BRIEF REPORT

Philadelphia Chromosome in Acute Lymphocytic Leukemia

By SIMON PROPP AND FRANK A. LIZZI

It is recognized that the Ph¹ chromosome anomaly is characteristic of chronic granulocytic leukemia. It has been shown however, to occur rarely in other myeloproliferative diseases. We have reported a 53-year-old roentgenologist with acute lymphocytic leukemia who showed a high percentage of Ph¹ chromosomes in his marrow cells. The possible role of radiation exposure in the production of the abnormal chromosome in this patient is discussed. The data presented is evidence against the specificity of the Ph¹ chromosome for myeloproliferative disorders.

THE PHILADELPHIA CHROMOSOME, which was discovered by Nowell and Hungerford[1] in 1960, occurs in most cases of chronic granulocytic leukemia and has been recognized as a specific cytogenetic anomaly in this disease. Other myeloproliferative disorders with the Philadelphia chromosome have been rarely reported. It was thought that the Ph¹ chromosome did not occur in lymphoproliferative disease. We are reporting a patient with acute lymphocytic leukemia who had the classic Philadelphia chromosome in a high percentage of his marrow cells.

CASE REPORT

M. L. was a 53-year-old white male radiologist who had been practicing diagnostic radiology, including fluoroscopy, for 11 years preceding his final illness. His family history was negative for leukemia or neoplasm. In early December 1968, he developed cough, fever and malaise. On December 14, physical examination showed an overweight white male with no lymphadenopathy and no palpable enlargement of liver or spleen. Blood examination showed: hematocrit 53 per cent, hemoglobin 17.9 Gm./100 ml., white blood count 111,500/cu. mm. with 47 per cent prolymphocytes and lymphoblasts (Fig. 1), 39 per cent lymphocytes, 6.5 per cent segmented neutrophils, 4.5 per cent band neutrophils, 1 per cent metamyelocytes, 0.5 per cent myelocytes, 1 per cent eosinophils and 0.5 per cent monocytes. The platelet count was normal. Bone marrow aspiration (Fig. 2) showed marked hyperplasia of the lymphocytic series with predominance of prolymphocytes and lymphoblasts. The erythrocytic and granulocytic series were markedly decreased and megakaryocytes were low normal. Peroxidase-Wright stains of the marrow smears showed few positive cells. Rare immature cells

From the Division of Hematology, Department of Medicine, Albany Medical College of Union University, Albany, N. Y.

Submitted August 27, 1969; revised April 1, 1970; accepted April 4, 1970.

Supported in part by a research grant from Leukemia Society of America, Inc.

SIMON PROPP, M.D.: *Professor of Medicine and Head, Division of Hematology, Albany Medical College of Union University, Albany, N. Y.* FRANK A. LIZZI, M.D.: *Fellow in Hematology, Albany Medical College of Union University, Albany, N. Y. Clinical Fellow of the American Cancer Society.*

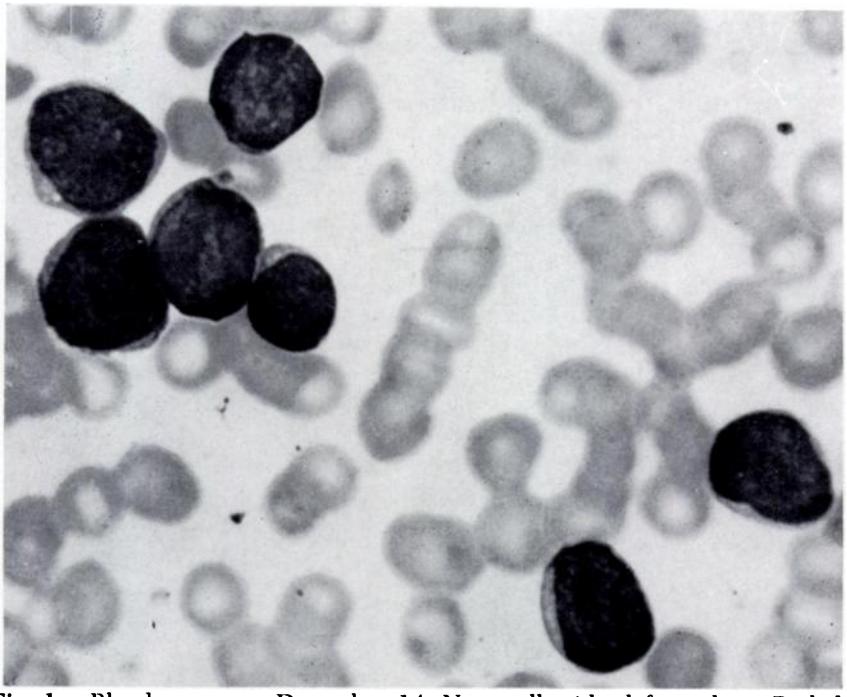

Fig. 1.—Blood smear on December 14. Note cell with cleft nucleus. Both large and small cells have similar nuclear immaturity.

