[CANCER RESEARCH 36, 3034-3038, September 1976]

Regulation of Bone Marrow Myeloblast Proliferation in Chronic Myeloid Leukemia[1]

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SUMMARY

The *in vitro* [^{3}H]thymidine-labeling index of bone marrow myeloblasts and myelocytes was determined for 9 hematologically normal individuals and 20 Ph^1-positive chronic myeloid leukemia (CML) patients in the chronic phase of their disease. The mean labeling index of myeloblasts from CML patients when the white blood cell (WBC) count was lower than 20,000/cu mm (42.4%) was not significantly different from that of normal myeloblasts (49.9). This index was found to be significantly ($p < 0.05$) decreased to an average of 20.9% when the WBC count was higher than 40,000/cu mm. The mean labeling index of CML myelocytes was not significantly influenced by the level of WBC.

The data presented indicate that such variations in the labeling index of the leukemic myeloblasts represent changes of their proliferative activity related to the level of WBC. It is concluded that the proliferation of CML myeloblasts is sensitive, to a certain degree at least, to the size of the myeloid cell population in the body or a subclass of it.

INTRODUCTION

Many observations have been made suggesting that the production of myeloid cells in CML[3] is still the object of some regulation (6, 11, 17, 25).

It has been shown by autoradiography that, after incubation of the bone marrow with [^{3}H]TdR, the myeloblasts of the bone marrow in CML, at diagnosis before any treatment, have a lower labeling index than the myeloblasts of hematologically normal individuals (1). In view of the decreased labeling index of the myeloblasts in acute myeloblastic leukemia as well, this finding in CML myeloblasts could be interpreted either as the expression of the leukemic nature of these cells during the chronic phase of CML (12) or as a decreased fractional production rate of cells resulting from a regulatory mechanism operating on the myeloblasts in the presence of an increased myeloid cell mass. To test the latter hypothesis, the [^{3}H]TdR labeling index of marrow CML myeloblasts when the WBC count was high was compared to the labeling index of CML myeloblasts when the WBC count was normal or close to normal, generally as a consequence of the treatment. The same comparison was made for CML myelocytes to determine whether the degree of maturation of the cells plays a role in their sensitivity to the hypothesized regulation mechanism.

MATERIALS AND METHODS

The proliferative activity of the myeloid cells was studied for 9 hematologically normal patients and 20 Ph^1-positive CML patients. The CML patients were investigated once or several times either before treatment (11 observations), during busulfan treatment (4 observations), off busulfan therapy for 35 to 189 days (7 observations), or off another form of chemotherapy for 14 to 21 days (8 observations). Bone marrow was drawn for all determinations of the labeling index except those performed during busulfan therapy. Those for which only peripheral blood was examined are reported separately in Chart 2.

All the samples added to EDTA were incubated for 45 to 60 min in the presence of [^{3}H]TdR (40 μCi/ml) at room temperature. Smears were then made, dipped in NTB2 Kodak liquid nuclear track emulsion, and exposed in the dark for 11 days at 4° before being developed. On autoradiographs stained with May-Grünwald-Giemsa, the myeloid precursors were classified as myeloblasts, promyelocytes, myelocytes, and metamyelocytes. Large and small myelocytes were considered together as a single morphological compartment.

The myeloblasts were identified as cells with a delicate chromatine structure, basophilic cytoplasm, and no granules. The myelocytes were defined as cells with a diameter of between 12 to 20 μm and a more condensed chromatin and a less basophilic cytoplasm than the myeloblasts and promyelocytes. The chromatin structure, rather than the shape of the nucleus, was used to distinguish myelocytes from metamyelocytes, since kidney-shaped CML cells frequently showed uptake of [^{3}H]TdR. The criteria for distinction between myelocytes and metamyelocytes may thus have differed somewhat from those used by other investigators and may explain differences in the labeling index of the myelocytes observed between our nonleukemic patients and those described in the literature.

The percentage of labeled cells was determined separately for the myeloblasts and myelocytes. A background of 3 autoradiographic silver grains was arbitrarily adopted.

