The New England Journal of Medicine

Owned and Published by the Massachusetts Medical Society

PROSPECTIVE authors should consult "Information for Authors." which appears in the first issue of every volume and may be obtained lrom the *Journal* office.

ARTICLES with original material arc accepted for consideration with the understanding that. except for abstracts. no part of the data has been published. or will be submitted for publication elsewhere. before appearing hcrc.

STATEMENTS or opinions expressed in the *Journal* reflect the views of the author(s) and do not represent official policy of the Massachusetts Medical Society unless so stated.

NOTICES should be sent at least 30 days before publication date.

ALTHOUGH all advertising material accepted is expected to conform to ethical medical standards, acceptance doer nor imply endorsement by the *Journal*.

REPRINTS: Reprints are available in minimum quantities of 50. To order. please contact the Business Office at the address provided below.

SUBSCRIPTION PRICES: CSA: $60 per year (interns. residents $35 per year; students $30 per year). Outride USA (U.S. funds only): $75 prr year (interns. residents $55 per year; students $45 per year). Remit payments and correspondence to NEJM Subscriber Service Dept., 1440 Main St., Waltham, MA 02254-0803. Please include current mailing label with renewal order.

EDITORIAL Omm: 10 Shattuck St., Boston. MA 02115.

BUSINESS AND SUBSCRIPTION OFFICES: 1440 Main St., Waltham, MA 02254.

ACUTE LEUKEMIA AND CELL-DIFFERENTIATION

ACCUMULATING evidence for a unicellular (clonal) origin of leukemia, based on the studies of Fialkow and coworkers employing isozymes of the X-linked gene, glucose-&phosphate dehydrogenase (G6PD), as clonal markers,[1] combined with evidence from rigorous karyotyping,[2] leaves little doubt that acute nonlymphocytic leukemia (ANLL), chronic myelogenous leukemia. and acute lymphoblastic leukemia originate in the transformation and subsequent clonal expansion of a hematopoietic precursor, often (at least in the myeloid leukemias) a precursor with multipotent developmental capabilities. The goal of antileukemic therapy seems clear enough: to destroy the transformed blastic clone and encourage repopulation by residual normal precursors. Common sense dictates that the disappearance of leukemic blast cells from blood and marrow and the reemergence of adequate numbers of functioning erythrocytes, platelets, and granulocytes constitute irreducible evidence of therapeuric success. whether short-lived, prolonged, or even permanent. Reports of chemotherapy-induced remissions of ANLL accompanied by disappearance of the chromosomal markers of the malignant clone and reappearance of functionally normal blood cells of polyclonal origin[1] reinforce the wisdom of the common-sense view. Yet, some disquieting evidence has also accumulated. Two years ago in these pages, Fialkow and his colleagues[3] described a patient with ANLL. a chromosomal abnormalitv, and enzymatic evidence of cionality of the leukemic blasts, in whom complete remission was characterized by a normal karyotype but persistent evidence for a clonal origin of the repopulating normal blood cells. This strongly suggests that a leukemogenic clonal population of blood precursors can generate apparently normal progeny, as well as serve as a source of an overtly leukemic. chromosomally abnormal blast-cell population. Yet. the implications of this observation remained uncertain; is this a rare event or the common underlying biologic pattern in ANLL?

Much of the uncertainty is dispelled by a report in this issue of the *Journal,* by Fearon et al.,[4] which provides further compelling evidence for the developmental capabilities of clonal leukemogenic blood-cell precursors. this time based on recombinant-DNA techniques. The new genetic tool provided by DNA restriction-fragment–length polymorphism,[5] which has been so powerful in mapping the human genome and detecting elusive genetic traits, has been brought to bear decisively on the somatic-cell genetics of leukemic cells. Restriction-fragment–length polymorphisms are produced by small local variations in DNA sequence in the vicinity of identifiable genetic loci; these variations are recognized because they change the size of DSA fragments generated by the cleavage of DNA by restriction enzymes. which have very specific base-pair sequences as their cutting-site tar-

gets. A ratnction-fragment-length polymorphism is Gassed, like any other genetic feature, in germ-line and somatic cells; heterozygosity can be detected, and the analysis is independent of the actual expression of any particular gene; In any population of nucleated cells, whether dividing or quiescent, the restriction-fragment–length polymorphism will bc revealed to the investigator armed with the appropriate molecular probes. To detect clonality in leukemic blast cells and the normal blood cells of patients with ANLL, whether at diagnosis or during remission, Fearon et ai. used molecular techniques and restriction-fragment–length polymorphisms according to three strategies: (1) to detect X-linked markers entirely analogous to the G6PD isozymes studied by Fialkow et al., but without the need to detect expression of an enzymatic gene product; (2) to detect clonal aneupioidies, but without the need for a dividing cell population to generate visible chromosomes: and (3) to detect the immunoglobulin gene rearrangement that normally occurs early during E-cell differentiation and that for unknown reasons may occasionally occur during the evolution of a myeloid leukemia clone.

Three major conclusions can be drawn from these studies. First of all. clonality in at least a large majority of cases of ANLL seems certain: 7 of 10 patients who were heterozygous for the X-linked restriction-fragment–length polymorphism locus had the telltale ıonoclonal pattern. Second, clones of leukemic preıursors seem quite frequently capable of sustaining apparently normal differentiation to mature granulocytes during active disease; five of six patients evaluated according to one or another of the three strategies had this phenomenon. Third, in elegant confirmation of Fialkow's prediction. 3 of 13 evaluable patients had evidence that their remission-stage granulocyte populations were of clonal origin and presumably (although not proved) of the same clone as the blasts of their active-stage disease.

Taken together. these studies. the work with G6PD isozvmes, and the results of karyotype analysis begin to point out a more comples pathophysiology for AKLL than might have been supposed. As proposed by both Fialkow and Fearon et al., emergence of a clone of hematopoietic preleukemic precursors capable of essentially normal differentiation, but with variable levels of proliferative advantage over normal precursors, may constitute an early event in leukemogenesis. Such a stage could be hematologically undetectable except by analysis of clonality. Overt blastic disease would supervene with a second somatic genetic event. perhaps signaled by a chromosomal abnormality. Successful chemotherapy may suppress the blast population (and the chromosomal marker), leaving both the clonal preleukemic cell lineage and normal precursors. in variable proportions, to reemerge s hematologically normal blood-ceil elements.

Exciting questions and uncertainties remain. Would it be clinically useful. if it were actually possibic. to be able to detect the presence of a hematologically normal but preleukemic clonal population? At present there seems little likelihood chat such a population could be detected before the onset of overt hematologic disease. either blastic leukemia or dyshematopoiesis (such as refractory anemia). Bur a remission-stage clonal population is fair game for detection by using — if necessary — additional genetic markers for clonality. Could the behavior of such a clone and its potential, on the one hand, to support normal hematopoiesis or, on the other, to allow the reemergence of blastic leukemia in relapse be modified by therapeutic intervention? It is clear from studies with experimental leukemia cell lines of both murine and human origin[6,7] that both chemical cytodifferentiation agents and natural growth factors can modify the expression of developmental potential. The possible role of simultaneous and sequential combinations of cytocidal chemotherapy and cytodifferentiation agents must surely be considered in the light of these findings, as must the future role of ablative therapy followed by bone marrow transplantation. Whatever the practical outcome, the studies. provide powerful new insights into the natural history of a hematologic neoplasm — insights that may apply equally to nonhematopoietic cancers.

Memorial Sloan–Kettering
Cancer Center
New York. NY 10021

RICHARD A. RIFKIND, M.D.

REFERENCES

1. Fialkow PJ, Singer JW, Adamson JW, et al. Acute nonlymphocytic leukemia: heterogeneity of stem cell origin. Blood 1981: 57:1068-73.
2. Pearson MG. Larson RA. Golomb HM. Clinical significance of chromosome patterns in malignant diseases: from molecules to man. In: Rowley JD. Ultmann JE. eds. Chromosomes and cancer: from molecule, to man. New York: Academic Press. 1983:311-31.
3. Jacobson RJ. Temple MJ. Singer JW. Raskind W. Powell J. Fialkow PJ. A clonal complete remission in a patient with acute nonlymphocytic leukemia originating in a multipotent stem cell. N Engl J Med 1984: 3101513-7.
4. Fearon ER. Burke PI, Schiffer CA. Zehnbauer BA. Vogelstein B. Differentiation of leukemia cells to polymorphonuclear leukocytes in patients with acute nonlymphocytic leukemia. N Engl J Med 1986 315:15-24.
5. Boatein D. White RL. Skolnick M. Davis RW. Construction of a generic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 1980: 32:314-31.
6. Rifkind RA. Sheffery M. Marks PA, Induced differentiation of murine erythroleukemia cells: cellular and molecular mechanisms. In: Klein G. Weinhouse S. eds. Advances in cancer research. Vol. 42. Orlando. Fla.: Academic Press. 1984:149-66.
7. Collins SJ. Bodner A. Tinp R. Gallo RC. Induction of morphological and functional differentiation of human promyelocytic leukemia cells (HL-60) by compounds which induce differentiation of murine leukemia cells. Int J Cancer 1980; 25:213-8.

ADVANCES IN CORNEAL TRANSPLANTATION

DISEASE of the cornea is the most common cause of blindness in the world.' The cornea is the normally transparent anterior structure of the eye that functions similarly to the lens of a camera and has the appearance of a watch crystal. The normal cornea is a layered. avascular structure in which an outer epithelium and an inner endothelium surround the central stroma. The stroma imparts its opticai properties to

Seminars in Hematology

VOL. XI, NO. 2 APRIL 1974

Current Concepts in Chronic Myelogenous Leukemia

By P. A. Stryckmans

A 50% 5-YR LEUKEMIA-FREE SURVIVAL and possible cure rate can be expected in children with acute lymphocytic leukemia treated with the best treatment schedules available today.[62,65] This is not the case for chronic myelogenous leukemia in which the median duration of survival is still of the order of 3–3.5 yr, which is almost the same as for the patients with no treatment. described by Minot in 1924.[104] Most reviews dedicated to the treatment of chronic myelogenous leukemia in recent years have emphasized this point. It must be recognized that. on the other hand. many important new facets of the disease. related to its pathogenesis. are continuously described.

New forms of treatment such as more aggressive chemotherapy. maneuvers to decrease physically the total mass of myeloid cells (early splenectomy and leukapheresis) and others to increase the immunologic defense of the patient have been proposed in the last few years. Whether these new approaches will significantly improve the survival of the patients is not clear yet, but the new experimental facts certainly constitute a logical and ethical basis for the use of more aggressive therapeutic procedures in a disease still rapidly fatal.

The basic concepts and some of the emerging concepts will be discussed first, and the classical and the more recent therapeutic procedures will be reviewed thereafter. Only the classical Ph^1-positive CML will be considered in this article because the evaluation of new therapeutic procedures is easier in this form of the disease than in the Ph^1-negative form. The reasons therefore are: that the Ph^1-positive CML is more frequent. that it represents probably a nosologic entity distinct from the Ph^1-negative CML[38] and finally that eradication of the disease by therapy may be indicated by disappearance of the marker.

THE PERSISTENCE OF REGULATORY MECHANISMS FOR MYELOPOIESIS IN CML

Although Ph^1-negative myeloid cell lines have been so far described in only a few patients with Ph^1-positive CML,[20,49,41,85] it is conceivable that they do exist in all these patients. If so, they probably represent what is left of the normal nonleukemic population.

From the Service de Médecine Interne et d'Investigation Clinique de l'Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Brussels, Belgium.

Supported in parr by the Fonds Cancérologique de la Caisse Générale d'Epargne et de Retraite de Belgique and by the Lady Tata Foundation.

The obvious proliferative advantage of the Ph¹-positive cell lines could be inherent to the cell or related to feedback mechanisms. The second possibility would be realized if the normal cell line were depressed because its normal sensitivity to regulatory mechanisms which would not affect, to the same extent. the Ph¹-positive cells. In fact, this Ph¹-positive cell line produces large numbers of mature cells which, directly or indirectly, by feed-back effect could depress the Ph¹-negative cells more than the Ph¹-positive cells. This hypothesis implies partial retention of normal homeostatic control. Granulopoiesis has been proposed as a negative feed-back system containing a time delay and therefore may show oscillations under appropriate circumstances.[105] The cyclic oscillations of the granulocytes blood level which occurs in CML spontaneously[106,153] or during chemotherapy given at a constant dose[75] suggest therefore that a homeostatic control is still acting on myeloid cell proliferation in CML.