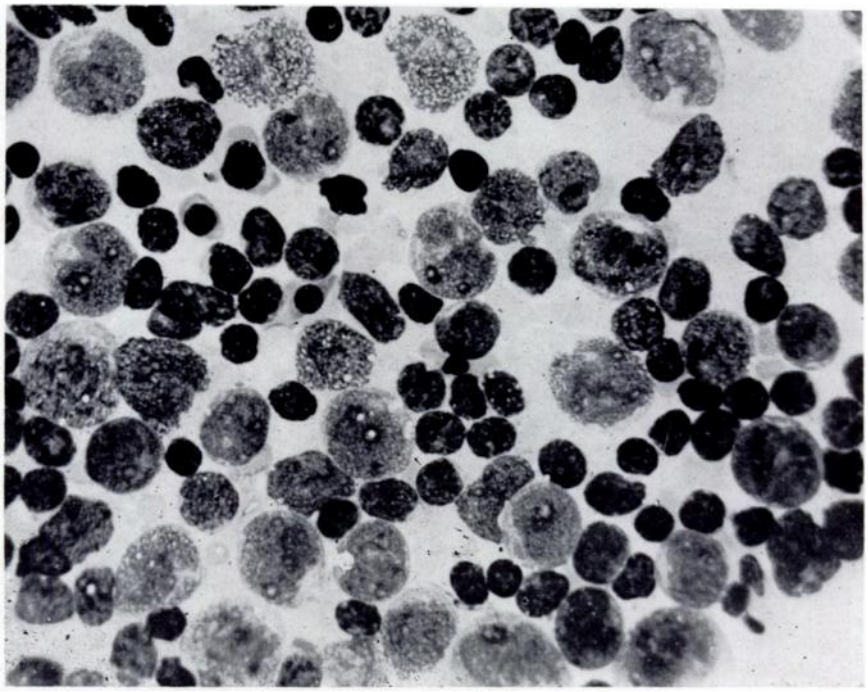

Fig. 2.—Bone marrow aspiration smear on December 14. Mature lymphocytes and lymphoblasts predominate. A few late forms of erythrocytic series but no granulocytes are present in this area.