[1] Presented in part at the Symposium on "Classification of Unclassifiable Leukemias," Paris, October 1974. Supported by the "Fonds Cancérologique de la Caisse Générale d'Epargne et de Retraite de Belgique" and by Euratom Grant 088.72. I BIAC and realized under contract of the Ministère de la Politique Scientifique within the framework of the Association Euratom-University of Brussels-University of Pisa.

[2] This service is a member of the European Organization for Research on Treatment of Cancer.

[3] The abbreviations used are: CML, chronic myeloid leukemia; [^{3}H]TdR, tritiated thymidine; CFC, colony-forming cells; PMN, polymorphonuclear cells.

Received June 23, 1975; accepted May 20, 1976.

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Therefore, in most instances, a cell was considered labeled when more than 3 silver grains were seen over its nucleus. However, a cell with 4 to 6 grains over the nucleus in an area of high background was not necessarily scored as labeled. Whenever possible, at least 100 myeloblasts were examined for determination of their labeling index. However, in most cases with normal or slightly increased WBC counts, due to the low percentage of myeloblasts in the bone marrow, the labeling index was determined on less than 100 cells (Table 1). The labeling index of the myelocytes was determined on 250 cells in most cases. In some cases, it was difficult to distinguish myelocytes from metamyelocytes or promyelocytes. In these instances, the labeling index of the myelocytes was not determined.

The data were analyzed by means of the Kruskal Wallis H test (K sample Wilcoxon test).

RESULTS

Table 1 shows the individual blood leukocyte levels, treatment status, and labeling indexes. Table 2 shows the mean and median labeling index of myeloblasts and myelocytes separately for: (*a*) 9 nonleukemic patients; (*b*) the CML patients with less than 20,000 WBC/cu mm (in the pretreatment phase or, more often, in a posttreatment phase of their disease); (*c*) the CML patients with 20,000 to 40,000 WBC/cu mm; (*d*) the CML patients with more than 40,000 WBC/cu mm; (*e*) the CML patients never treated; and (*f*) all the CML marrows examined. The labeling index was determined more than once for certain patients. The mean value of these patients was used when determining the mean labeling index of Groups *b*, *d*, and *f*.

Table 2 shows that in the group with fewer than 20,000 WBC/cu mm, the average labeling index of the marrow myeloblasts was 42.4, thus not significantly different from the average value observed in the group of hematologically normal patients (49.9%). In the group with more than 40,000 WBC/cu mm, the mean labeling index of the myeloblasts was 20.9, significantly ($p < 0.01$) lower than in the group of nonleukemic patients. In these comparisons, statistical significance, or its lack, remains unchanged even if Patient R. A. with CML and 11,000 WBC/cu mm were excluded be-

Table 1
Clinical and experimental data

	Patient	WBC/cu mm	Previous chemotherapy	Days off therapy	No. of myeloblasts counted	% myeloblasts labeled	No. myelocytes counted	% myelocytes labeled
Hematologically normal (9)[a]	H. O.	7,200			73	41	250	17.6
	V. D.	5,600			75	49.3	250	19.1
	B. R.	8,300			88	48.8	250	12.2
	D. N.	9,000			34	44	150	15.2
	V. L.	6,700			41	58.5	250	20.6
	D. G.	7,900			100	38	250	20.4
	C. E.	4,900			30	53.3	250	22.8
	B. M.	7,000			50	46		
	P. S.	8,200			50	70	250	20.4
CML (17)	G. A.	5,200	HU[b]	21	100	40		
	R. A.	6,400	VCR	14	42	66.5	250	11.1
	C. U.	7,300	Busulfan	119	142	36	250	25.3
	T. L.	7,900	CA and TG	17	100	39	250	21
	M. A.	9,900	CA and TG	25	100	34	250	13.9
	R. A.	11,000	Busulfan	36	8	50		
	C. O.	14,100	Busulfan	35	32	47	250	12
	M. A.	23,600	Busulfan	97	122	40	250	16
	V. Y.	30,600			125	29.6	250	17.3
	M. K.	31,000	Busulfan	49	100	62	250	31.8
	D. R.	45,000	HU	41	50	16	250	7.2
	B. O.	51,500			50	14		
	R. I.	68,000	CA	75	100	15		
	V. W.	72,000	Busulfan	189	100	22	250	22.8
	R. I.	82,000	VCR	27	100	14		
	M. A.	82,000	Busulfan	161	100	23.5	250	10.7
	R. I.	87,000			100	11		
	P. A.	118,000			105	31.4		
	L. A.	122,000			126	20.6	250	21
	M. A.	166,000			100	14		
	C. U.	196,000			150	28	250	21.3
	T. L.	225,000	HU	28	132	24.6	250	6.2
	C. A.	229,000			118	19	250	6.8
	G. A.	244,000			120	18.3	250	22.8
	S. C.	280,000			113	25.7	250	15.7
	V. E.	680,000			148	21.6	250	19.3