A humoral factor has been proposed for the homeostatic control of granulopoiesis operating in normal individuals[50] and the colony-stimulating factor (CSF) found in serum and urine which brings about differentiation of myeloid precursors cells in vitro, could very well be the humoral factor responsible for the regulatory mechanism operating in vivo.[50,102] Gatti et al.[51] found an inverse relationship between the level of CSF and the peripheral count in their patient with CML who had oscillating leukocyte counts. This is an additional argument for the persistence of some regulatory mechanisms in CML.

Two characteristics of the leukocyte oscillations seen in CML should be pointed out; the great amplitude of individual cycles (some exceeding 100,000/cu mm) and the length of the cycles: 30.40.50. 110,[106] 66,[153] and 72 days.[51] These amplitudes and cycle lengths are considerably greater than those found in cyclic neutropenia and normal individuals.[105] Cronkite and Vincent[11] found that the cycle length of 20 days seen in normal individuals and in cyclic neutropenia fitted what we know of humans on the time for a stem cell to produce polymorphonuclear cells (PMN). This time is normally around 10 days. Such a delay of 10 days from maximal signal from the blood (the level of PMN) to maximum response from bone marrow will produce oscillations of 2 x 10 days. Therefore the observation of long oscillation periods in CML suggest that in this disease more time may be needed to produce a mature PMN. One possible explanation for this long delay could be an increased number of successive divisions, which, in turn, could explain the great amplitude of the oscillations. On the other hand, the high amplitude of oscillation could also result from an abnormal sensitivity of the Ph¹-positive stem cells to the regulation system. How this fits with the observation of high levels of colony-stimulating activity found in the urine of CML patients[125] is not yet clear.

THE TRANSIT TIME IN THE BLOOD OF POLYMORPHONUCLEAR CELLS (PMN) IN CHRONIC MYELOGENOUS LEUKEMIA

A remarkable observation was the fact that the transit time of PMN of CML in the blood is considerably longer than in normal individuals during the active phase of the disease and tends to become normal when the blood leukocyte count decreases as a consequence of treatment.[1,44,122] In order to reduce the artifacts due to the presence, in the blood. of immature myeloid cells in the determination of the $T_{1/2}$ of the PMN, irradiation of the labeled cells when the blood contained a large proportion of myelocytes was performed to stop the

maturation of these cells into labeled PMN.[45] This technique has given more accurate values for the $T_{1/2}$ of PMN in CML. Around 210,000 WBC/cu mm, a $T_{1/2}$ of 26 hr was found, instead of 6.4 hr as for normal subjects. This study thus confirmed that the PMN of CML had a longer transit time in the blood than normal PMN. This prolonged transit time, although it potentiated the leukocytosis once it had developed, was of secondary importance in the pathogenesis of the increased number of blood PMN.

The conclusion is that the increased number of PMN in CML is due to an increased production. This would suggest that the chances are small of inducing a good remission only by removing the excess of myeloid cells in the peripheral blood.

FUNCTIONAL CAPACITY OF POLYMORPHONUCLEAR CELLS (PMN)

The prolonged transit time of PMN of CML in the blood has been shown to be due to cellular rather than to extracellular factors.[1,44] This suggests that these cells may be deficient and may not function normally. As a matter of fact, the capacity of CML mature neutrophils to egress from the blood to the tissues has been found to be decreased in CML.[4,120] By means of plastic chambers at the site of mechanical abrasion, a defect in cell migration was found in all stages of CML. It was inversely correlated with the clinical state.[4] Moreover, once at the site of inflammation, the PMN of CML may be less active than normal.[11,119] Pedersen and Hayhoe[115] have compared leukocyte alkaline phosphatase activity (LAP) and phagocytic capacity in PMN from CML patients and have observed that the cells with demonstrable enzyme content were more active phagocytes than the LAP negative ones. This is in agreement with an improved phagocytic ability found during remission." However, although deficient, PMN of CML were found useful in the treatment of severe infections in neutropenic non-CML recipients.[37] This suggests that their decreased functional capacity observed in vitro may be due to their immaturity but that with time, in vivo, they are able to mature into normally functioning cells.

THE PHILADELPHIA CHROMOSOME

The Philadelphia or Ph' chromosome, discovered in 1960,[108] is still the only chromosomal marker consistently found in a human neoplasm. It represents a deletion of the long arm of chromosome 22.[21,112] It has been shown recently that it probably results from a translocation between the long arm of chromosome 22 and the long arm of chromosome 9.[126] It persists throughout the course of CML both in relapse and during remission.[146,154] The myeloid, erythroid and the megakaryocytic cell lines presumably have the marker,[129,150,154] whereas other cells such as skin cell lymphocytes and bone marrow fibroblasts" do not have the anomaly. CML is apparently an acquired disease since concordance among identical twins is exceptional. In only one pair did both twins have Ph'-positive CML.[148] In the other pairs, there was no Ph' chromosome in the normal twins whose leukemic twins showed typical Ph'-positive CML.[32,54,55,66,67] This strongly supports the postulate that the Ph' chromosome is an acquired abnormality rather than a hereditary defect of the chromosome. It seems to be acquired long before the appearance of clinical symptonis as in a patient described by Canellos[15] in whose marrow cells rhe Ph' chromosome was consistently detected for almost 6 yr in the absence of hematologic disease.

All these observations are compatible with the concept that eradication of the Ph[1]-positive cells could be a way to eradicate the disease. However, the Philadelphia chromosome is said to be neither constant nor specific for CML. As a matter of fact, it is found in approximately 85% of the cases of CML, and, on the other hand, it has been described occasionally in all sorts of myeloproliferative disorders. It should be stressed however that several studies[38,149,155] have indicated that the clinical features of the Ph[1]-negative cases are somewhat different from those of Ph[1]-positive cases. The Ph[1]-negative cases are more frequent in infancy and in the older adults, they have usually enlarged lymph nodes and lower leukocyte and platelet counts. They respond poorly to treatment, they transform earlier and have a much shorter median survival than the Ph[1]-positive cases. The Ph[1]-negative cases therefore probably represent a nosologic entity distinct from a classical CML associated with the Ph[1] chromosome.[38,149,155]

On the other hand, a low percentage of Ph[1]-like chromosomes have been found in occasional cases of acute myeloblastic leukemia,[81,94,111,156] erythroleukemia,[17,81] thrombocytopenia,[] polycythemia,[83] myelofibrosis,[77,158] agnogenic myeloid metaplasia,[43] untreated pernicious anemia.[] For several reasons, all these observations do not rule out with certainty the possibility that the Ph[1] is still specific of CML. First, most of these diseases share clinical features with CML and therefore some of these cases may have been particular forms or phases of a true CML mistakenly labeled: secondly, most of these data should be reviewed today since they were obtained between 1965 and 1970, at the time when the more accurate banding techniques were not then available; thirdly, because these cases may have been CML in their preclinical stage since we know that the Ph[1] chromosome may be present many years before the clinical onset of CML.[15] The finding that the Ph[1] is probably constant and specific for CML reinforces the idea that its eradication may be of therapeutic value in this disease.

A crucial finding which has important therapeutic implications is that although in most cases of CML, all or practically all cells in the bone marrow have the Ph[1] chromosome, Ph[1]-negative myeloid cells also exist. Occasionally, in a few patients who had been intensively treated or at least deeply myelodepressed, clearly Ph[1]-negative myeloid cells have been seen in vivo.[41,49,85,150] The existence of Ph[1]-negative myeloid cells in Ph[1]-positive CML was confirmed on bone marrow grown in vitro. This technique allows observation, in bone marrow cultured in a petri dish of many granulocytic colonies each arising from a single granulocyte precursor. It was found that all metaphases from a single colony were either Ph[1]-positive or Ph[1]-negative suggesting that leukemic and normal cells may coexist in CML.[20]

THE CLONAL ORIGIN OF CML

The Ph[1] chromosome being acquired and limited to the myeloid, erythroid, and megakaryocytic series, strongly suggests but does not prove however that the disease is originated from one single transformed cell. G6PD heterozygote patients with CML provide an additional strong argument for the theory of the clonal origin of CML. G6PD heterozygotes have two distinct populations of cells: one producing the type A isoenzyme only and the other the type B iso-

enzyme only. It was found that when these patients who have type A and type B enzyme-producing fibroblasts, get CML, that their red cells and granulocytes had either type A or type B enzyme but not both.[5,40] The single enzyme phenotypes in leukemic cells of CML most probably reflect a clonal origin of the disease. Studies on experimental animals indicate that the concept of clonal origin is not incompatible with a viral origin of CML which is suggested by the finding of reverse transcriptase associated with 70 S-RNA in the leukocytes of three CML patients.[6]

Nevertheless, one observation exists which does not support that CML is originated from one single cell. It concerns a patient with Klinefelter's syndrome and CML who had a sex chromosome mosaicism of the XY/XXY pattern in the blood cells and was found to have the Ph¹ chromosome in some cells of both cell

PROLIFERATION OF MYELOID CELLS IN CML

As it was indicated in a previous paragraph. there is good evidence from studies on PMN blood transit times that the production of these cells is increased. This may be due either (1) to an increased rate of cell division, (2) to an increased number of successive divisions in the recognizable myeloid cells, (3) to an increased number of myeloid precursors at the stern cell level.

The first possibility can easily be tested by measurement of the ^{3}H-thymidine labeling indices. providing the duration of DNA synthesis is known. The duration of DNA synthesis during the chronic phase of the disease, whether it was measured by the double labeling technique[77] or by the stathmokinetic method,[2] was found to be in the normal range of 10–15 hr.[144] This information made it interesting to compare the labeling index of the myeloid cells in CML and normal individuals. The labeling index of the proliferating bone marrow myeloid cells taken as a whole was of the order of 23% in 12 studies on nine CML patients in the chronic phase of the disease. i.e., in the order of what was found in normal individuals.[145] This value was the same whether the patient had a high number of leukocytes/cu mm of blood or a nearly normal number as a consequence of treatment (Fig. 1). When only the granulated precursors (pro-

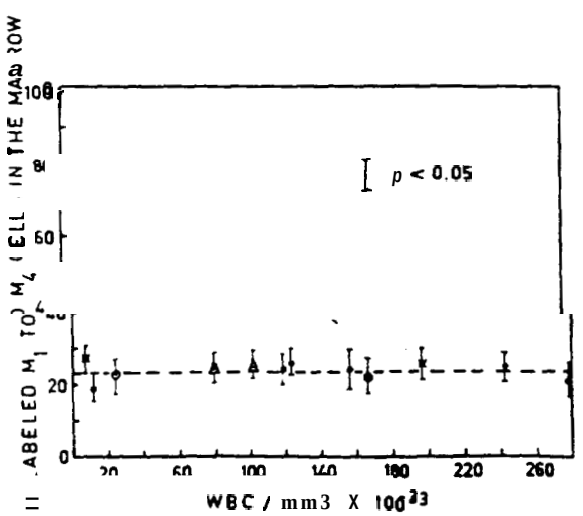

Fig. 1. Ordinate: the percentage of ^{3}HTdR labeled myeloblasts, promyelocytes, end myelocytes in the marrow of nine CML patients. Abscissa: the blood leukocyte counts. The bone marrow of three patients (e, ✱, and ▲) was examined twice, at different WBC levels.

myelocytes + myelocytes) were **examined** for their labeling index, the mean value was of the same order of magnitude, i.e., 24.7 in seven CML patients.[1] In contrast, when the myeloblasts in the bone marrow of these patients were examined separately, at the onset of the disease they showed a mean labeling index of 16.9, a value considerably lower than in normal individuals but of the same order of magnitude as in blastic transformation[2] and only slightly higher than in blasts of acute myeloblastic leukemia.[52,78,97] Thus CML myeloblasts, from the clinical onset of the disease on, seem to behave as the blast cells of acute myeloblastic leukemia. It is still not clear, however, whether the labeling index of the myeloblasts remains low when a remission with a normal spleen and a normal blood picture have been obtained. The answer to this would tell us whether the decreased labeling index is directly related to the disease or rather indirectly related through the effect of homeostatic mechanisms probably still operating in this disease. The answer to this question will have considerable implications for the treatment of the chronic phase of CML. These observations do not support the hypothesis of an increased rate of cell production in the recognizable myeloid cells.

