from acute granulocytic leukemia. Other patients suffer a myeloproliferative acceleration of their disease with progressive splenomegaly, myelofibrosis, abnormalities of platelet and erythrocyte morphology, and basophilia.[5,6] Although the chronic phase of the disease can be controlled satisfactorily with busulfan, radioactive phosphorus, and splenic radiation, the overall survival of patients with this disease has not significantly changed since the days of Minot, because of the inability to treat the eventual blastic transformation.[1,7,8]

The chemotherapeutic approach to the treatment of the latter phase of this disease has generally included the drugs and combinations of agents found to be effective in the treatment of acute granulocytic leukemia. The results have been disappointing, with only occasional and brief remissions.[9-13] The effectiveness of vincristine and prednisone alone would be unexpected, on the basis of their utility in acute myeloblastic leukemia. The pancytopenia that results from the use of some of the drugs effective in the therapy of acute leukemia may be more severe in patients with blastic transformation of CGL because previous extensive chemotherapy with cytotoxic agents such as busulfan, massive splenomegaly, and myelofibrosis, can limit their effectiveness.

The advantages of the use of vincristine and prednisone in this disease are analogous to the experience in acute lymphoblastic leukemia.[14] The relative lack of severe myelosuppression and the marked sensitivity of blast cells in at least a certain percentage of patients explains the higher remission rate attained with this combination. Some of the patients experienced a marked diminution of marrow and peripheral blood absolute blast counts without hematologic improvement. In this latter group of patients, a marked reduction of precursor cells capable of maturation may have precluded a remission, or, alternatively, the malignant transformation may have occurred in the earliest precursor cells, so that two populations of cells were not present.

The majority of patients with CGL have the Philadelphia chromosome marker, which remains throughout the course of the illness. It has been observed that additional chromosomal abnormalities can occur coincidentally with or shortly before the onset of blastic transformation.[15-19] The degree of aneuploidy that has been observed varies greatly from hypodiploidy to marked hyperploidy, including duplication of the Philadelphia chromosome. These aneuploid karyotypes in addition to the Philadelphia chromosome suggest that they are closely

related clones derived from a common ancestral cell.[20,21] The short survival of patients in whom aneuploidy is detected suggests that it is a valuable marker for incipient blastic transformation, even before a systemic hematologic transformation has occurred.[19]

The findings in this series of patients indicate that hypodiploid cell lines are significantly more sensitive to the cytotoxic effects of vincristine and prednisone therapy. This could be explained by a more rapid generation time and increased proliferating cell pool size, associated with a hypodiploid chromosomal constitution or, more speculatively, that hypodiploidy, per se, renders the cells more susceptible to a given dose of vincristine. One of the pharmacologic effects of the vinca alkaloids involves binding by the drug to microtubular proteins associated with mitotic spindles.[22] It is possible that hypodiploid cells, with potentially fewer binding sites, may be more sensitive to a given dose of vincristine. Relapse of the disease and progressive refractoriness to subsequent courses of vincristine occurred and was associated with the appearance of new aneuploid cell lines, usually of a higher chromosomal number. A probable mechanism for the acquisition of extra chromosomes by cells is the process of nondisjunction. It has further been suggested that cells of abnormal chromosomal content are more likely to develop nondisjunction.[24,25] The occurrence of further degrees of aneuploidy during the course of blastic transformation, especially in those instances where survival is prolonged by chemotherapy, is consistent with this proposition.

The achievement of hematologic remission of the disease was accompanied by a disappearance of the aneuploid cell line that previously characterized the blastic transformation with a return to the cytogenetic characteristics of the chronic phase of the disease. Thus, serial chromosome studies were useful in determining the status of the disease and effectiveness of chemotherapy used for remission induction. Since the inception of this study a brief cytogenetic and hematologic remission in a case of blastic crisis of CGL following the use of a combination of vincristine, prednisone, methotrexate, and 6-mercaptopurine has been reported.[23]

Although a significant response rate of 30% and a complete remission rate of 20% are far from ideal, the use of vincristine and prednisone alone allows for a relatively nontoxic remission, when obtained. If no indication of an effect is apparent after 3 wk of therapy, it should be abandoned, since the prolonged administration of vincristine and prednisone can be associated with considerable morbidity. The numbers are too small for a definitive comment on maintenance therapy. It would seem that intermittent reinduction therapy, interspersed during chronic, continuous, oral antimetabolite maintenance, may have more to offer the patients in remission than continuous oral antimetabolites alone.

These results further suggest that, in the future, the type of therapy selected might be based on the chromosomal nature of the leukemic cell, since the response rate to vincristine and prednisone in this series of patients appear to be closely related to the cytogenetic findings.

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