[a] Numbers in parentheses, number of patients.
[b] HU, hydroxyurea; VCR, vincristine; CA, cytosine arabinoside; TG, 6-thioguanine.

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Table 2
[^{3}H]TdR labeling indexes of myeloblasts and myelocytes

Status at time of labeling		Myeloblasts					Myelocytes			
Hematological	(WBC/cu mm)	*n*	Mean	Median	*p*		*n*	Mean	Median	*p*
Nonleukemic	<10,000	9	49.9	48.8	NS[a]	<0.01	8	18.5	19.7	NS
CML	<20,000	7	42.4	39.5			5	16.6	13.9	
	20,000-40,000	3	43.9	40	0.05		3	21.7	17.3	NS
	>40,000	16	20.9	20.6			10	15.4	17.5	
	Never treated	11	21.2	21.6			7	17.7	21.3	
	All	26	29.3	22.7			18	16.8	16.6	

[a] NS, not significant; *n*, number of determinations.

cause of the fact that the labeling index of his myeloblasts was determined on 8 cells only.

The labeling index of CML marrow myelocytes, on the other hand, was not influenced by the WBC count. Its mean value was 16.6% in CML patients with low WBC counts and 15.4% in CML patients with high WBC counts. Only 3 CML patients were studied while the WBC count was between 20,000 and 40,000/cu mm. The average value of their labeling indexes was 43.9% for the myeloblasts and 21.7% for the myelocytes. This groups of patients is too small to be compared with the other groups.

Chart 1 shows that most patients with a high WBC count and a low myeloblast labeling index (and vice versa) had never been treated. It also indicates that the relationship between WBC and labeling index is not a linear one. Chart 2 shows the labeling index of the myeloblasts during 4 courses of busulfan given to 3 CML patients; these were the only determinations of the labeling index made on peripheral blood. The chart indicates that the labeling index either stayed stable (in 2 studies) or decreased (in 2 other studies), but never increased significantly during this treatment.

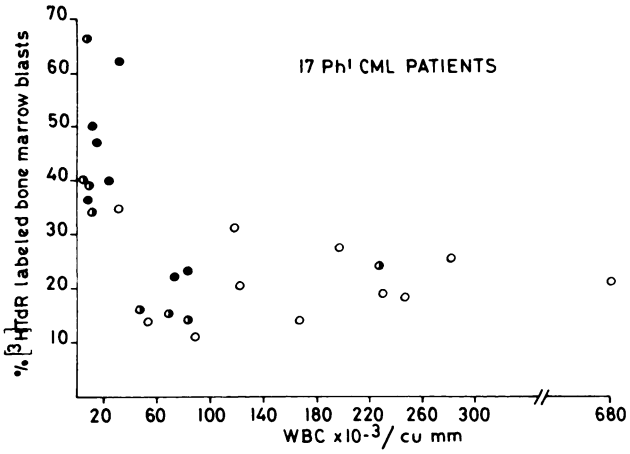

Chart 1. The percentage labeled bone marrow myeloblasts as a function of the WBC/cu mm in untreated CML patients (○), in CML patients off busulfan therapy (●), and in CML patients off other chemotherapy (◑).