The second possibility, namely an increased number of successive divisions in

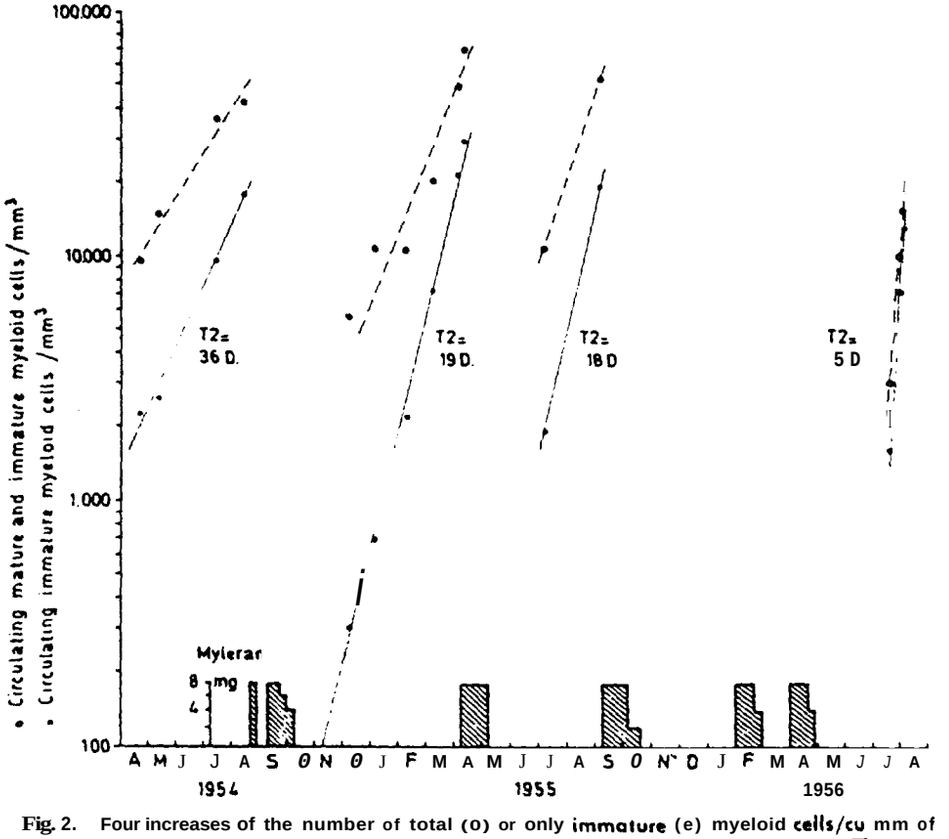

Fig. 2. Four increases of the number of total (o) or only immature (e) myeloid cells/cu mm of blood after discontinuation of busulfan during periods with no maintenance treatment. The doubling times (T_2) got progressively shorter. The last before death under blastic transformation was 5 days. The T_2s were always shorter far immature myeloid cells than for the total of mature and immature.

the recognizable myeloid cells, could be suggested. as explained above. by the long period of the oscillations seen in the patients who exhibited cyclic variations of their leukocyte counts. However, as Bresciani[12] has pointed out. an additional division would increase the number of cells produced. but in order to increase it continuously. the number of divisions should also increase continuously, a modification which seems unlikely. For the reasons explained below. the last alternative is the most probable namely an increased pool of precursor cells. The fact that the immature myeloid cells in the blood between two successive courses of busulfan increase progressively faster and faster after each course of myleran up to the blastic transformation (Fig. 2), suggests that the total mass of immature myeloid cells in the body also increases faster and faster. in view of the traffic existing between marrow. blood, and spleen. This observation and the fact that no real change of the labeling index in the bone marrow occurs during the course of the disease (Fig. 1). suggest a progressive maturation defect of the self-perpetuating precursors. The consequence of this could be an exponential expansion of the precursor compartment which in turn would explain a more and more rapid increase of the number of recognizable precursors in the blood after discontinuation of busulfan.[11]

THE BLASTIC TRANSFORMATION OR BLAST-CELL CRISIS

This event is the main threat of CML. Sixty to seventy per cent of the patients die in this stage of the disease which is clinically and hematologically reminiscent of acute myelobiastic leukemia (AML).[10] There is almost no effective treatment for this complication once it has arisen; it is more resistant to therapy than AML. Prevention of this acute phase of the disease should therefore be the principal aim of all our efforts. However, so far most of our classical treatments of the chronic phase of CML have failed in delaying significantly the onset of the blast crisis.

Several modifications which have been found to precede and herald the blastic transformation could shed some light on the pathogenesis of this event: (1) myelofibrosis in the bone marrow biopsy;[56] (2) additional chromosomal abnormalities on top of the Ph1,[3,7,8,11,13,17,19,149,151] (3) a marked increase of the LAP;[57] (4) a short doubling time of the immature myeloid cells in the blood.[8,47,145]

In 181 patients with CML, myelofibrosis was found in seven patients at time of initial diagnosis of CML.[56] Four of them died from blastic transformation with a mean survival of 8.8 mo. Thirty-two patients who developed myelofibrosis late in the course of CML had a mean survival of 4.9 mo after the diagnosis of myelofibrosis was established. In 27 of them, the cause of death could be attributed to blastic transformation. This report thus stresses that myelofibrosis was an ominous prognostic sign. This observation raises an important question concerning the pathogenesis of blastic transformation namely, is this complication exclusively related to a modification of the leukemic cells or also the consequence of changes in the microenvironment of these cells? The last possibility is suggested by reports on exceptional cases such as the five cases in whom blastic transformation started with destructive-lytic lesions in the bones.[11] the patients with typical blood and marrow features of the chronic phase in whom

blastic transformation was seen only in lymph nodes (two patients) or in the cerebrospinal fluid (two patients)[53] and the patient in whom blastic transformation started in a post-traumatic hematoma.[79]

The Ph¹ anomaly persists during the blastic transformation. hut all sorts of additional chromosomal abnormalities appear on top of it in about two thirds of the cases.[22,38,57,71,87,113,130,137,149,151,155] In most cases, abnormalities of several autosomes are seen often associated with the appearance of aneuploidy. Hyperploidy represents the most frequent finding but hypoploidy may also hc seen. The appearance of two or more Ph¹ chromosomes either heralds or accompanies the development of the blastic phase.[29,33,73] It may appear a considerable period of time before the blastic phase develops.[35]

Serial investigations have provided evidence that progression of the disease is accompanied by a karyotype evolution which leads to chromosome complements with increasing numbers of numerical abnormalities.[29,42,114] It has been proposed that the nondiploid cells differentiate less successfully than diploid Ph¹-positive cells.[118] This will result in a more dedifferentiated appearance of the marrow and is supported, not only by the increased proportion of myeloblasts in the marrow and the blood. but also by findings suggesting disorganized differentiation with nucleo-cytoplasmic asynchrony. namely, premature development of PAS[60] and Sudan black B[142] positivity as well as peroxidase activity[92] in blast crisis myeloblasts.

Serial caryotypes during chemotherapic treatment of the blast crisis have demonstrated the heterogeneity of the leukemic cell population. Several leukemic clones could he recognized which responded differently to chemotherapy since some decreased as a consequence of treatment while others were recorded for the first time after chemotherapy.[117] The cell line not seen before treatment may have been dormant and therefore resistant to therapy. This observation supports the idea to treat intensively CML in the chronic phase since it suggests that if a Ph¹-negative cell line in a dormant state exists in all CML patients. an assumption for which we have arguments, it may survive to the treatment better than the Ph¹-positive cells and. by proliferation, become predominant thereafter.

An important question which is intimately related to the treatment of CML and which has been extensively discussed in the last few years is whether CML in its chronic phase is a true leukemia or rather a preleukemic condition. In other words. is the acute blastic transformation "built into" the disease from the very beginning[79] or is it rather an additional event[118] which does not necessarily occur but has only a high risk to occur in a disease characterized by a chromosomal abnormality and therefore predisposing to leukemia.[''] The fact that the development of cytologic and PAS anomalies in the myeloblasts late in the disease is positively correlated with karyotype alterations and the Fact that the leukocyte alkaline phosphatase dccrcasc seen early *in* the disease. may be due to incomplete maturation of granulocytic end-cells on early release from the bone marrow rather than to a maturation defect,[116] have been argued for the theory of CML being a preleukemic state.[''] However, strong arguments exist for the opposite theory of CML being leukemic from the very beginning. One is the correlation found by Bergsagel between the duration of remission

following the first course of busulfan and total survival from diagnosis.[1] Another is the fact that the doubling time (T_d) of the white blood cells after each course of busulfan was found to get shorter with time.[47] In the same line,[48] in 10 out of 12 cases of CML, the T_2 of the number of immature myeloid cells in the blood between successive courses of intermittent treatment with busulfan and/or ^{32}P has been found to become progressively shorter with time, up to the blastic transformation (Fig. 3). In one additional patient followed for more than 10 yr and still in the chronic phase of her disease 20 yr after its onset, a very slow shortening of the T_2 could be seen. The shortening of T_2 was not found to be related to the treatment but appeared rather as the natural evolution of the disease. It strongly suggests that, at the time of diagnosis, the disease was already progressing towards its terminal phase. This concept is

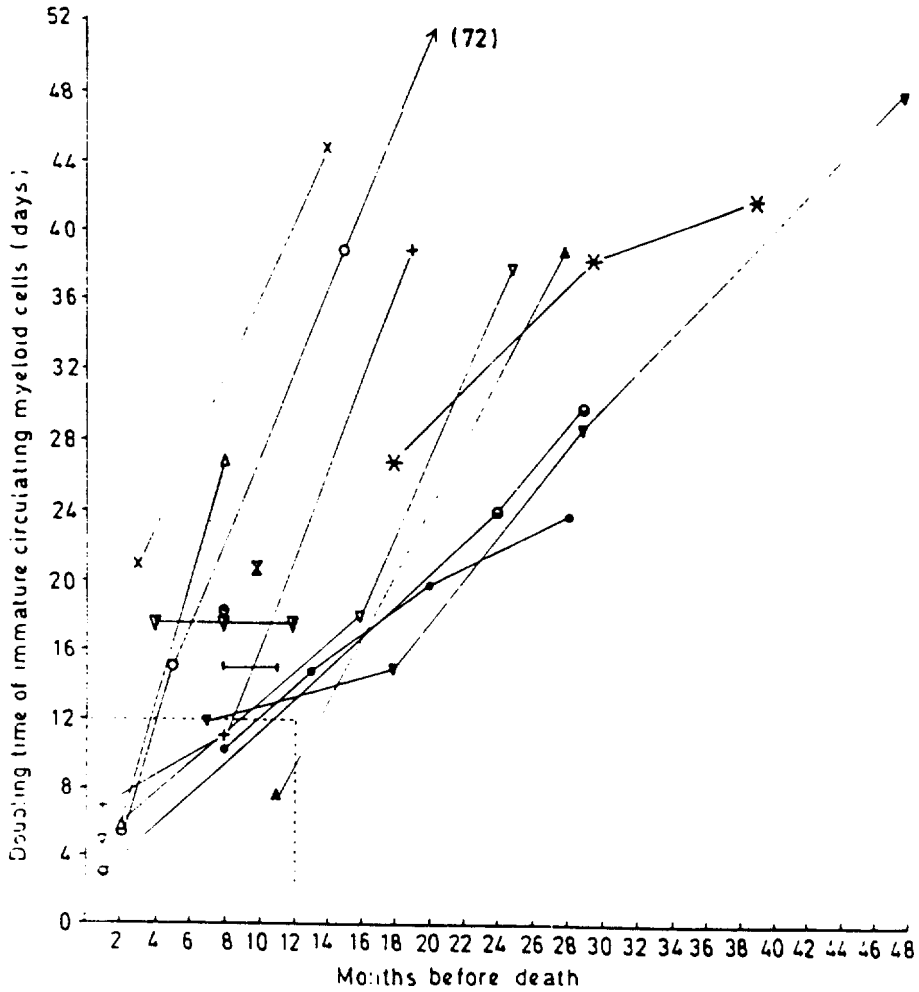

Fig. 3. Shows for 12 patients deceased the correlation between the doubling time (T_2) of the number of immature myeloid cells/cu mm of blood (in ordinate) and the time at which this was determined before death (in abscissa). A different symbol is used for each patient, and each line joins the successive estimations of T_2 for each patient. The eight cases whose T_2 became shorter than 12 days all died within a year (dotted square).

compatible wiih the finding that, at the very beginning of the disease, the 'H-thymidine labeling index of the myeloblasts was considerably lower than in normal myeloblasts and as low as it is seen in acute transformation or in acute myeloblastic leukemia.* These findings strongly support the hypothesis that the blastic crisis is the fully expressed end of a continuous process which is progressing, more or less rapidly. from the very beginning of the disease and is not affected by our actual treatments.

