Canad. Med. Ass. J.
June 24, 1967, vol. 96

The Chronic Leukemias:
A Review of Disease Manifestations and the Aims of Therapy

DANIEL E. BERGSAGEL, M.D., D.Phil.,* *Toronto*

Certain aspects of the chronic leukemias that may influence future therapeutic trials are reviewed. In chronic lymphocytic leukemia (CLL), there is minimal mitotic activity in lymphoid tissues; indolent, long-lived lymphocytes, unresponsive to antigenic or phytohemagglutinin (PHA) stimulation accumulate. In many patients, erythroid precursors fail to proliferate despite the stimulus of a severe anemia, but a proliferative response can be initiated by prednisone. We need to know how the normal proliferative responses of these cells are modified, because the correction of these abnormalities would relieve most of the disease manifestations. CLL may not be a neoplastic disorder. In chronic myelogenous leukemia (CML), the leukocyte doubling time shortens as the disease duration lengthens; a significant correlation between this time and survival is demonstrated. Before therapy designed to eliminate the Ph¹-positive (Philadelphia chromosome) stem cell is tried, we need to know whether a normal hematopoietic stem cell exists in Ph¹-positive CML.

L'auteur passe en revue certains aspects des leucémies chroniques qui peuvent influencer les essais thérapeutiques ultérieurs. Dans la leucémie lymphoïde chronique (LLC), la mitose a une activité minimum dans le tissue lymphoïde; il y a accumulation des lymphocytes indolents, à vie prolongée et qui ne réagissent pas à la stimulation des antigènes ou à la phyto-hémoagglutinine (PHA). Chez de nombreux malades, les érythroblastes ne prolifèrent pas malgré le stimulus d'une anémie grave. La prednisone parvient cependant à déclencher une réaction de prolifération. Il importe de cannaître la réaction normale de prolifération de ces cellules, car la correction de ces anomalies supprimerait la majorité des manifestations pathologiques. La LLC peut ne pas être une maladie néoplasique. Dans la leucémie myéloïde chronique (LMC), la période de temps nécessaire pour doubler le nombre de leucocytes s'abrège à mesure que la durée de la maladie s'allonge: on a prouvé l'existence d'une nette corrélation entre ce temps de prolifération leucocytaire et la survie du malade. Avant d'essayer un traitement destiné à éliminer la cellule-souche contenant le chromosome de Philadelphie (Ph¹-positive), il est indispensable de savoir si une cellule-souche hématopoïétique normale existe dans la cellule-souche Ph¹-positive de la LMC.

THE prognosis of patients with acute leukemia has improved markedly within the past four years, as a result of improved therapy. In 1924, Minot and Isaacs[1] reported that 50% of patients with acute lymphocytic leukemia died less than two months after the onset of symptoms, while the median survival of patients with both forms of chronic leukemia was about three and one-half years. Today, if patients with acute childhood leukemia receive the treatment described by Freireich,[2] at least 50% will live for more than 30 months, and a few will have such prolonged disease-free intervals that the possibility of cure in some of these patients is considered with cautious optimism. In contrast, the prognosis for patients with the chronic leukemias has changed very little. The moderate improvement which has occurred in the median survival of patients with chronic lymphocytic leukemia (CLL) should probably be attributed to improved supportive care, especially to antibiotics, rather than to the activity of any antileukemia agent. The life expectancy of patients with chronic myelocytic leukemia (CML) has changed very little in the past 50 years.

Treatment of patients who have CML with irradiation by x-rays or busulfan (Myleran*) causes regression of most of the manifestations of the disease and improves the quality, if not the duration, of the patient's life. The ability of radiation and chemotherapeutic agents to induce clinically significant regression of disease in patients with CLL is less well established.

During the past 10 years several new observations have been made in the clinic and laboratory that suggest new concepts regarding the pathogenesis of the chronic leukemias. In the future, these concepts should have an important influence on the therapy advocated for the chronic leukemias. Co-operative group studies have not yet contributed much useful information because the basic information needed for the design of a good clinical trial has not been available. For this reason I will not review the results of such group studies but will concentrate on certain new attitudes toward the chronic leukemias which should influence the design of studies of therapy in the future.