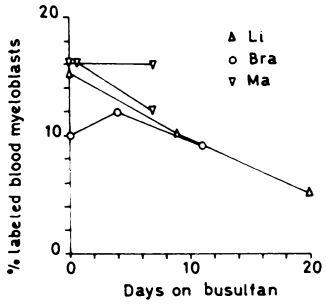

Chart 2. The percentage labeled blood myeloblasts as a function of time on busulfan therapy.

DISCUSSION

The problem of regulatory mechanisms eventually acting on leukemic cells was studied in CML for the following reasons: (*a*) the blastic transformation and the appearance at the same time of CFC of lighter density as seen in acute myeloblastic leukemia strongly suggest that CML, already in its chronic phase, is at least a "subleukemic" disease; (*b*) remissions (almost normal hematological parameters) and relapses (high WBC counts) can be observed repeatedly if treatment is intermittent (21); (*c*) the persistence of the original abnormal cell line during remission is indicated by the persistence of the Ph¹ chromosome (26); (*d*) at diagnosis of CML, the labeling index of the myeloblasts in the marrow has been found to be decreased in comparison to that of normal cells, although not so much as in acute myeloblastic leukemia (1, 2); (*e*) the cyclic oscillations of the granulocyte blood level that may occur spontaneously in CML (17, 25) or during chemotherapy with drugs given at a constant dosage (11), as well as a case with cyclic oscillations in which the colony-stimulating factor levels showed an inverse relationship to the peripheral leukocyte count (6), provide indirect evidence for the activity of positive and negative regulatory influence operating on myeloid cell proliferation in CML.

The results obtained raise many questions. The 1st concerns the value of the labeling index of the myeloblasts in hematologically normal individuals, since published values vary from 15 to 85% (5). This variability may be due to the difficulties in defining exactly the morphology of the myeloblast. In the present study, this problem was partially overcome by the fact that 1 person did all the counting of cells. Nevertheless, in our 9 hematologically normal individuals, the labeling index still ranged from 38 to 70%. The

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mean labeling index for normal myeloblasts obtained in the present work (49.9%) was very close to the mean (46.2%) obtained from the literature (4, 5, 9, 13, 19, 23). It is thus conceivable that the variability of the labeling index of the myeloblasts reflects a true variability of the proliferative activity of these cells.

The next question concerns the reality of the low labeling index of marrow myeloblasts in CML when the WBC count is high. To answer this, one should rule out the possible role played by the contamination of bone marrow by peripheral blood every time a marrow aspirate is done. As a matter of fact, since the labeling index of the myeloblasts in CML is generally much lower in the peripheral blood than in the bone marrow (18) with higher WBC counts, a greater contamination of marrow by blood and, consequently, an apparently lower marrow labeling index are to be expected. Such a contamination cannot be ruled out. However, it is probably not the explanation for the results obtained for the myeloblasts, since in that case the marrow myelocytes also would have been affected, which was not the case. Indeed, a difference between marrow and blood, although less marked than for myeloblasts, was found also for the labeling index of the myelocytes (18). It is therefore concluded that the marrow myeloblasts do, in fact, have a lower labeling index when the number of myeloid cells increases in the periperal blood.

Another question is whether the variations in the labeling index of the CML myeloblasts represent differences in proliferative activity or only variations in the duration of DNA synthesis (S phase). It is unlikely that the variations in the labeling index of the myeloblasts were related only to changes of the S phase. As a matter of fact, in 3 CML patients with 54,000 to 158,000 and 323,000 WBC/cu mm, the duration of the S phase for marrow myelocytes was around 13 hr (24), which is similar to the S phase for normal myelocytes (22).