THE TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA

A large number of drugs have been found to have sonic activity in CML. All these studies were different in their aims and methods and will therefore be classified as follows:

(1) The studies which indicate that a drug is able to induce a remission (decrease of WBC. spleen, and platelets and eventual increase of hemoglobin). The drugs only studied in this way are the following: nitrogen mustard,[69] uracil mustard,[157] vinblastine,[61] thioguanine,[84] TEM,[127] melphalan,[133] Colcemid,[90] etc.

(2) The studies in which a drug in its capacity to induce remissions was compared to a reference drug (usually busulfan) in a randomized way for a limited period of time. Such studies have shown that 6MP,[64] chlorambucil[128] and cyclophosphamide[70] are less active than mylerari in inducing a remission.

(3) The studies in which one mode of treatment was given over most or all the duration of the disease. These studies make it possible to evaluate the median survival of the patients so treated. Splenic irradiation.[7,9] ^{32}P,[124] busulfan.[7,9,10] hydroxyurea,[76] and dibromomannitol[28] have been studied in this way (Table 1).

(4) Only rare studies of this type exist. The MRC study compared on a random basis, the median survival obtained with two different modes of treatment namely busulfan and splenic irradiation[7,9] (Table 1).

BUSULFAN (MYLERAN)

Busulfan has been considered up to now as probably the best treatment available in CML.[48] This view may be challenged, now that new approaches

Table 1. Survival of Patients Treated for CML

Treatment	Number of Patients	Median Survival (mo)	Survival From	Ret.
None	52	31	—	104
^{32}P	118	32	EO	124
Busulfan	30	42	EO	58
Busulfan	48	40	BT	100
Splenic irrodiotion	54	28	BT	100
Dibromomannitol	73	43	D	28
Hydroxyurea	10	50	D	76
Hydroxyurea (+ or − splenectomy)	43	39.6	—	132
Chemo. + immunotherapy	15'	>84	—	13c

EO. estimated onset; BT, beginning of treatment; D, diagnosis

'Selected patients.

to treatment of CML seem promising. The method of administration. the advantages and the hazards of myleran in CML, which have been remarkably described by Galton in a 1969 issue of this journal,[48] have practically not changed since then. Therefore, major points only will be stressed here.

The individual daily dose seems more important in terms of toxicity than the total dose. The daily dose should not exceed 6 mg or even 4 mg.[48] Since the rate of fall is dose related. with these relatively low doses, the decrease of the leukocyte count in the blood is slow and weekly blood controls are therefore sufficient to adapt the dose and avoid overdosage. The decrease of the leukocyte count is generally a good indicator of the therapeutic effect of the drug since this decrease is usually accompanied by a decrease of the size of spleen and by correction of anemia. A decrease of the leukocyte count without effect on the spleen or without correction of the anemia often announces blastic transformation. The leukocyte count still increases during the first 10 15 days of the treatment.[72] This occurs in most patients and should not be considered as resistance to the drug and the daily dose should thus not be increased. The leukocyte count may continue to fall more than 1 mo after treatment has been stopped. Therefore, busulfan should be stopped around 15,000/cu mm and not only when a normal leukocyte count has been reached. When a normal leukocyte count is reached. no more immature myeloid cells are generally seen in the blood. At that time. smears of blood and marrow may be undistinguishable from normal, a result rarely obtained with other chemotherapeutic agents.

When the platelet count falls below the limits of normal during treatment. vigilance is required since persistent thrombocytopenia and irreversible marrow aplasia have been encountered in these cases when treatment was continued. A trend for platelets to decrease faster than expected may herald blastic transformation.

In most cases. at least in the beginning of the disease, the anemia is completely corrected by the intake of busulfan. Transfusion is not part of the treatment and should be given only in exceptional cases of severe anemia. As for the platelets. a tendency for red cells to fall. should make one suspect either overdosage or blastic transformation.

Busulfan appears to be as effective as irradiation of the spleen in its capacity to shrink the spleen and to maintain it in a state of regression for long periods.[100]

Whether a maintenance treatment with myleran should be given or whether the treatment should be discontinued when the leukocytes are nearly normal is a question which has never been definitely settled. The possibility to re-treat with busulfan with the same efficacy several times over many years is an argument for intermittent treatment. In this case. the patient may not be treated until the WBC reaches 50,000 or even 100,000/cu mm, since the majority of patients start to complain of fatigue, perspiration, and headache only at these levels of WBC. Evidence has been put forward for a better maintenance of normal or subnormal levels of hemoglobin in the case of continuous therapy.[48] However. no controlled study answered the question in terms of survival. Anyway, either type of treatment seems compatible with a long survival: Galton[48] describes a case surviving more than 13 yr on almost continuous therapy

whereas one of our patients treated intermittently with busulfan only, is still in the chronic phase of her disease after 20 yr. When resistance to busulfan is achieved, several other drugs (dibromomannitol, hydroxyurea, melphalan, etc.) have still been found active.

Only a few controlled studies exist in which busulfan has been compared with other sorts of treatment. The classical study which was conducted by the Medical Research Council[100] has very clearly demonstrated the superiority of busulfan over splenic irradiation. This was true for quality of remission and for survival. Another randomized study has shown clearly the superiority of busulfan over cyclophosphamide in its ability to normalize or to decrease the leukocyte count, to normalize the platelet count, to increase the hemoglobin, to reduce the spleen, and to improve the performance status of the patient." Cyclophosphamide was also considerably less active in maintaining the remission. Moreover, early development of resistance was seen with cyclophosphamide. Busulfan was also found superior to 6-MP for the over-all control of CML during a 12-wk course of study[64] and considerably superior to chlorambucil as evaluated after a period of 12 wk.[128]

Among the several toxic effects of busulfan, the bone marrow depression is certainly the most constant one. In most patients, this toxic effect seems dose-related and can thus be avoided if the daily dose is maintained low and is reduced in time. It has been pointed out[9,48,58,99,121] that a few patients appear much more sensitive than the others and are brought into deep marrow aplasia very rapidly, usually, with doses well tolerated by the large majority of patients. The mortality of these cases was high. Interestingly, survivors seemed to have long remissions some lasting one or several years. Whether busulfan should be administered deliberately at a dose inducing aplasia in order to obtain longer remissions has been questioned several times.[48,121]

Rare forms of busulfan toxicity include: amenorrhea,[46] skin pigmentation,[46] germinal cell atrophy,[72] fetal malformations,[27] and a wasting syndrome.[86] The most dangerous of these rather rare complications is probably the "busulfan lung syndrome."[88,110,134] This is seen in patient on busulfan therapy generally for more than 1 year or much longer. The clinical picture is characterized by progressive dyspnea, cough, fever, weakness, and bilateral crepitations in the lungs. The chest x-rays show characteristic widespread mottling. A needle biopsy of the lung is useful for differential diagnosis with miliary tuberculosis, mycotic infection, leukemic infiltration, etc. Pulmonary function studies show an alveolar capillary block. Cessation of the drug led to progressive clinical and radiologic improvement in some cases." Some cases responded to large doses of prednisone.[110] Most cases developed progressively increasing dyspnea and died.[68,134] Histology of the lungs shows cellular atypia, fibrinous edema, hyaline membranes and interstitial and intraalveolar fibrosis.[59,91] It should be noted that anyone of these are frequently seen in symptomless CML patients whether they were treated with myleran or not."' The cellular atypia of long-term administration of busulfan are not restricted to lung; generalized nuclear abnormalities have been reported."

DIBROMOMANNITOL (DMB)

Dibromomannitol, a polyalcohol derivative synthesized in 1961, was found to be active in the treatment of CML but to have no advantages over busulfan.

The daily dose in most clinical studies on CML patients was 250 mg/day decreasing to 250 mg every 2 or 3 days. Given in this way. partial or complete remissions were obtained in 60% 80% of cases[16,34,95,123,141] instead of 85% SO% response rate for busulfan. The leukocytes tend to dccrcasc from the beginning as opposed to busulfan which causes an increase for IO IS days. The time required to normalize the WBC depends on the daily dose. Using the dose indicated, 80% of the patients were in remission within 3 mo of treatment. Only 13% though achieved a complete remission, i.e., normalization of cell blood parameters and complete regression of splenomegaly.[16] The beneficial effect on anemia was differently appreciated in the different studies. DBM given in doses over 5 mg/kg per day causes considerable and long-lastins granulo- and thrombocytopenias. Neither erythropoiesis nor lymphopoiesis are severely affected. The toxicity appears to be restricted to the hemopoietic system. The gastrointestinal side-effects are rather insignificant. Complete remissions may last 1–9 mo.

In a retrospective study the Dibromomannitol Co-operative Study Group" has shown that the median survival of a group of 122 patients treated with DBM was 43.3 mo or 3.6 yr. The median survival of the 73 patients treated with DBM only was not significantly different from the one of the 49 patients who received DBM for more than half the duration of the disease. This is of the same order of magnitude as that obtained by busulfan in other studies. The 25% survival is at 62 mo for DBM: perhaps somewhat better than for all the other groups (48 mo). The analysis of the causes of death in this group indicated that the blast crisis occurred with the same frequency as in the other studies, i.e., 68.7% and more probably 74.8% depending on the definition of blast crisis. As in other series. death due to marrow toxicity was not negligible: 8.4% of the cases.

HYDROXYUREA

Hydroxyurea. synthesized a century ago, is a cell cycle-phase-specific agent: a specific inhibitor of DNA synthesis. Since more than 90% is excreted within 12 hr,[7] it was recommended to give the drug every 12 hr. Given to hematologically normal individuals. it causes several hematologic anomalies such as anemia, neutropenia, thrombocytopenia, megaloblastosis. Howell-Jolly bodies, macrocytes, and hypersegmented PMN.[74]

A clinical study was performed on 20 cases of CML[76] who received 50 mg/kg/day in two divided doses until the WBC was below 10,000/cu mm. The WBC was rapidly normalized within a week or two in all of the cases. An increase in hemoglobin to more than 12 g/100 ml occurred in the 12 of the 16 patients with anemia. Splenomegaly which was present in the 20 patients was reduced by more than 90% in 18. In the 11 instances of thrombocytosis, a reduction of platelets to normal values occurred following the decrease of WBC. These excellent results were obtained despite the fact that ten patients were no longer responsive to busull'an. The bone marrow returned to a near normal differential count in all patients studied serially unless myelofibrosis had developed. Subsequently, a maintenance dose of approximately 20 mg/kg/day was employed. This dose was found to produce a continuing suppression of the WBC. The median duration of improvement was 41 mo. and the median sur-

vival from diagnosis to death was 50 mo for ten patients who had not had previous chemotherapy.