Presented at a Clinical Conference on Leukemia sponsored by the Ontario Cancer Treatment and Research Foundation (London Clinic), London, Ontario, November 4, 1966.

*Chief of Medicine, Princess Margaret Hospital, Toronto, and Associate Professor of Medicine, University of Toronto.

Reprint requests to: Dr. Daniel E. Bergsagel, The Princess Margaret Hospital, 500 Sherbourne Street, Toronto 5, Ontario.

*Burroughs Wellcome.

Canad. Med. Ass. J.
June 24, 1967, vol. 96

CHRONIC LYMPHOCYTIC LEUKEMIA

Chronic lymphocytic leukemia develops very slowly. Galton[4] followed the evolution of the disease in 39 untreated patients, and observed two main trends (patterns) of lymphocyte count changes. *Type I trend:* In 17 patients the lymphocyte counts rose slowly and progressively throughout an observation period which had to be terminated in each case because the patients developed symptoms and required treatment. *Type II trend:* (a) In 11 patients there was a slow increase in the lymphocyte counts during which the lymphocytes doubled in number every 250-300 days and then "levelled off" and remained relatively constant for a long period of time. (b) The lymphocyte counts in the remaining 11 patients remained relatively constant throughout the observation period, a pattern which probably represents the stationary phase of the type IIa trend. Type IIa was associated with slower progression of disease and longer survival.[4]

Galton followed up one patient for more than 13 years; during this period, four treatments with nitrogen mustard analogues were given.[5] The first two treatments had very little effect on the slow, progressive increase in the lymphocyte count which occurred during the first six years; the lymphocyte doubling time during this period was 660 days. During the next two years the lymphocyte counts "plateaued" at levels between 150,000 and 200,000 per mm.^{3} After the third course of treatment the lymphocyte counts dropped temporarily, then returned to the previous plateau, with a doubling time of 100 days. The count remained at that level for another year, until the fourth course of therapy was given, following which the lymphocyte count returned to the pre-treatment level with a doubling time of about 150 days.

These observations demonstrate that therapy with alkylating agents did not influence the rate of progression of the disease, and did not alter the level at which the lymphocyte count was maintained in this patient. The shorter lymphocyte count doubling time following the third and fourth treatments suggests that lymphocyte production in this patient was under the control of a readjusted homeostatic mechanism which maintained the lymphocyte count between 150,000 and 200,000 per mm^3.

ABNORMALITIES IN IMMUNE MECHANISM

The abnormalities in the immune mechanism that have been observed in patients with CLL are listed in Table I. The reported incidence of

TABLE I.—THE IMMUNE SYSTEM IN CHRONIC LYMPHOCYTIC LEUKEMIA

1. Hypogammaglobulinemia
2. Antibody formation:
   a. Primary stimulation—severely impaired response
   b. Secondary stimulation—impaired response
3. Delayed hypersensitivity:
   a. Primary
      (i) 2,4-dinitrochlorobenzene—severely impaired response
      (ii) Skin transplants—impaired rejection
   b. Secondary—PPD; mumps; *Candida albicans*—normal response to at least one antigen in 80%
4. Lymphocyte transformation—impaired

hypogammaglobulinemia varies from 19%[5] to 75%.[6] This variation results from a progressive decrease in globulin levels with progression of the disease,[7-11] and also reflects different techniques of determination and criteria of hypogammaglobulinemia. Immunoelectrophoresis studies on 10 patients demonstrated a marked decrease in macroglobulin (γM) in nine, and a decrease in immunoglobulin A (γA) in seven.[12]

Patients with CLL are poor producers of antibodies. The response to primary stimulation is more severely impaired[12] than the secondary response to antigens to which the patient has previously been exposed.[10, 13-15] Primary delayed hypersensitivity was induced in only one of the nine CLL patients tested with 2, 4 dinitrochlorobenzene,[12] and delayed rejection of skin homografts was found in seven of 16 CLL patients.[16] In contrast, 25 of 31 CLL patients had normal secondary skin hypersensitivity to at least one of three common antigens (tuberculin, mumps and *Candida albicans*).[17] Thus, with respect to both antibody formation and delayed hypersensitivity, the primary response is more severely impaired than the secondary response.