The next question is whether the variations in the labeling index represent a process of regulation. Since most CML patients with WBC count below 40,000/cu mm and a normal labeling index of their marrow myeloblasts had previously been treated (see Table 1 and Chart 1), the normal labeling index could therefore be related directly to the treatment rather than to a negative feedback mechanism triggered by the decrease of the WBC level. In order to elucidate this point, 3 CML patients were studied during busulfan treatment. Since it appeared unlikely that marrow and blood myeloblasts would behave in an opposite fashion, this part of the study was made on peripheral blood myeloblasts in order to avoid repeated bone marrow aspirations to the patients. Chart 2, which shows that the labeling index of myeloblasts never increased during busulfan therapy, indicates thus that the increased labeling index observed after discontinuation of busulfan must be an indirect consequence of the treatment. Moreover, the fact that Patients C. U. and M. A. had a myeloblast labeling index in the normal range of values, 119 and 97 days, respectively, after cessation of busulfan, also argues against the direct effect of this drug on the normalization of their myeloblast labeling index.

Rather than triggering a feedback regulation, the treatment could instead select a leukemic subline proliferating faster than the others. Again, the observation that the labeling index did not increase during busulfan therapy makes such a selection unlikely.

The 6th question concerns the leukemic nature of the cells that are the target of this regulation. In 3 CML patients (C. U., R. A., and M. K.) with relatively low WBC counts (7,300, 11,100, and 31,000 per cu mm, respectively) and normal myeloblast labeling indexes (39, 50, and 62%), a chromosomal analysis was made at the same time. In the 3 cases, 100% Ph^1 chromosomes were found on 20, 20, and 26 mitoses, respectively. This observation rules out the possibility that the normal labeling index was that of Ph^1-negative cells sometimes seen in increased proportion during remission.

The next question concerns the maturation stage of the leukemic cells sensitive to this regulation mechanism. The absence of significant variability in the labeling index of CML myelocytes reflects the absence of regulation of the cell cycle length at that stage of maturation, at least in CML. In regard to myeloid cells less differentiated than myeloblasts, Moore *et al.* (16) have shown that in active CML the percentage of CFC in DNA synthesis with the use of [^{3}H]TdR suicide technique is lower than normal and also increases to normal values during remission. In Table 3, their results with CFC (16) are compared to our results with myeloblasts and myelocytes. This comparison suggests that the proposed regulatory mechanism acting on CML myeloblast proliferation is also acting on CML CFC. This is not surprising, in view of the observation that the CFC in CML, as opposed to normal CFC, are morphologically indistinguishable from the myeloblast (16). The observation that the fraction of CFC in DNA synthesis was greater during the nadir and lower during the peak level of mature granulocytes in the blood of a patient with cyclic neutropenia (10) suggests that this regulation of myeloid precursor proliferation by the level of peripheral blood PMN is not restricted to leukemia. Finally, all these observations suggest that the proposed regulatory mechanism acting on CML myeloblast proliferation represents one of the controls of normal granulopoiesis.

The next question concerns the trigger and the possible mediator of this negative feedback. Normal human PMN cells were shown to inhibit colony formation (7), a result also found for PMN cells from CML patients (14). Such inhibition is probably mediated by the release of a cell product. This mediator could be the lipoprotein, detected in

Table 3
Percentage cells in DNA synthesis

Patients	CFC[a]	Myeloblasts[b]	Myelocytes[b]
Normal and miscellaneous diseases	39 ± 14	49.9 ± 9.8[c]	18.5 ± 3.4
CML remission	53-48	42.4 ± 9	16.6 ± 6.1
CML active	21 ± 11	20.9 ± 5.2	15.4 ± 7

[a] Results obtained by Moore *et al.* (16), with the [^{3}H]TdR suicide technique. There were only 2 patients in remission with 6,000 and 12,000 WBC/cu mm; 13 patients had active CML or chronic myelomonocytic leukemia.

[b] Present study: CML remission, less than 20,000 WBC/cu mm; CML active, more than 40,000 WBC/cu mm.

[c] Mean ± S.D.

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leukemic and in normal serum, which inhibits myeloid colony formation *in vitro* (3). It is therefore likely that the PMN is playing a role in the phenomenon described here. On the other hand, the myeloblast itself cannot be ruled out since, in the case of acute myeloblastic leukemia, at least, this cell was also found capable of releasing such an inhibitor (15). Moreover, an inverse correlation was found in CML between the labeling index of marrow myeloblasts and the percentage of myeloblasts in the bone marrow at least above 10% (8). Since all myeloid cells from the myeloblast to the mature neutrophil appear to be present in increased numbers in the body during active CML, it is impossible at the present time to attribute to a specific one the exclusive or principal role in this regulation.