In another study.[74] lower doses were given: 1500 2500 mg/day for induction. A complete remission. i.e., a complete disappearance of all blood and marrow signs of CML (except the chromosomal abnormality). as it is often obtained with busulfan. was only rarely seen. In [act. in 43 cases so treated, a "complete remission" was observed only once. Another difference with busulfan. probably related to the previous one. was that a rapid increase of the WBC was observed as soon as HU was stopped. The median survival (40 mo) appeared similar to that obtained by the other classical treatmenls. It should be pointed out that this last study does not allow a precise evaluation of the antileukemic activity of HU. since for 15 of these patients. the treatment consisted of splenectomy followed by HU and repeated leukapheresis. However. this study probably indicates that when a complete remission or nearly normal whitecell count is not maintained most of the time. the results are not worse in terms of survival. An important information which has to be kept in mind when using HU is that. in certain patients. even when the dose is kept constant. cyclic oscillations of the leukocyte count may be observed.[75] In one single cycle the WBC was found to vary from normal to 15 or 80 or even 170,000/cu mm. The cyclic periods usually ranged from 30 to 50 days with considerable similarity in each individual. Similar oscillations with the same periodicity as the leukocyte count were observed for the platelet counts. The recognition of this phenomenon during HU treatment is of course of great practical importance for the dosage regulation.

Despite a mechanism of action apparently very different from the one of the other treatments effective in CML, the median survival obtained in these two studies appeared similar to that obtained by the other classical treatments. It is clear that as all the other agents tested so far. on one hand, it does not prevent the development of myelofibrosis and blast crisis, and, on the other hand, it may be responsible for fatal bone marrow aplasia. Besides the bone marrow suppression. systemic reactions were seen. They consisted in mild anorexia and dermatologic alterations such as partial alopecia, pigmentation. dry skin. atrophy of the skin and subcutaneous tissue, nail changes, and erythema of the face and hands which occurred mainly with long-term continuous maintenance therapy.[76]

The mode of administration of HU has not been entirely examined and could probably be improved. For instance. there are studies on solid tumors. suggesting that larger doses (80 mg/kg) given every third day in a single dose are better tolerated and might have greater antitumor effects.[89]

SPLENIC IRRADIATION

This method of treatment has been somewhat-abandoned at the moment. although it has been extensively used in the past. At a dose of 600-1000 rads, it is effective not only to shrink the spleen but also to normalize the peripheral blood and the bone marrow.[63] Several observations give an explanation for the systemic effect of this local treatment. The myeloid metaplasia seen in the spleen of these patients has suggested for a long time that an abundant cell production

occurs in this organ. This impression was confirmed by kinetic studies conducted by Clarkson et al.[109] who showed. using infusions of 'HTdR into the splenic artery, that the great majority of the immature myeloid cells in the blood of these patients originate in the spleen. However. splenectomy is not very efficient in decreasing the WBC. and the arrest of production in the spleen is probably not the only way of action of splenic irradiation. The observations made by Moxley[107] and Galbraith[44] may explain the normalization of blood and bone marrow after irradiation of the spleen only. These studies indicate that the immature myeloid cells in CML circulate from blood to marrow. from blood to spleen and from spleen to marrow. However, they still do not give a complete explanation for the bone marrow aplasia sometimes observed after splenic irradiation.[77] Such clinical observations would support the theory of the release into the circulation by the irradiated spleen cells of an inhibitor of the myeloid cell proliferation.[98]

Splenic irradiation is capable of maintaining a state of regression for long periods of several months. However, progressively, courses of radiotherapy using successively higher doses are required and cause less regression. Splenic irradiation as the sole form of treatment of CML has been rather abandoned. This was due to the publication of the results of the controlled clinical trial by the Medical Research Council's Working Party for Therapeutic Trials in Leukemia conducted on 102 untreated CML patients who were allocated at random for treatment by either splenic x-irradiation or busulfan.[100] It has shown that busulfan was significantly better at obtaining and maintaining a higher level of hemoglobin concentration. Moreover, the median survival time was longer in the group allocated to myleran (170 wk) than in the group allocated to splenic irradiation (120 wk). The incidence of blast crisis being the same in both groups (70%), suggests that treatments may be different in their capacity to delay or to enhance the blast crisis. An important conclusion from this clinical study is thus that it seems possible by the treatment to influence the time of onset of the blast crisis. Splenic irradiation has, since then, no longer been used alone for the treatment of CML. However, it remains a practical way to obtain a rapid decrease of the size of the spleen even in some cases resistant to myleran.

EXTRACORPOREAL IRRADIATION OF THE BLOOD (ECIB)

First described by Cronkite et al.[23,131] and Thomas et al.,[147] this technique permits the irradiation of the peripheral blood cells only. It takes advantage of the radioresistance of the erythrocytes and in the case of CML of the existence of a rapid traffic of myeloid cells existing between marrow and spleen via the blood. A chronic arteriovenous shunt inserted in the forearm is connected to an extracorporeal circuit at the time of irradiation. The blood then flows through a radiation source, and the system is built so that the amount of radiation (500 rads) received by a segment of blood as it passes completely through the irradiation one time (the transit dose) is sufficient to kill the cell. Only few patients wiih CML were treated by this method. For instance two patients'[77] with 90.000 and 140,000 WBC and 36% and 14% immature white cells, respectively, were submitted to 2–4 hr ECIB sessions, respectively, 14 and 32 times. The re-

sult of this treatment was a decrease of the WBC (to 21,000 and 12,000). a decrease of the per cent of immature cells in the blood (to 24% and 3%) and a considerable decrease of the size of the spleen in the second patient. Complete remissions were not obtained. Moreover, after cessation of ECIB, relapse of leukemic process occurred in a matter of days to several weeks. Whether this was due to increased cell production has not been elucidated. There are no studies on the proliferative capacity of the bone marrow cells before and after ECIB in CML patients. However, studies on the bone marrow of patients with acute leukemia using tritiated thymidine labeling indices and mitotic indices before and after ECIB suggest that it may be increased as a consequence of the irradiation of the blood.[19,36] An increased proliferative activity of the myeloid precursors in CML may explain the disappointing results obtained with this technique. Obviously, this form of therapy is not a cure for CML and therefore in view of the existence of other much more convenient forms of treatment, its use should be restricted to very special cases such as pregnancy, a situation in which all chemotherapeutic agents should be suppressed at least in the first months.

LEUKAPHERESIS

As a consequence of the rapid traffic of cells existing between marrow and spleen via blood,[44,107] a depletion of the blood should theoretically affect the bone marrow also. The continuous flow centrifuge (NCI-IBM Blood Cell Separator) has been used in CML in order to investigate the possibility to use CML patients as granulocyte donors and the possibility to improve CML patients by subtracting regularly large amounts of blood myeloid immature cells. The efficiency of leukocyte separation and collection was variable from patient to patient with median yields of 18%–53%.[13] In 98 separate centrifugations completed in 12 CML patients, the average decrease of WBC expressed in per cent of the pretreatment value was only 16.5% (range 45 to 0). Moreover, in most patients, 2–3 days only were required for the WBC to return to baseline level. Since, moreover, red blood cells were always lost during continuous flow centrifugation and technical problems encountered in more than 25% of the sessions, it appears that this technique is not very efficient and practical as the only treatment of all CML patients.

The explanation for these disappointing results may be the same as the one suggested for ECIB. A complete evaluation of the role of leukapheresis in the treatment of CML has still to be done since some patients appear to respond better than others. This was the case of a patient. described recently, in whom intensive leukapheresis (12 and 18 sessions) appeared able, by reducing to total amount of leukemic cells, to come back on two occasions to a previous, apparently more benign, stage of the disease.[51] Whether the patient who exhibited this promising response to leukapheresis had a very peculiar form or a rather classical form of CML is important to know but still unsettled.

SPLENECTOMY

The constancy of splenomegaly in CML has suggested for more than a century to remove the spleen as a therapeutic procedure. This was followed by an opera-

tivc mortality of 25%[143] or more which was due partly to the difficulties still existing at that time for blood transfusions, anesthesia. and treatment of infections. Since splenectomy was. in most cases. performed in order to control the chronic phase of the disease and since it did not generally modify greatly either the blood picture or the late outcome of the disease. it fell into disrepute as CML became easily controlled by progress in radiotherapy and chemotherapy. Strumia[111] reviewed 34 cases published in the literature between 1940 and 1964 including live of his own cases and found still an operative and postoperative mortality of the order of 25% as it was during the period 1902–1939. Great improvement was noticed in five patients out of 34, minor improvement in nine. It should be pointed out that, at that time. splenectomy was generally performed because of loss of control of the disease in its chronic phase and. therefore, on large spleens when the leukocyte count was high[101,143] and that the transient decrease following splenectomy in some of these cases could have been unrelated to splenectomy but rather due to undisturbed cycling of the leukocyte count. This explanation is certainly not valid in all the cases who improved after splenectomy. For instance, it seems improbable in a case[26] who had a remission lasting 8 mo.

Three indications for splenectomy have been suggested but have not been fully evaluated. Decreased platelet production due to busulfan in one of them. It has been shown on a small group of six patients that splenectomy may considerably improve thrombocytopenia consecutive to long-term myleran therapy.[138] In this study, splenectomy was performed in the chronic phase of the disease. Although the platelet count was generally very low (6000/cu mm in one case), no operative mortality was observed. There was an immediate postoperative rise in platelet count in all six patients which was sustained in five patients. In these five patients with bleeding and thrombocytopenia. no further hemorrhagic problems or platelet transfusions were required as long as they remained in the chronic phase of CML. Splenectomy as a part of the treatment of blastic transformation is a second possible indication. It has been advocated only rarely. It was performed in eight cases in blastic transformation, caused no operative death. and apparently improved most patients.[135]

Splenectomy as prevention of blast crisis is the third possible indication still the subject of an unsettled debate. It consists of removing the spleen during the chronic phase of the disease when the size of the spleen and the hematologic picture have been normalized or almost by a conventional treatment, in order to reduce, as much as possible, the operative risk. Late in the course of the disease the surgical procedure is certainly more difficult. The rationale is that the spleen contains Ph^1-positive cells.[111] and constitutes therefore a large reservoir of cells susceptible to transformation. As a matter of fact, abnormal sublines of Ph^1-positive cells have been found in the spleen at the time of blastic transformation,[139] and it is conceivable that this transformation may start in the spleen (cited by Galton[11]). Early splenectomy per se is certainly an inadequate treatment. It is proposed as an adjuvant procedure in combination with an intensive chemotherapy in order to get rid of the Ph^1-positive cell line or at least to decrease the number of abnormal myeloblasts seen in the bone marrow already at the time of diagnosis.[1]

Single cases have already shown us that blast crisis may still occur after early splenectomy. Moreover, no randomized study has so far demonstrated that splenectomy performed in the chronic phase during a period of remission is certainly a useful procedure in CML. In a clinical study performed by the group of Villejuif, splenectomy was performed as soon as the first remission had been obtained by chemotherapy.[11] This study suggests that the blast crisis may be delayed by splenectomy. since the median survival of 15 splenectomized patients (43 mo) was 6 mo longer than the niedinn survival of 25 patients who refused splenectomy (37 mo). However. the incidence *of* the blast crisis (13/15) was of the same order as in the reference patients (19/24) and the morbidity. mainly venous thrombosis due io thrombocytosis. was not negligible. We also experienced this in one of our early CML who reached more than 2,000,000 platelets/cu mm. Finally, mortality in the last years, although in decrease. remains prohibitive as it can be seen in Table 2.

Whether early splenectomy is a useful procedure for the patient in terms of survival cannot be settled from the available studies. Controlled clinical trials are needed to evaluate the effect of splenectomy performed early in CML. Such a trial is pre :ntly conducted by the Medical Research Council's Working Party on Leukemia in Adults and one has been started by the European Organization for Research and Treatment of Cancer (EORTC), but conclusive results have not yet been obtained. Studies on the usefulness of splenectomy in early CML might have to be repeated in association with different regimens of chemotherapy.