In short-term tissue cultures, phytohemagglutinin (PHA) stimulates the majority of lymphocytes from normal blood to transform into large "blast-like" cells.[18, 19] During PHA stimulation, normal lymphocytes synthesize new DNA, produce γ globulin and divide.[20, 21] Specific antigens, to which the lymphocyte donor is sensitive, will also stimulate normal lymphocytes to transform and divide in tissue culture.[22] Lymphocyte transformation induced by specific antigens is believed to be an expression of the immunologic competence of the cell; the mechanism of action of PHA is still unknown. The failure of CLL lymphocytes to transform normally after stimulation by common antigens or PHA[18, 22, 23] suggests that the immunologic unresponsiveness of CLL patients is secondary to this cellular defect.

The two mechanisms primarily responsible for the anemia of CLL are illustrated in Table II. An autoimmune hemolytic anemia, characterized

TABLE II.—THE ANEMIA OF CHRONIC LYMPHOCYTIC LEUKEMIA

	"Packed-marrow" syndrome	*Autoimmune hemolytic*
Reticulocytes.....	Reduced	Elevated
Direct Coombs'.....	Negative	Positive
RBC survival.....	Normal or reduced	Reduced
Marrow.....	Minimal erythropoiesis	Moderate erythropoiesis
Serum iron.....	Elevated	Reduced

by a positive direct Coombs' test, is seen in about 15% of patients with CLL. The anemia is more frequently secondary to impaired erythropoiesis; these patients have a negative direct Coombs' test, low reticulocyte counts, a red cell survival which may be normal or reduced, minimal erythroid activity in the marrow and an elevated serum iron.[24] In a study of the non-autoimmune anemia of 11 CLL patients, Bergsagel and Giannopoulos[24] found evidence of reduced erythropoiesis in every patient and shortened red cell survival in nine. In most patients with congenital or acquired hemolytic anemias the plasma iron clearance rate, which serves as an index of erythropoiesis, increases as the red cell survival shortens and the stimulus for erythropoiesis becomes greater. In contrast, the plasma iron clearance rate of anemic CLL patients was found to be lowest in the patients who had the most marked shortening of red cell survival (Fig. 1). This finding indicates that the marrow in these patients is unable to respond to the erythropoietic stimulus of anemia, and also suggests that the factors responsible for the increased red cell destruction and reduced erythropoiesis are interrelated. It is unlikely that the reduced erythropoiesis was caused by crowding of the erythroid marrow by lymphocytes, for there was no evidence of extramedullary hematopoiesis in any of these patients.

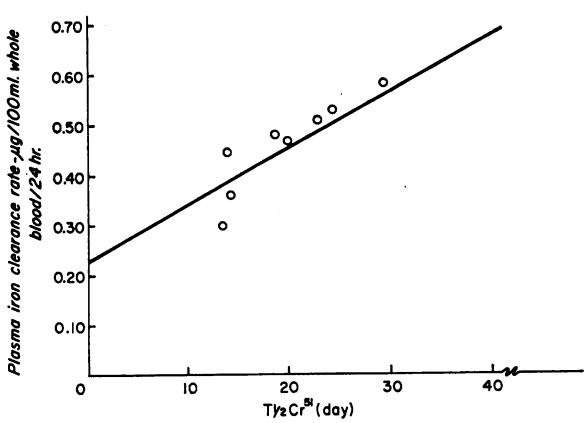

Fig. 1.—The relationship between the T½ ^{51}Cr-RBC survival and the plasma iron clearance rate in patients with the anemia of chronic lymphocytic leukemia.