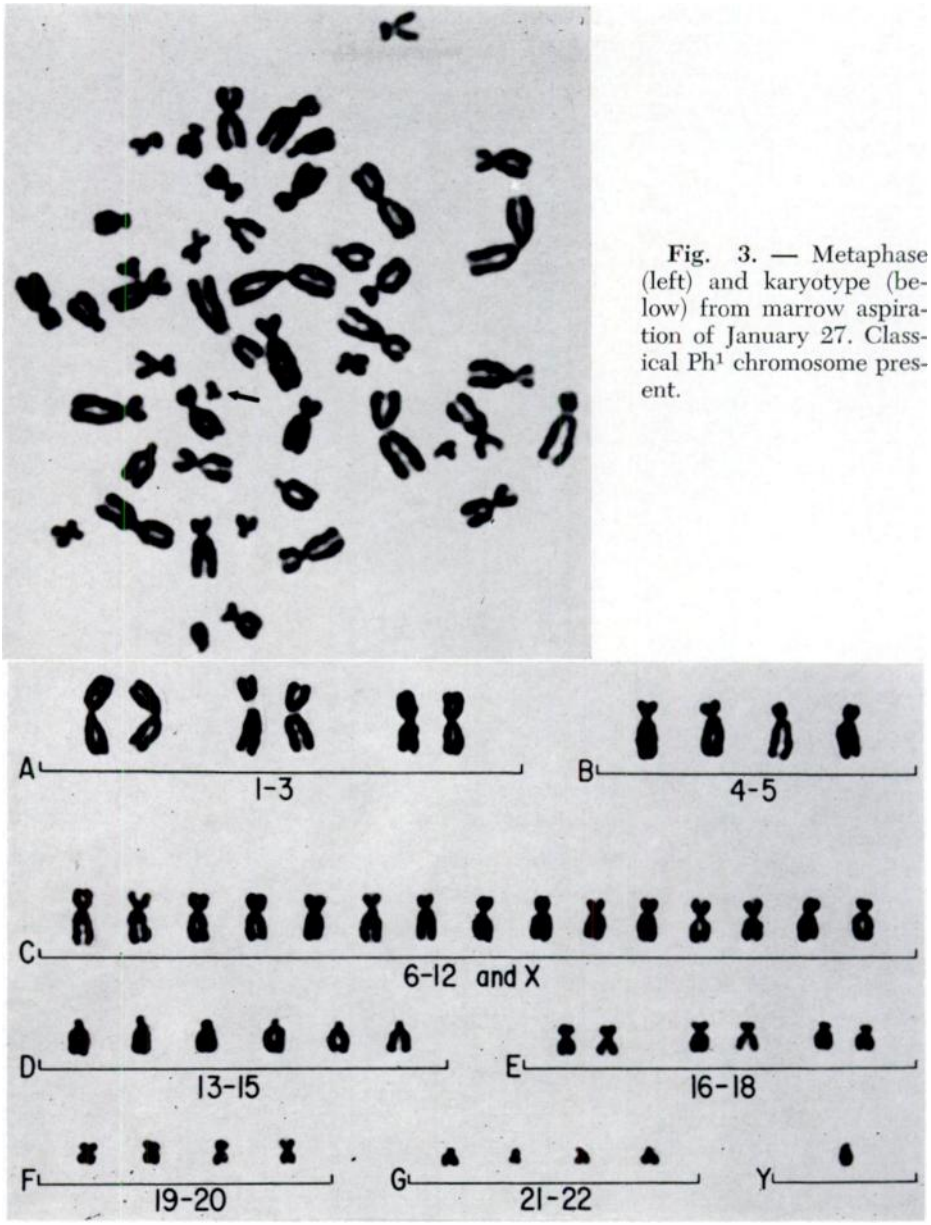

Fig. 3. — Metaphase (left) and karyotype (below) from marrow aspiration of January 27. Classical Ph[1] chromosome present.

showing a few peroxidase-positive granules could be well correlated morphologically with the early progranulocytes rarely observed in the Wright-Giemsa stained smears. All blasts and cells of the prolymphocyte type were peroxidase-negative.

The diagnosis of acute lymphocytic leukemia was made and the patient was started on prednisone, 6-mercaptopurine, and isoniazid (for prophylaxis against tuberculosis). There was an initial good response with a decrease in the white blood count to normal and a disappearance of lymphoblasts from the blood smear. Venous blood chromosome cultures on January 6, 1969, using the Moorhead technique,[2] failed to show any countable cells in metaphase. On January 27, a repeat bone marrow aspiration was per-

Table 1.—Chromosome Studies of Patient (M.L.)

Date	Cells Counted	Chromosome Count < 43	44	45	46	47	Polypl.	Total Ph¹	Per Cent Polypl.	Aneupl.	Ph¹	Structural Defects
1/27/69												(1)* 46† with
Marrow	50		1	3	44	2	0		0	12	64	4 CB and
Cells with Ph¹				3	27	2		32				1 IF
2/14/69												
Marrow	100	1	4	12	82		1		1	18	63	
Cells with Ph¹		1	3	11	48			63				(1) 40 with CB (2) 45 with CB (1) 46 with IF
2/25/69												
Marrow	100		2	7	88		3		3	12	49	(1) 45 with CB (2) 46 with CB
Cells with Ph¹			1	3	44		1	49				(1) 45 with CB and Qua (3) 46 with CB (1) 46 with IF
3/7/69												
Blood	50				49		1		1	1	1	(1) 46 with CB (1) End 92
Cells with Ph¹								0				

*Number of cells.

†Chromosome number.

CB, chromatid break; IF, isochromatid fragment; Qua, quadriradius; End, endoreduplication.

formed and marrow chromosomes were prepared using a direct technique.[3] The Ph¹ was present in 64 per cent of the metaphase counted (Fig. 3 and Table 1). The marrow smears showed no evidence of remission of the leukemia. On January 31, methotrexate was added to the therapy because of the apperance of 40 per cent lymphoblasts in the blood smear. Ten days later the white cell count was reduced to 2800. The 6-mercaptopurine was stopped and the dosage of methotrexate was decreased. Bone marrow aspiration on February 14 showed a marked increase in the lymphocytic series with groups of lymphoblasts (Fig. 4). The granulocyte series was markedly reduced, but the erythrocytic series was cellular in areas. Megakaryocytes were normal. The Ph¹ chromosome was observed in 63 per cent of the cells counted. Another bone marrow aspiration on February 25 showed a marked increase in the lymphocytic series with a predominance of lymphoblasts. The Ph¹ chromosome was found in 49 per cent of the metaphases counted. Venous blood chromosome cultures performed on February 28 were not successful. On March 7, cultures without phytohemagglutinin did not show any metaphases, but with PHA, after 72 hours, 50 metaphases were counted. No Ph¹ chromosomes were found (Tables 1 and 2).