PROPHYLACTIC TREATMENT OF THE BLASTIC TRANSFORMATION OF CML

The observation that at the clinical onset of CML the bone marrow already contains a population of abnormal myeloblasts having kinetic features highly reminiscent of those of the blasts of acute myeloblastic leukemia[2] suggests the

Table 2. Mortality of Splenectomy in CML

Authors	Indications	No. of Cases	Operative or Postoperative Death	Evolution	Ref.
Strumia 1940–1964	Resistance. severe pain	34	8 (23%)	5 great improvemeni 9 minor improvement	143
Meeker et al. 1967	Hypersplenism, splenomegaly	15	3	8 improved	101
Sokal et al. 1971	Hypersplenism, blast crisis	17	0	Immediate benefit 1/2 Lasting improvement 1/4	135
Canellos et al. 1972	Thrombocytopenia after busulfan	6	0	5 improved	14
Schwartzenberg et al. 1973	Prevention of BC in early CML	18	3	6 mo increased median survival?	132
Spiers 1973	Prevention of BC in early CML	(17)	(0?)		140
Stryckmons et al. 1973	Prevention of BC in early CML	3	*0*		
Total 1967–1973		*59*	6 (10%)		

prevention of their accumulation by destroying them as soon as possible during the chronic phase of the disease. Since busulfan appears to have more tendency than the other agents to produce long-lasting cytopenias and since, moreover, it is not a cell cycle-phase specific agent, it does not seem reasonable to recommend its use at higher doses. In the present state of our knowledge, it is logical to believe that the best chemotherapeutic regimens for acute myeloblastic leukemia could also be the best candidates for this prophylactic chemotherapy. Arabinosyl-cytosine is therefore probably a drug of first choice. There are data suggesting that this drug might act selectively on myeloblasts since it has been shown, in CML. to be more active on myeloblasts than on promyeiocytes and myelocytes.' There are presently several clinical studies going on in order to test the validity of this sort of chemotherapy for delaying the blast crisis.

In the same line as studies carried out in acute lymphoblastic[Y6] and acute myeloblastic[''] leukemia, another approach has been to stimulate the immunologic defenses of the organism presumably against the leukemic blasts. Sokal et al.[136] have recently published the results of a study of CML patients who were subjected to repeated vaccination with BCG and allogeneic cultured cells. Fifteen patients with uncomplicated Ph[1]-positive CML were treated with conventional chemotherapy whenever it was found necessary and moreover, except for one receiving BCG alone, they were immunized with one or both of two cultured cell lines mixed with BCG organisms. One of the cell lines was established from the peripheral blood of a patient with acute myeloblastic leukemia, the other was established from the blood of a patient in blastic crisis of Ph[1]-positive CML. Most patients received their first three vaccinations at 3–6 wk-intervals. The interval was extended gradually so that they received three to four vaccinations per year when the maintenance schedule was reached. These 15 patients were compared to 28 unimmunized historical controls whose initial prognosis and chemotherapeutic management were comparable to those of the immunized patients. A survival curve for the immunized patients was constructed despite the fact that they entered the immunotherapy program at widely varying times after diagnosis (0.5–7 yr). The median survival of the immunized patients (more than 7 yr) was strikingly better than that of the selected controls (about 3.5 yr). This study certainly suggests a favorable effect ofimmunotherapy but does not prove it, firstly, because the treatment was not randomly assigned and, secondly, because immunized patients were admitted in the study any time after diagnosis which may have resulted in the selection of patients who survived longer than the average. A controlled trial on a random basis has been started by the same group (personal communication). If their initial results are confirmed, many questions would still remain unanswered. Is the mechanism of action immunologic? If so, is it a specific or nonspecific immunologic stimulation? What is the part played, respectively, by BCG and leukemic blasts? What are the hazards of BCG administration?

ERADICATION OF THE LEUKEMIC CELLS

This term will be used here indifferently for eradication of the Ph[1]-positive cell line. There are important facts which justify the concept of eradication.

The first is that all the chromosomal analyses of blast metaphases during

acute transformation have shown the persistence of the Ph¹ chromosome, implying that it is always in this cell line that transformation will occur.

The second fact is that in vitro studies clearly indicate the coexistence of Ph¹-positive and Ph¹-negative myeloid cells in the bone marrow.[20] The absence of Ph¹-negative cells as it is observed in most direct karyotype analyses, probably reflects the inhibition of proliferation of these cells rather than the absence of these cells.

The third fact is that in a few cases, as a consequence of chemotherapy, the Ph¹-negative cells became predominant in the bone marrow. In most cases it was unexpected and occurred as a consequence of a busulfan-induced aplasia. Finney[41] has described a patient who, after a busulfan-induced bone marrow insufficiency, has had a remission lasting 10 yr. During this period of time, seven chromosome studies on bone marrow cells have shown that 70%–100% of the mitoses were Ph¹-negative. Galton[49] has recently reviewed several of his cases of CML treated with busulfan who showed a preponderance of normal Ph¹-negative cells with persistance of only some Ph¹-positive cells. All these cases were found among patients whose bone marrow had become severely hypoplastic after treatment with busulfan. Within this subgroup, the few who showed regeneration of apparently normal bone marrow with Ph¹-negative cells and restoration of an apparently normal blood picture had received relatively small amounts of busulfan, treatment having been discontinued or reduced rapidly because of an exceptional sensitivity to the drug. Maurice et al.[99] have reported a case of CML, who, after busulfan treatment, had severe pancytopenia for 1 yr and had still, 9 yr later, a completely normal hematologic status with Ph¹-negative cells. This study was only very suggestive of eradication of the Ph¹-positive cells since chromosomal analysis was not yet available at the time of diagnosis. It should be kept in mind that the long duration of a normal hematologic status only does not imply "cure" since relapses have been observed after more than 6 yr complete remission."

These clinical observations allow us to draw two conclusions and to make certain working assumptions. Firstly, in some cases at least, preferential cytocidal effect can be obtained on the leukemic cells and it may be assumed that is not intrinsic to the cell and specific to busulfan but, instead, a consequence of a greater proliferative activity of the leukemic cells and therefore obtainable by other drugs. Secondly, these observations also indicate that the Ph¹-positive cells have probably to be considerably decreased in number before the "normal" cell line is able to reappear. This second finding may be explained by a negative feed-back exerted by the abnormal cell line, on the normal cell line. The persistence of a regulatory mechanism in CML, strongly suggested by the cyclic oscillations of the leukocyte counts in CML, is compatible with this hypothesis. Thus, excellent arguments do exist to induce deliberately an intense marrow aplasia. It should be of short duration to be able to give the patient the best supportive treatment available if necessary. Moreover, cycle phase-specific agents should probably be used in order to protect the "dormant" Ph¹-negative cells. Therefore, the long duration and the unpredictability of the aplasia induced by busulfan and the absence of cell cycle phase-specificity of this agent make it a bad candidate for this sort of eradication treatment.

A prospective study based on this concept of therapy of CML has been started in an attempt to determine if intensive treatment might alter the course of the disease by elimination of the leukemic stemline.[85] The regimen consisted of radiotherapy of the spleen followed by splenectomy, then an intensive course of chemotherapy with arabinosylcytosine and thioguanine, and usually further maintenance therapy with various chemotherapeutic agents. In eight of the patients studied. two showed disappearance of the Ph^1 chromossme. In one of those, it reappeared in 6 wk whereas the second was still free of the chromosome abnormality more than 2 yr after its disappearance.

The information available indicates clearly that the Ph^1-negative cells occasionally seen in Ph^1-positive patients must have been present before treatment was begun, although they could not be found in chromosome analyses. Since eradication has so far been rather exceptional, it raises the questions whether Ph^1-negative myeloid stem cells are present in all the cases of CML. The difficulty of obtaining a Ph^1-negative bone marrow by chemotherapy in the great majority of the cases. does not exclude the universal presence of Ph^1-negative myeloid cells in CML. This difficulty could only reflect the inability of our treatments to affect preferentially the Ph^1-positive cells.[49] If this is the case, it holds out the hope that with better Chemotherapy the return to a Ph^1-negative bone marrow is possible in all the patients. Unfortunately. so far the return to a Ph^1-negative state usuaily was neither complete nor permanent; nevertheless, it appeared to be associated with a long survival.[41,49,85,99]

SUMMARY

None of the classical treatments have so far significantly increased the survival of CML patients. This general statement includes all the treatments suitably tested, namely, ^{32}P, splenic irradiation, busulfan, hydroxyurea, or dibromomannitol. However, there is no doubt that all these treatments have all, each in a different way, improved the quality of the survival. Moreover, the possibility to choose among several treatments makes it more and more possible to find in individual cases, with toxicity or resistance, another more suitable treatment. Other therapeutic procedures which are not able to control the disease by themselves have to be evaluated for their capacity. as adjuvant. to improve the results obtained by chemotherapy. These procedures include early splenectomy, extracorporeal irradiation of the blood, leukapheresis, etc.

The therapeutic effect of immunotherapy, which appeared in humans to be restricted to acute myeloblastic and lymphoblastic leukemia, could perhaps include CML. This deserves additional evaluation.

Finally. the few patients in whom intensive chemotherapy was able to induce a normal hematologic state with disappearance of the Ph^1-positive cell line allow us to hope that, with better drug administration schedules. such results could be obtained on more patients and maintained indefinitely.

REFERENCES

1. Athens JW, Raab SO. Haab OP. et al: Leukokinetic studies. X. Blood granulocyte kinetics in chronic myelocytic leukemia. J Clin Invest 44:765, 1965

2. Baccarani M. Killmann SA: Cytokinetic studies in chronic myeloid leukaemia: Evidence for early presence of abnormal myeloblasts. Scand J Haematol 9:283, 1972

3. Baccarani M. Santucci MA. Zaccaria A. et al: Kinetics of granulocyte precursors in chronic myeloid leukemia: The effect of arabinosyl cytosine. Second Meeting of the European and African Division of the International Society of Haematology, Prague. August 1973, p 89.

4. Banerjee TK. Hansjoerg S. Holland JF: Comparative studies on localized leukocyte mobilization in patients with chronic myeloid leukemia. Cancer 29:637, 1972

5. Barr RD. Fialkow P: Clonal origin of chronic myelocytic leukemia. N Engl J Med 289:307, 1973

6. Baxt W. Hehlmann R. Spiegelman S: Human leukaemic cells contain reverse transcriptase associated with high molecular weight virus-related RNA. Nature [New Biol] 240:72, 1972

7. Beckloff GL, Lerner HJ. Frost D, et al: Hydroxyurea (NSC 32065) in biologic fluids: Dose-concentration relationship. Cancer Chemother Rep 48:57, 1965

8. Bergsagel DE The chronic leukemias: A review of disease manifestations and the aims of therapy. Can Med Assoc J 96:1615, 1967

9. Bernard J. Mathe G, Najean Y: Traitement de 50 leucémies myéloïdes chroniques par Misulban. Scm Hop Paris 31:3083, 1955

10. Bernard J. Seligmann M, Kvicala R: La transformation aiguë de la leucémie myéloïde chronique. Rev Fr Etud Clin Biol 4:1024, 1959

11. Brandt L: Phagocytic activity of neutrophilic leukocytes in chronic myeloid leukemia. Scand J Hacmatol [Supply]2:35, 1967

12. Bresciani F Cell proliferation in cancer. Eur J Cancer 4:343, 1968

13. Buckner D, Graw RG, Eisel RJ, et al: Leukapheresis by continuous flow centrifugation in patients with chronic myelocytic leukemia. Blood 33:353, 1969

14. Canellos GP. Nordlund J. Carbone PP: Splenectomy for thrombocytopenia in chronic granulocytic leukemia. Cancer 29660, 1972

15. Canellos GP. Whang-Peng J: Philadelphia-chromosome-positive preleukaemic state. Lancet 2:1227, 1972

16. Casazza AR, Cahn EL, Carbone PP: Preliminary studies with dibromomannitol (NSC-94100) in patients with chronic myelogenous leukemia. Cancer Chemother Rep 51: 91, 1967

17. Castoldi G. Yam LT. Mitus WJ. et al: Chromosomal studies in erythroleukemia and chronic erythremic myelosis. Blood 31:202, 1968

18. Castoldi GL., Scapoli GL. Dallapiccola B. et al: Analyse des anomalies chromosomiques dans l'anémie pernicieuse. Nouv Rev Fr Hematol 9:769, 1969

19. Chan BWB, Hayhoe FGJ, Bullimore JA: Effect of extra corporeal irradiation of the bood on bone marrow activity in acute leukaemia. Nature 221:972, 1969