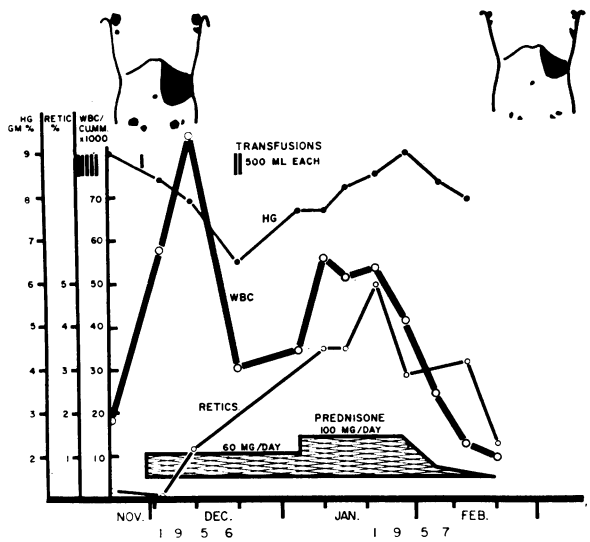

Fig. 2.—The response of an anemic patient with chronic lymphocytic leukemia to prednisone (Meticorten*) therapy.

The reduced erythropoiesis in these patients was not due to a lack of marrow erythroid precursors because corticosteroid therapy frequently causes a reticulocytosis and a rise in hemoglobin.[24] Fig. 2 illustrates the response of an anemic CLL patient to prednisone therapy. Before prednisone was started, the patient required frequent transfusions to maintain the hemoglobin between 8.0 and 9.0 g. %; the reticulocyte count was low, the serum iron was elevated, the plasma iron clearance rate was reduced to 0.37 mg./100 ml. blood/24 hours (normal: 0.58 ± 0.062), and the half-life (T½) of the patient's ^{51}Cr-tagged erythrocytes (T½ ^{51}Cr RBC) was 14 days (normal: 31 ± 3.9 days). Following prednisone therapy the reticulocyte count rose to a peak of 5.0% on the 51st day, the serum iron fell, the T½ ^{51}Cr-RBC increased to 28 days and the hemoglobin rose from 6.4 to 9.0 g. %, but began to fall when prednisone was discontinued. During prednisone administration the spleen and lymph nodes regressed, and a marked lymphocytosis developed in the peripheral blood, with a rise in the count from 20,000 to 86,000 lymphocytes/mm^3. The lymphocytosis appeared to be the result of the mobilization and re-circulation of tissue lymphocytes, as has been observed by others.[25]

The doubling time of the peripheral lymphocyte count is very slow in patients with CLL, and the mitotic index of lymphocytes in lymph nodes and marrow is also low, in contrast to the acute leukemia and other neoplastic disorders. The CLL lymphocyte appears to be normal morphologically, but it is indolent and its re-

*Schering.

Canad. Med. Ass. J.
June 24, 1967, vol. 96

sponse to antigenic or PHA stimulation is reduced. On the basis of these observations, Galton[3] has speculated that aged lymphocytes gradually accumulate in the peripheral blood and tissues in CLL because they are abnormal and cannot respond to antigenic stimulation. The pathogenesis of the suppressed erythropoiesis in most anemic patients with CLL is unknown, but this defect may be due to a deficiency of erythropoietin or to a cellular defect, similar to the lymphocyte defect, which prevents the erythroid precursors from responding to erythropoietic stimulation. The suppressed erythropoiesis is not due to a reduced number of erythroid precursors because a reticulocyte response and a rise in hemoglobin can be stimulated in most patients by corticosteroid therapy.