The existence of a negative feedback that continues to regulate the proliferation of the myeloid cells in CML raises the question of how the myeloid cell mass is increased in CML. First, it is conceivable that the regulation that persists is set at an abnormal level. The mediator(s) shown to be produced by CML-PMN (14) and to act on leukemic myeloid cells (20) could be quantitatively or qualitatively insufficient, or the myeloid progenitors could be less sensitive than normal to the mediator(s). In this case, the decrease of the labeling index of the myeloblasts should be more pronounced and/or appear for less-elevated WBC counts in a nonleukemic individual than in a CML patient. This is presently being investigated in our laboratory. Second, even if the postulated regulatory mechanism were functioning normally, this would not be incompatible with the increased cell mass in CML. As a matter of fact, the increased myeloid cell mass has been attributed partly to an increased input of stem cells in the proliferating compartment and partly to an increased number of cell divisions, 3 to 4 rather than 2, in the myelocyte compartment.[4] Conceivably, these mechanisms of overproduction could exceed the capacity of a normally functioning regulatory system.

ACKNOWLEDGMENTS

We thank G. Flowerdew and R. Sylvester of the European Organization for Research on Treatment of Cancer Data Center for statistical assistance, Dr. T. Peltzer for her continuous help in the hematological follow-up of the patients, and Dr. A Verhest (Cytogenetics Department, Institute Jules Bordet) for the cytogenic analysis.