20. Chervenik PA. Ellis LD. Pan SF. et al: Human leukemic cells: In vitro growth of colonies containing the Philadelphia (Ph') chromosome. Science 174:1134, 1971

21. Chicago Conference. Standardization in human cytogenetics. The National Foundation March of Dimes, 1966

22. Court Brown WM. Tough IM: Cytogenetic studies in chronic myeloid leukemia. Adv Cancer Res 7:351, 1963

23. Cronkite EP: Extracorporeal irradiation of the blood and lymph in the treatment of leukemia and for immunosuppression. Ann Intern Med 67:415, 1907

24. Cronkite EP, Vincent PC: Granulopoiesis. Ser Haematol 2:3, 1969

25. Crowther D. Powles RL, Batcman CJT. et al: Management of adult acute myelogenous leukaemia. Br Med J 1:131, 1973

26. Cutting HO: The effect of splenectomy in chronic granulocytic leukemia. Arch Intern Med 120:356, 1967

27. Diamond L. Anderson MM. McCready SR: Transplacental transmission of busulfan (myleran) in a mother with leukemia. Production of fetal malformation and cytomegaly. Pediatrics 25:85, 1960

28. Dibromomannitol Cooperative Study Group. Survival of chronic myeloid leukemia patients treated by dibromomannitol. Eur J Cancer 9:583, 1973

29. De Grouchy J. De Nava C. Cantu JM. et al: Models for clonal evolutions: A study of chronic myelogenous leukemia. Am J Hum Genet 18:485, 1966

30. Djaldetti M. Padeh B. Pinkhas J. et al: Prolonged remission in chronic myeloid leukemia after one course of busulfan. Blood 27: 103. 1966

31. Dougan L. Onesti G, Woodliff HJ: Cytogenetic studies in chronic granulocytic leukaemia. Aust Ann Med 16:52, 1967

32. Dougan L, Scott ID. Woodliff HJ: A pair of twins. one of whom has chronic granulocytic leukemia. J Med Genet 3:217, 1966

33. Duvall CP, Carbone PP, Bell WK. et al: Chronic myelocytic leukemia with two Philadelphia chromosomes and prominent peripheral lymphadenopathy. Blood 29:642, 1967

34. Eckhardt S, Sellei C, Horvath FP, et al: Effect of 1,6-dibromo-1,5-dideoxy-D-mannitol on chronic granulocytic leukemia. Cancer Chemother Rep 33:57, 1963

35. Engel E, McKee LC: Double Ph' chromosomes in leukaemia. Lancet 2:337, 1966

36. Ernst P, Andersen J, Killmann SA: Cell cycle effect of extracorporeal irradiation of the blood in acute myeloid leukemia. Scand J Haematol 8:21, 1971

37. Eyre HJ, Golstein IM, Perry S, et al: Leukocyte transfusions: Function of transfused granulocytes from donors with chronic myelocytic leukemia. Blood 36:432, 1970

38. Ezdinli EZ, Sokal JE, Crosswhite L: Philadelphia-chromosome-positive and -negative chronic granulocytic leukemia. Ann Intern Med 72:175, 1970

39. Feingold ML, Koss LG: Effects of long-term administration of busullan. Arch Intern Med 124:66, 1969

40. Fialkow PI, Gartler SM, Yoshida A: Clonal origin of chronic myelocytic leukemia in man. Proc Natl Acad Sci (Wash) 58:1468, 1967

41. Finney R, McDonal GA, Baikie AG, et al: Chronic granulocytic leukemia with Ph'-negative cells in bone marrow and a ten-year remission after busulfan hypoplasia. Br J Haematol 23:283, 1972

42. Ford CE, Clarke CM: Cytogenetic evidence of clonal proliferation in primary reticular neoplasms. Can Cancer Conf 5:129, 1969

43. Forresier RH, Louro JM: Philadelphia chromosome abnormality in agnogenic myeloid metaplasia. Ann Intern Med 64:622, 1966

44. Galbraith RR: Studies on the longevity, sequestrations and release of the leukocytes in chronic myelogenous leukemia. Can Med Assoc J 95:511, 1966

45. Galbraith PR, Abu-Zahra HT: Granulopoiesis in chronic granulocytic leukemia. Br J Haematol 22:135, 1972

46. Galton DAG: Myleran in chronic myeloid leukemia. Lancet 1:208, 1953

47. Galton DAG: Treatment of the chronic leukemias. Br Med Bull 15:79, 1959

48. Galton DAG: Chemotherapy of chronic myelocytic leukemia. Semin Hematol 6:323, 1969

49. Galton DAG: Thyroid tumours, lymphomas granulocytic leukemia. Proceedings of the Fifth Padua Seminar on Clinical Oncology. Padua: Piccin Medical Books. October 1972.

50. Gatti RA, Deinard HS, Nesbit ME, et al: Chronic myelogenous leukemia; cyclic leukocytosis and identical twins discordance. Proceedings Ann Pediatries Soc Meeting, Atlantic City NJ, May 1970, p 80

51. Gatti AH, Robinson Wh, Deinard AS, et al: Cyclic leukocytosis in chronic myelogenous leukemia: New perspectives on pathogenesis and therapy. Blood 41:771, 1973

52. Gavosto F, Pileri A, Bachi C, et al: Proliferation and maturation defect in acute leukaemia cells. Nature 203:92, 1964

53. Gee TS, Dowling MD, Clarkson BD, et al: Blastic transformation occurring in extramedullary sites in controlled chronic myelogenous leukemia (CML). Proceedings of the Am Assoc for Cancer Research. April 1973. Abstract 217

54. Goh K, Swisher SN: Identical twins and chronic myelocytic leukemia. Arch Intern Med 115:475, 1965

55. Goh K, Swishcr SN, Herman EC Jr: Chronic myelocytic leukemia and identical twins. Additional evidence of the Philadelphia chromosome as postzygotic abnormality. Arch Intern Med 120:214, 1967

56. Gralnick HR, Harbor J, Vogel C: Myelofibrosis in chronic granulocytic leukemia. Blood 37:152, 1971

57. Hammouda F, Quaglino D, Hayhoe FGJ: Blast crisis in chronic granulocytic leukemia. Cytochemical, cytogenetic and autoradiographic studies in 4 cases. Br Med J 1:1275, 1964

58. Haut A, Abott WS, Wintrobe MM, et al: Busulfan in the treatment of chronic myelocytic leukemia. The effect of long term intermittent therapy. Blood 17:1, 1961

59. Heard BE, Cooke RA: Busulfan lung. Thorax 23:187, 1968

60. Heckner F: Polysaccharide in Blut- und Knockenmarks-Zellen. Zyto- und Histochemie in der Hämatologie Neutes Freiburger Symposium. Berlin. 1963, p 408

61. Hill JM, Loeb E: Treatment of leukemia, lymphoma and other malignant neoplasms with vinblastine. Cancer Chemother Rep 15:41, 1961

62. Holland JF, Glidewell O: Oncologists' reply: Survival expectancy in acuie lymphocytic leukemia. N Engl J Mcd 287:769, 1972

63. Hotchkiss DJ, Block MH: Effect of splenic irradiation on systemic hematopoiesis. Arch Int Mcd 109:695, 1962

64. Huguley CM Jr, Grizzle J, Rundles RW, et al: Southeastern Canc. Chemother. Coop. Study Group: Comparison of 6-mercaptopurine and busulfan in chronic granulocytic leukemia. Blood 21:89, 1963

65. Hustu HO. Aur KJA. Verzosa MS, et al: Prevention of central nervous system leukemia by irradiation. Cancer 32:585, 1973

66. Jackson EW, Norris FD, Klauber MK: Childhood leukemia in California-born twins. Cancer 23:913, 1969

67. Jacobs EM. Luce JK. Cailleau R: Chromosome abnormalities in human cancer. Report of a patient with chronic myelocytic leukemia and his non leukemic monozygotic twin. Cancer 19:869, 1966

68. Jeanmart L, Klastersky J, Verhest A. et al: Les pneumopathies diffuses au cours de la chimiothérapie par le busulfan et la bléomycine. J Belge Radiol 55:425, 1972

69. Karnofsky DA: Summary of results obtained with nitrogen mustard in the treatment of neoplastic disease. Ann NY Acdd Sci 68: 899. 1958

70. Kaung DT. Close HP. Whittington KM. et al: Comparison of busulfan and cyclophosphamide in the treatment of chronic myelocytic leukemia. Cancer 27:608, 1971

71. Kemp NH, Stafford JL, Tanner R: Chromosome studies during early and terminal chronic myeloid leukaemia. Br Med J 11:1010, 1964

72. Kenis Y, Dustin P Jr. Henry JA. et al: Action du Myleran dans 22 cas de leucémie myéloïde chronique. Rev Fr Etud Clin Biol 1:435, 1956

73. Kenis Y. Koulisher L: Etude clinique et cytogénétique de 21 patients atteints de leucémie mycloide chronique. Eur J Cancer 3:83, 1967

74. Kennedy BJ. Yarbro JW: Metabolic and therapeutic effects of hydroxyurea in chronic myeloid leukemia. JAMA 195:1038. 1966

75. Kennedy BJ: Cyclic leukocyte oscillations in chronic myelogenous leukemia during hydroxyurea therapy. Blood 35:751, 1970

76. Kennedy BJ: Hydroxyurea therapy in chronic myelogenous leukemia. Canccr 29: 1052, 1972

77. Khan MH. Martin H: Multiple chromosomal aberrations in a case of malignant myelosclerosis. Acta Harmalol (Basel) 39:299, 1968

78. Killmann SA, Cronkite EP, Robertson JS. et al: Estimation of phases of the life cycle of leukemic cells from labeling in human beings in vivo with tritiated thymidine. Lab Invest 12:671, 1963

79. Killmann SA: Chronic myelogenous leukemia: Preleukemia or leukemia'! Proceedings of an International Symposium. Tura S. Baccarani M (ed) Edirione di Haematologica: Pavia 1972.

80. King Smith EA. Morley A: Computer stimulation of granulopoiesis: Normal and impaired granulopoiesis. Blood 36:254. 1970

XI. Kiossoglou KA. Mitus WJ. Dameshek W: Chromosomal aberrations in acute leukemia. Blood 26610. 1965

82. Kirschner KH. Esterly JK: Pulmonary lesions associated with busulfan therapy of chronic myelogenous leukemia. Cancer 27: 1074, 1971

83. Koulischer L, Fruhling J. Henry J: Observations cytogénétiques dans la maladie de Vaquez. Eur J Cancer 3:193, 1967

84. Krakoff IH, Ellison RR. Tan CTC: Clinical evaluation of thioguanosine. Cancer Res 21:1015, 1961

85. Krakoff IH. Dowling MD. Gee T. et al: A perspective of intensive treatment aiming at prolonged control and/or eradication of chronic granulocytic leukemia. Thyroid Tumours Lymphomas Granulocytic Leukemia. Proceedings of the Second Padua Seminar on Clinical Oncology. October 1972. Padua. Piccin Medical Books. 1973. p 113

86. Kyle RA. Schwartz RS. Oliner HL. et al: A syndrome resembling adrenal cortical unsufficiency associated with long term busulfan (Myleran) therapy. Blood 18:497, 1959

87. Lawler SD, Galton D: Chromosome changes in the terminal stages of chronic granulocytic leukemia. Acta Med Scand (Suppl) 179:312, 1966

88. Leake E. Smith WG. Woodliff HJ: Diffuse interstitial pulmonary fibrosis after busulfan therapy. Lancet 2:432, 1963

89. Lerner HJ. Beckloff GL: Hydroxyurea administered intermittently. JAMA 192:1168, 1965

90. Lessmann EM. Sokal JE: A colchicine derivative in therapy of chronic myelocytic leukemia. JAMA 175:741, 1961

91. Litter WA, Kay JM. Hasleton PS. et al: Busulfan lung. Thorax 24:639, 1969

92. Lortholary P. Lejeune F. Tanzer J. et al: Etude cytochimique des leucoblastes au cours de la transformation aiguë dc la leucémie myéloïde chronique. Presse Med 74:1237, 1966