In view of the low mitotic index in chronic lymphocytic leukemia, one would anticipate that agents such as vinblastine and methotrexate, which kill cells entering mitosis and DNA synthesis respectively, would not be very effective in the treatment of this disease. The ineffectiveness of vinblastine and methotrexate in the limited trials that have been conducted tend to confirm this prediction. Following treatment with x-rays, radioactive phosphorus (^{32}P) and certain alkylating agents, the lymphocyte count frequently falls, and there is some regression of lymphadenopathy and splenomegaly, but all the disease manifestations rarely regress completely. There is no evidence that any of these agents alters the course of the disease or increases survival significantly. In view of these considerations, we should alter our approach to the treatment of chronic lymphocytic leukemia and search for agents that will restore the immunological competence of lymphocytes and the ability of erythroid precursors to proliferate.

CHRONIC MYELOCYTIC LEUKEMIA

In the majority of patients with chronic myelocytic leukemia (CML), 90 to 100% of the marrow cell metaphases contain the abnormal Philadelphia (Ph^1) chromosome. In about 15% of patients who present with all of the other characteristic clinical and laboratory manifestations of CML,[26] however, this chromosome is not found—Ph^1-negative patients. The clinical and laboratory features, the results of chemotherapy and survival of Ph^1-positive and Ph^1-negative patients with chronic myelocytic leukemia have been studied by Tjio *et al.*;[26] Table III summarizes the results of this study.

The median age of the Ph^1-positive and -negative patients was similar, but three of the 13 Ph^1-negative patients were less than 7 years of age, whereas all of the Ph^1-positive patients were older than 9 years. The leukocyte and platelet counts were significantly lower in the Ph^1-negative patients. It is of great interest that complete or partial regression of disease manifestations was noted in only three of 10 Ph^1-negative patients treated with chemotherapy. In contrast, 95% of the Ph^1-positive patients benefited from chemotherapy. The median survival of the Ph^1-negative patients was significantly shorter than for the Ph^1-positive group ($p = 0.01$). At the time the data was analysed, 15 of 60 Ph^1-positive and eight of 13 Ph^1-negative patients were dead; a "blast crisis" was the cause of death in all but two of the deceased patients.

TABLE III.—A COMPARISON OF Ph^1-POSITIVE AND Ph^1-NEGATIVE PATIENTS WITH CHRONIC MYELOCYTIC LEUKEMIA (TJIO *et al.*, 1966)

	Ph^1-positive	*Ph^1-negative*
Number	47	13
Age: Median	46	44
Range	9.5-77	0.75-76
No. under 7 years	0	3
Total leukocytes x 10^3/mm.^{3}		
Median	133.7	41.5
Range	1.5-377	2.1-177
Platelets x 10^3/mm.^{3}		
Median	388	180
Range	4.0-1835	67-803
Results of chemotherapy (complete or partial remission/total treated)	20/21	3/10
Survival from diagnosis (months)		
Median	45	18
Chief cause of death	Blast crisis	Blast crisis

In view of the differences in the response to chemotherapy and the survival of these two groups of patients, the co-operative groups studying CML now feel that chromosome studies should be done on all patients so that they can be classified and grouped properly.

The observation that 95 to 100% of direct marrow metaphases from patients with CML contain the Ph^1 chromosome, whereas metaphases from lymphocytes, skin and other tissues do not, and the successful establishment of a Ph^1-positive marrow homograph on a patient with acute leukemia being treated with immunosuppressive drugs[27] lead to the conclusion that the myeloid, erythroid and megakaryocytic series are derived from the same stem-cell. It seems likely that the cell which undergoes a malignant transformation in CML is a hematopoietic stem-cell. An important problem, which needs to be solved before much progress can be made in the treatment of this disease, is whether there are two types of hematopoietic stem-cells in CML. If a leukemic "stem-line" containing the Ph^1 chromosome, and a normal stem-cell with a normal chromosome complement can be demonstrated,