REFERENCES

1. Baccarani, M., and Killmann, S. A. Cytokinetic Studies in Chronic Myeloid Leukemia: Evidence for Early Presence of Abnormal Myeloblasts. Scand. J. Haematol., *9:* 283-292, 1972.
2. Baccarani, M., Santucci, M. A., and Tura, S. Observations of the Cytokinetics of Chronic Myeloid Leukemia. *In:* S. Tura and M. Baccarani (eds.), Chronic Myeloid Leukemia, pp. 76-89. Pavia, Italy: Edizione Haematologica, 1972.
3. Chan, S. H., and Metcalf D. Inhibition of Bone Marrow Colony Formation by Normal and Leukemic Human Serum. Nature, *227:* 845-846, 1970.
4. Cronkite, E. P. Enigmas Underlying the Study of Hemopoietic Cell Proliferation. Federation Proc., *23:* 649-661, 1964.
5. Cronkite, E. P., and Vincent, P. C. Granulocytopoiesis. Ser. Haematol., *4:* 3-43, 1969.
6. Gatti, A. R., Robinson, W. A., Deinard, A. S., Nesbit, M., McCullough, J. F., Ballow, M., and Good, R. A. Cyclic Leukocytosis in Chronic Myelogenous Leukemia: New Persepectives on Pathogenesis and Therapy. Blood, *41:* 771-782, 1973.
7. Haskill, J. S., McKnight, R. D., and Galbraith, P. R. Cell-Cell Interaction *in Vitro* Studied by Density Separation of Colony-forming, Stimulating, and Inhibiting Cells from Human Bone Marrow. Blood, *40:* 394-399, 1972.
8. Gavosto, F. Granulopoiesis and Cell Kinetics in Chronic Myeloid Leukaemia. Cell Tissue Kinet., *7:* 151-163, 1974.
9. Gavosto, F., Maraini, G., and Pileri, A. Proliferative Capacity of Acute Leukemia Cells. Nature, *187:* 611-612, 1960.
10. Greenberg, P. L., and Schrier, S. L. Granulopoiesis in Neutropenic Disorders. Blood, *41:* 753-769, 1973.
11. Kennedy, B. J. Cyclic Leukocyte Oscillations in Chronic Myelogenous Leukemia during Hydroxyurea Therapy. Blood, *35:* 751-760, 1970.
12. Killmann, S. A. Chronic Myelogenous Leukemia. Preleukemia or Leukemia. *In:* S. Tura, and M. Baccarani (eds.), Chronic Myeloid Leukaemia, pp. 45-53. Pavia, Italy: Edizione Haematologica, 1972.
13. Kuroyanagi, T., and Saito, M. Proliferative Capacity of Leukemic Cells Studied with Tritiated Thymidine *in Vitro*. Tohoku J. Exptl. Med., *80:* 168-174, 1963.
14. Moberg, C., Olofsson, T., and Olsson, I. Granulopoiesis in Chronic Myeloid Leukemia. I. *In Vitro* Cloning of Blood and Bone Marrow Cells in Agar Culture. Scand. J. Haematol., *12:* 381-390, 1974.
15. Moore, M. A. S. Communication at the Symposium on Classification of Unclassifiable Leukemias, Paris, October 1974. Blood Cells, *1:* 249, 1975.
16. Moore, M. A. S., Williams, A. N., and Metcalf, D. *In Vitro* Colony Formation by Normal and Leukemic Human Hematopoietic Cells: Characterization of the Colony-forming Cells. J. Natl. Cancer Inst., *50:* 603-623, 1973.
17. Morley, A. A., Baikie, A. G., and Galton, D. A. G. Cyclic Leucocytosis as Evidence for Retention of Normal Homeostatic Control in Chronic Granulocytic Leukaemia. Lancet, *2:* 1320-1323, 1967.
18. Ogawa, M., Fried, J., Sakai, Y., Strife, A., and Clarkson, B. D. Studies of Cellular Proliferation in Human Leukemia. VI. The Proliferative Activity, Generation Time and Emergence Time of Neutrophilic Granulocytes in Chronic Granulocytic Leukemia. Cancer, *25:* 1031-1049, 1970.
19. Pileri, A., Masera, P., Pegoraro, L., Bachi, C., and Gavosto, F. Studio della Capacità Proliferative delle Cellule Sanguine Mediante l'Impiego di Acido Desossicitidilo in Luogo di Timidina. Bol. Soc. Ital. Biol. Sper., *41:* 744-747, 1965.
20. Rytömaa, T. Role of Chalone in Granulopoiesis. Brit. J. Haematol., *24:* 141-146, 1973.
21. Stryckmans, P. Current Concepts in Chronic Myeloid Leukemia. Seminars Hematol., *11:* 101-127, 1974.
22. Stryckmans, P., Cronkite, E. P., Fache, J., Fliedner, T. M., and Ramos, J. DNA Synthesis Time of Erythropoietic and Granulopoietic Cells in Human Beings. Nature, *211:* 717-720, 1966.
23. Todo, A. Proliferation and Differentiation of Hematopoietic Cells in Hematologic Disorders. *In Vitro* Radioautograph Study of Leukemia Cells. Acta Haematol. Japon., *31:* 932-946, 1968.
24. Vincent, P. C., Cronkite, P. C., Greenberg, M. L., Kirsten, C., Schiffer, L. M., and Stryckmans, P. A. Leukocyte Kinetics in Chronic Myeloid Leukemia. I. DNA Synthesis Time in Blood and Marrow Myelocytes. Blood, *33:* 843-850, 1969.
25. Vodopick, H., Rupp, E. M., Edwards, G., Groswitz, F. A., and Beauchamp, J. J. Spontaneous Cyclic Leukocytosis and Thrombocytosis in Chronic Granulocytic Leukemia. New Engl. J. Med., *286:* 284-290, 1972.
26. Whang, J., Frei, E., III, Tijo, J. H., Carbone, P. P., and Brecher, G. The Distribution of the Philadelphia Chromosome in Patients with Chronic Myelogenous Leukemia. Blood, *22:* 664-673, 1963.

[4] P. C. Vincent, E. P. Cronkite, T. M. Fliedner, M. L. Greenberg, C. Hunter, C. Kirsten, J. S. Robertson, L. M. Schiffer, and P. A. Stryckmans. Leukocyte Kinetics in Chronic Myeloid Leukemia. II. Size Distribution of Myelocytes, and Initial Labeling Pattern of Cells in Blood and Marrow, in preparation.

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