93. Maniatis AK. Amsel S. Mitus WJ: Chromosome pattern of bone marrow fibroblasts in patients with chronic granulocytic leukaemia. Nature (London) 2221278. 1969

94. Mastrangelo R. Zuelzer WW. Thompson R: The significance of the Ph' chromosome in acute myeloblastic leukemia: Serial cytogenetic studies in a critical case. Pediatrics 40:834, 1967

95. Mathe G. Schneider M. Cattan A. et al:

Essai de traitement de la leucémie myéloïde chronique par le dibromomannitol. Presse Med 72:2135, 1964

96. Mathe G, Amiel JL, Schwarzenberg L, et al: Active immunotherapy for acute lymphoblastic leukaemia. Lancet 1:697, 1969

97. Mauer AM, Fisher V: Characteristics of cell proliferation in four patients with untreated acute leukemia. Blood 28:428, 1966

98. Maurice PA, Williner B, Gindrat JJ, et al: Effect of splenic X-irradiation on bone marrow function. II. Experimental studies in patients with chronic myelocytic leukaemia. Br J Haematol 17:543, 1969

99. Maurice PA, Alberto P, Ferrier S, et al: Leucémie myélocytaire chronique: "Guérison" apparente depuis plus de 9 ans, consecutive à une hypoplasie médullaire thérapeutique. Schweiz Med Wochenschr 101:1781, 1971

100. Medical Research Council's Working Party for Therapeutic Trials in Leukaemia. Chronic granulocytic leukaemia: Comparison of radiotherapy and busulfan therapy. Br Med J 1:201, 1968

101. Meeker WR, Jr, dePerio JM, Grace JT, et al: The role of splenectomy in malignant lymphoma and leukemia. Surg Clin North Am 47:1163, 1967

102. Metcalf D: The colony stimulating factor (CSF). Aust J Exp Biol Med Sci 50547. 1972

103. Miller RW: Relation between cancer and congenital defects in man. N Engl J Med 275:87, 1966

104. Minot GR, Buckman JE, Isaacs R: Chronic myelogenous leukemia: Age incidence: duration and benefit derived. JAMA 821486. 1924

105. Morley AA: A neutrophil cycle in healthy individuals. Lancet 2:1220, 1966

106. Morley AA, Baikie AC, Galton DAG: Cyclic leucocytosis as evidence for retention of normal homeostatic control in chronic granulocytic leukaemia. Lancet 2:1320, 1967

107. Moxley JH, Perry S, Weiss GH, et al: Return of leucocytes to the bone marrow in chronic myelogenous leukaemia. Nature (London) 2011 1281, 1965

108. Nowell PC, Hungerlord DA: A minute chromosome in human granulocytic leukemia. Science 132: 1497. 1960

109. Ogawa M, Fried J, Sakai Y, et al: 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, 1970

110. Oliner H, Schwartz R, Rubio t-, Interstitial pulmonary librosis following busullan therapy. Am J Med 31:134, 1961

111. Onesti J, Woodliff HJ: The Philadelphia chromosome in a patient with acute leukemia. Med J Aust 1:544, IY70

112. Paris Conference. Standardization in human cytogenetics. The national Foundation March of Dimes. 1972

113. Pedersen B, Videbaek A: Several cell lines with abnormal karyotypes in a patient with chronic myelogenous leukaemia. Scand J Hacmatol 1:129, 1964

114. Pedersen B: Karyotypes profiles in chronic myelogenous leukaemia. Influence of therapy and progression of disease. Acta Pathol Microbiol Scand 67:463, 1966

115. Pedersen B, Hayhoue FGJ: Relation between phagocytic activity and alkaline phosphatase content of neutrophils in chronic myeloid leukaemia. Br J Haematol 21:257, 1971

116. Pedersen B, Hayhoe t-GJ: Cellular changes in chronic myeloid leukaemia. Br J Haematol 21:251, 1971

117. Pedersen B, Killmann SA: Leukaemic subclones in chronic myelogenous leukemia. Acta Med Scand 190:61, 1971

118. Pedersen B: The blastic crisis of chronic myeloid leukaemia: Acute transformation ol a preleukaemic condition? Br J Haematol 25: 141, 1973

119. Penny R, Galton DAG: Studies on neutrophil function. II. Pathological aspects. Br J Haematol 12:633, 1966

120. Perille PE, Finch SC: The local exudative cellular response in leukemia. J Clin Invest 391353. 1963

121. Perreau P, Gardais J: La survie de la leucémie myiloide chronique après aplasie par misulban. Sem Hop Paris 15:964, 1969

122. Perry S, Moxley JH III, Weiss GH, et al: Studies of leukocyte kinetics by liquid scintillation counting in normal individuals and in patients with chronic myelocytic leukemia. J Clin Invest 45:1388, 1968

123. Ramanan CV, Israels MCG: Treatment of chronic myeloid leukemia with dibromomannitol. Lancet 2:125, 1969

124. Reinhard EH, Neely CL, Samples DM: Radioactive phosphorus in the treatment of chronic myelocytic leukemia over a period of 15 years. Ann Intern Med 50:942, 1959

125. Robinson WA, Pike BL: Leukopoietic activity in human urine. The granulocytic leukemia. N Engl J Mrd 282:1291, 1970

126. Rowley JD: A new consistent chromosomal abnormality in chronic myelogenous leu-

kemia identified by quinacrine fluorescence and Giemsa staining. Nature. 243:290. 1973

127. Rundles RW. Coonrad EV. Willard NL: Summary of results obtained with TEM. Ann NY Acad Sci 68:926, 1958

128. Rundles RW. Grizzle J. Bell WN. et al: Southeastern Canc Chemother Corp Study Group: Comparison of Chlorambucil and Myleran in chronic lymphocytic and granulocytic leukemia. Ani J Med 27:424, 1959

129. Sandberg AA. Ishihara T. Crosswhite LH. et al: Cornparison of chromosome constitution in chronic myelocytic leukemia and other myeloproliferative disorders. Blood 20 393. 1962

130. Sandberg AA. Hossfeld DK: Chromosomal abnormalities in human neoplasia. Ann Kev Mcd 21:379, 1970

131. Schiffer LM, Chanana AD, Cronkite EP. et al: Extracorporeal irradiation of the blood. Semin Hematol 3:154, 1966

132. Schwarzenberp L. Mathe G, Pouillart P. et al: Hydroxyurea. leucopheresis and splenectomy in chronic myeloid leukaemia at the problastic phase. Br Med J 1:700, 1973

133. Seeler RA. Hahn KO: Chronic granulocytic leukemia responding to Melphalan. Cancer 27284. 1971

134. Smalley RV, Wall RL: Two cases of busulfan toxicity. Ann Intern Mcd 64:154. 1966

135. Sokal JE. Elias EG, Mittelman A: Splenectomy in chronic myelocytic leukemia (CML) and related states. Proc Am Assoc Cancer Res 12:Abstract 37, 1971

136. Sokal JE. Aungst CW, Grace JJ: Immunotherapy in well-controlled chronic myelocytic leukemia. NY State J Med 73:1180, 1973

137. Speed DE. Lawler SD: Chronic granulocytic leukaemia. the chromosomes and the disease. Lancet 1:403, 1964

138. Spiers ASD, Baikie AG: Chronic granulocytic leukaemia: Demonstration of the Philadelphia chromosome in culture of spleen cells. Nature 208:497, 1965

139. Spiers ASD, Baikie AG: Cytogenetic evolution and clonal proliferation in acute transformation of chronic granulocytic leukaemia. Br J Cancer 22192, 1968

140. Spiers ASD: Surgery in management of patients with leukaemia. Br Mcd J 3:528, 1973

141. Staeuber PG, Gerhartz H. Liss E: Die Chemotherapie Lcukamie-Kranker mit Myelobromol. Verh Dtsch Ges Inn Mcd 71:733, 1965

142. Storti E. Perugini S Cytochemical researches on the lipids of the hematic cells with particular attention to thox of acute leukosis. Acta Haematol 5:321, 1951

143. Strumia MM. Strumia PV. Bussert D: Splenectomy in leukemia: Hematologic and clinical effects on 34 patients and reviciw of 299 published cases. Cancer Res 26:519, 1966

144. Stryckmans P. Cronkite EP. Fache J. et al: Deoxyribonucleic acid synthesis time of erythropoietic and granulopoietic cells in human beings. Nature (London) 211:717, 1966

145. Stryckmans P. Manaster J. Peltzer T. et al: Cell proliferation in chronic myeloid leukemia. in Grigoletto E (ed): Thyroid Tumors Lymphomas Granulocytic Leukemia. Padua. Piccin Medical Books. 1972. p 99

146. Tjio JH. Carbone PP. Whang J. et al: The Philadelphia chromosome and chronic myelogenous leukemia. J Natl Cancer Inst 36: 567, 1966

147. Thomas ED. Epstcin RB. Eschbach JW Jr. et al: Treatment of leukemia by extracorporeal irradiation. N Engl J Mcd 273:6, 1965

148. Tokuhata GK, Neely CL, Williams DL: Chronic myelocytic leukemia in identical twins and a sibling. Blood 31:216, 1968

149. Tough IM, Court Brown WM. Baikie AG. et al: Cytogenetic studies in chronic myeloid leukaemia and acute leukaemia associated with mongolism. Lancet 1:411. 1961

150. Tough IM, Jacobs PA. Court Brown WM. et al: Cytogenetic studies on bone-marrow in chronic myeloid leukaemia. Lancet 1: 844. 1963

151. Trujillo JM. Fernandez MN. Schullenberger C. et al: Cytogenetic contributions to the study of human leukemias. Leukeinia Lymphoma Fourteenth Annual Clinical Conference on Cancer 1969 at the University of Texas MD Anderson Hospital and Tumor Institute. Houston. Texas. Chicago, Year Book. 1970. pp 105–122

152. Vincent PC. Cronkite PC. Greenberg ML. et al: Leukocyte kinetics in chronic myeloid leukemia. I. DNA synthesis time in blood and marrow myelocytes. Blood 33:843. 1969

153. Vodopick H, Rupp EM. Edwards CL. et al: Spontaneous cyclic leukocytosis and thrombocytosis in chronic granulocytic leukemia. N Engl J Med 286:284, 1972

154. Whang J. Frei E III. Tjio JH. et al: The distribution of the Philadelphia chromosome in patients with chronic myelogenous leukemia. Blood 22664. 1963

155. Whang-Peng J. Canellos GP. Carbone PP. et al: Clinical implications of cytogenetic variants in chronic myelocytic leukemia (CML). Blood 32:755. 1968

/, Basscrt **D:** atologic and nd review of 5:519, 1966
EP. Fache J. hesis time of tic cells in 211:717, 1966
. Peltzer T. et myeloid leu- yroid Tumors emia. Padua.

'hang J. et al: and chronic ancer Inst 36:

Eschbach JW mia by extra- J Med 273:6,

. Williams DL: identical twins

n WM, Baikie ies in chronic eukaemia asso- :411, 1961
, Court Brown i on bone-mar- mia. Lancet I:

MN. Schullen- contributions 10 nias. Leukemia I Clinical Con- e University of ind Tumor Insti- go. Year Book.

PC. Greenberg tics in chronic ynthesis time in s. Blood 33:843,

M, Edwards CL. eukocytosis and granulocytic leu- 1972
, Tjio JH. et al: adelphia chromo- myelogenous leu-

llos GP. Carbonc ns of cytogenetic leukemia (CML).

156. Whang-Peng J, Henderson ES, Knutsen T. et al: Cytogenetic siudics in acute myelocytic leukemia with special emphasis on the occurrence of Ph' chromosome. Blood 36:448, 1970

157. Wilkinson JF, Bourne MS, Israels MCG: Treatment of leukaemias and reticuloses with Uracil mustard. Br Med J 1:1563, 1963

158. Wolf U. Merker H. Bockelmann W: Chromosome-nuntersuchungen bei chronisch-myeloischer Leukämie. Klin Wochenschr 44: 12. 1966

159. Woodliff HJ. Onesti P. Dougan L: Karyotypes in thrombocythaemia. Lancet 1: 114, 1967