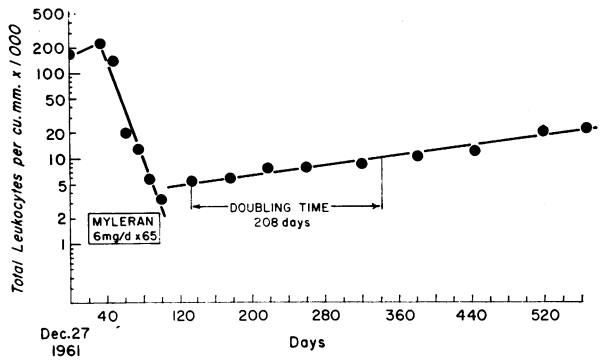

Fig. 3.—Changes in the leukocyte count of a patient with chronic myelocytic leukemia treated with busulfan.

studies will have to be initiated to elucidate the properties of the two types of stem-cells so that therapeutic measures can be developed to eliminate the leukemic stem-line, while preserving the normal stem-cells. On the other hand, if a normal stem-cell population cannot be demonstrated, therapy directed at eliminating the leukemic stem-line will have to wait until successful techniques of normal marrow homografting have been developed.

In CML patients who are not receiving any therapy and who have counts below 200,000/mm³., an exponential increase in leukocytes is noted. In Fig. 3 the leukocyte counts for a CML patient treated with busulfan (Myleran) have been plotted on a logarithmic scale versus time on an arithmetic scale to illustrate the response to busulfan and the exponential increase in the leukocyte count after busulfan therapy was stopped. Diagrams of this sort are valuable in following a patient's response to busulfan because, after three or four points have been established one can estimate, by extrapolation, how long therapy will have to be continued to lower the leukocyte count to between 5000 and 10,000/mm³. The leukocyte doubling time after busulfan was discontinued was 208 days. This figure is helpful in evaluating the patient's prognosis.

As the disease progresses, the patient receives several courses of therapy, and the leukocyte doubling-time shortens during each relapse period.[28] The reason for this progressive shortening of the leukocyte doubling time is unknown, but if this is a universal phenomenon in patients with CML, one would anticipate a correlation between the leukocyte doubling time and survival.

For 30 patients (Fig. 4), I have plotted on double logarithmic graph paper the leukocyte doubling time following the first course of therapy versus survival in months from diagnosis. It will be noted that the patients with the

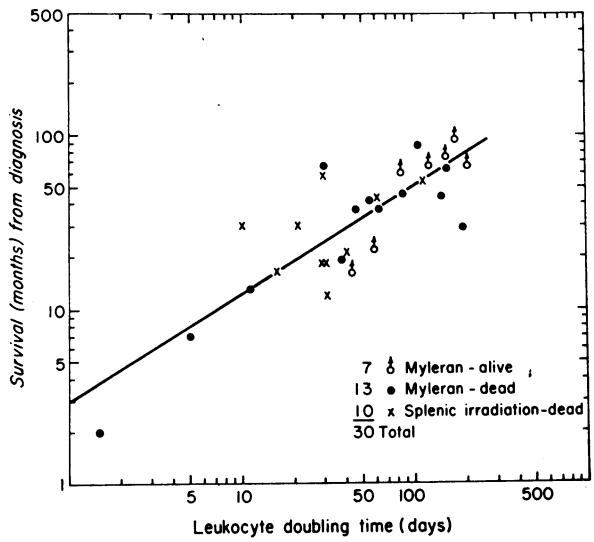

Fig. 4.—The relationship between the leukocyte doubling time of 30 patients with chronic myelocytic leukemia following the completion of the first course of busulfan or splenic irradiation, and their survival in months from diagnosis.

longer leukocyte doubling times also tended to survive for the longest period. The regression line for these points is shown. The significance of the correlation between the leukocyte doubling time and survival was tested. The T value was 1.936, indicating that the correlation was significant with $p < 0.05$.

The effect of therapy on survival in CML is shown in Fig. 5. We are fortunate to have the data of Minot, Buckman and Isaacs on the

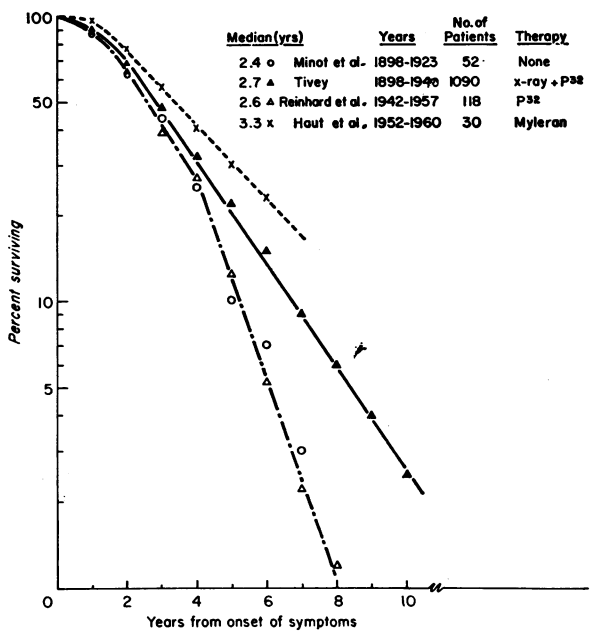

Fig. 5.—The survival of groups of patients with chronic myelocytic leukemia who received no therapy, x-irradiation, ^{32}P or busulfan as the primary form of treatment.

survival of 52 patients followed up to death without specific therapy before 1923.[29] The median survival of this group was 2.4 years, but it will be noted that the proportion of patients dying per year increased as the duration of disease lengthened, and only 10% of patients survived five years. The survival of 118 patients treated by Reinhard, Neely and Samples[30] with ^{32}P follows a pattern which is similar to that of the untreated group. The 1090 patients treated with x-ray and ^{32}P between 1898 and 1948 had a median survival of 2.7 years, but after the second year the proportion of patients dying per year remained constant and the survival curve became exponential, so that 10% of patients were alive at 6.75 years.[31] The curve for the busulfan-treated patients[32] also falls exponentially after the second year, but the slope is not as steep as for the group treated by x-rays. The median for the busulfan group is 3.3 years, and if the line continues to fall exponentially one would anticipate that 10% of patients would survive for 8.75 years.

These survival curves suggest that the late survival of x-ray-treated patients is better than that of the group receiving no therapy or ^{32}P, and that the survival of the busulfan-treated group is better than that of the irradiated group. However, it is difficult to compare the survival of patients treated in different centres at different times, because case selection and ancillary care may differ considerably. The conclusions suggested by these survival curves have been partially confirmed in a recent co-operative study by a Medical Research Council working party in Great Britain in which the survival of CML patients treated with busulfan or splenic irradiation was compared. The busulfan-treated patients in this study lived significantly longer than those treated by splenic irradiation.[33]

It would appear that we can finally state, with some authority, that busulfan is more effective than splenic irradiation in the treatment of CML.

SUMMARY

In CLL the minimal mitotic activity in lymphoid tissues, the slow accumulation of indolent, immunologically incompetent lymphocytes, and the evidence that lymphocyte production may be under the control of a readjusted homeostatic mechanism, cast doubt on our concept that CLL is a neoplastic disorder. In many untreated patients, erythroid precursors fail to proliferate in response to the stimulus of a severe anemia. We need to understand the pathogenesis of the failure of lymphocytes and erythroid precursors to undergo a proliferative response to normal stimuli, for the correction of these disorders would correct the most serious manifestations of CLL. In CML we need to know whether a normal hematopoietic stem-cell exists before attempts are made to eliminate the Ph^1-positive stem-cell.

I wish to thank Dr. G. D. Hart, Toronto East General Hospital, for the contribution of four busulfan-treated CML patients included in Fig. 4.

REFERENCES

1. MINOT, G. R. AND ISAACS, R.: *Boston Med. Surg. J.*, **191**: 1, 1924.
2. FREIREICH, E. J.: *Canad. Med. Ass. J.*, **96**: 1605, 1967.
3. GALTON, D. A. G.: *Ibid.*, **94**: 1005, 1966.
4. *Idem*: *Sci. Basis Med., Ann. Rev. 1962*: 152, 1962.
5. VIDEBAEK, A.: *Acta Haemat. (Basel)*, **24**: 54, 1960.
6. MILLER, D. G.: Cited by Scharff, M. D. and Uhr, J. W.: *Seminars Hemat.*, **2**: 65, 1965.
7. CREYSSEL, R. *et al.*: *Sang*, **29**: 383, 1958.
8. FAIRLEY, G. H. AND SCOTT, R. B.: *Brit. Med. J.*, **2**: 920, 1961.
9. HUDSON, R. P. AND WILSON, S. J.: *J. Lab. Clin. Med.*, **50**: 829, 1957.
10. SHAW, R. K. *et al.*: *A.M.A. Arch. Intern. Med.*, **106**: 467, 1960.
11. ULTMANN, J. E. *et al.*: *Ann. Intern. Med.*, **51**: 501, 1959.
12. CONE, L. AND UHR, J. W.: *J. Clin. Invest.*, **43**: 2241, 1964.
13. LARSON, D. L. AND TOMLINSON, L. J.: *Ibid.*, **32**: 317, 1953.
14. HEATH, R. B., FAIRLEY, G. H. AND MALPAS, J. S.: *Brit. J. Haemat.*, **10**: 365, 1964.
15. SASLAW, S., CARLISLE, H. N. AND BOURONCLE, B.: *Proc. Soc. Exp. Biol. Med.*, **106**: 654, 1961.
16. MILLER, D. G., LIZARDO, J. G. AND SNYDERMAN, R. K.: *J. Nat. Cancer Inst.*, **26**: 569, 1961.
17. MILLER, D. G.: *Ann. Intern. Med.*, **57**: 703, 1962.
18. ELVES, M. W. AND WILKINSON, J. F.: *Exp. Cell Res.*, **30**: 200, 1963.
19. TANAKA, Y. *et al.*: *Blood*, **22**: 614, 1963.
20. JACKSON, J. F. AND KILLANDER, D.: *Exp. Cell Res.*, **33**: 459, 1964.
21. BACH, F. AND HIRSCHHORN, K.: *Ibid.*, **32**: 592, 1963.
22. OPPENHEIM, J. J., WHANG, J. AND FREI, E., III: *Blood*, **26**: 121, 1965.
23. QUAGLINO, D. AND COWLING, D. C.: *Brit. J. Haemat.*, **10**: 358, 1964.
24. BERGSAGEL, D. E. AND GIANNOPOULOS, P. P.: Studies of the anemias associated with chronic lymphocytic leukemia and Hodgkin's disease using Fe^{59} and Cr^{51}. *In*: Radiation biology and cancer, 12th Annual Symposium on Fundamental Cancer Research, Houston, Texas, 1958. M. D. Anderson Hospital and Tumor Institute, University of Texas Press, Austin, 1959, p. 443.
25. FREYMANN, J. G.: *Brit. J. Haemat.*, **6**: 303, 1960.
26. TJIO, J. H. *et al.*: *J. Nat. Cancer Inst.*, **36**: 567, 1966.
27. LEVIN, R. H. *et al.*: *Blood*, **26**: 587, 1965.
28. GALTON, D. A. G.: *Brit. Med. Bull.*, **15**: 78, 1959.
29. MINOT, G. R., BUCKMAN, T. E. AND ISAACS, R.: *J. A. M. A.*, **82**: 1489, 1924.
30. REINHARD, E. H., NEELY, C. L. AND SAMPLES, D. M.: *Ann. Intern. Med.*, **50**: 942, 1959.
31. TIVEY, H.: *Amer. J. Roentgen.*, **72**: 68, 1954.
32. HAUT, A. *et al.*: *Blood*, **17**: 1, 1961.
33. GALTON, D. A. G.: Personal communication.