On March 10, the patient's temperature was 101°F. The methotrexate was stopped and antibiotics were started. Fever persisted and a chest X-ray showed a left lower lobe infiltrate. On March 15, lymphoblasts had increased to 74 per cent and segmented neutrophils were only 1 per cent. He was given vincristine 0.025 mg./Kg. and daunomycin 30 mg./sq. M. I.V., and the prednisone was changed to cortisone acetate 75 mg. I.M. every six hours daily. He was also given fresh blood transfusions and platelet transfusions. The fever persisted and on March 18, the white cell count had fallen to

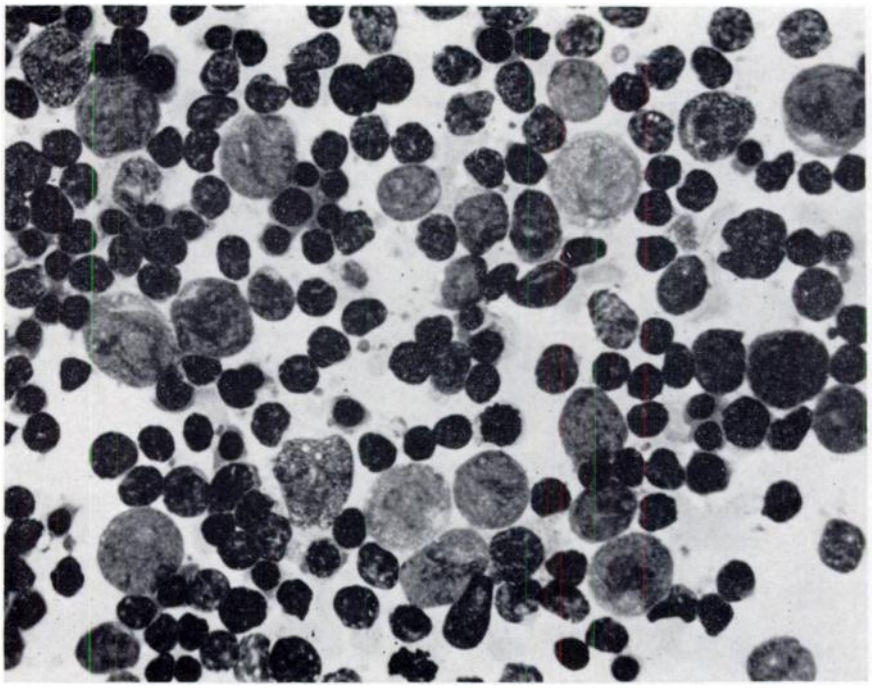

Fig. 4.—Bone marrow aspiration smear on February 14. Many mature lymphocytes with discrete and grouped lymphoblasts. Late cells of erythrocytic series present but only rare granulocyte seen.

880 per cu. mm. and the platelet count to 11,600 (indirect method). Later that day, the patient developed a high fever, became hypotensive and expired. Blood cultures taken at that time showed growth of coliform bacilli. Post mortem examination could not be obtained.

The course of the leukemia was four months from the onset of symptoms. The Ph[1] chromosome was demonstrated in three separate bone marrow aspirations. No Ph[1] chromosome was observed in a 72-hour blood culture using phytohemagglutinin in 50 metaphases counted.

Discussion

In addition to chronic granulocytic leukemia and the blast crises of CGL, the Ph[1] chromosome has been found in patients with myelofibrosis and myeloid metaplasia,[4,5] myeloproliferative syndrome,[6,7] acute granulocytic leukemia,[8-11] polycythemia vera,[12] thrombocythemia (megakaryocytic myelosis),[13,14] eosinophilic leukemia,[7,15-17] and erythroleukemia (di Guglielmo syndrome).[8] In a study of institutionalized mentally retarded males, the Ph[1] chromosome was demonstrated in one subject by blood culture technique.[18] In addition, a family with a high incidence of CGL has been reported in which hematologically normal members have been found to have the Ph[1] chromosome.[19]

The Ph[1] chromosome has been demonstrated in the granulocytic, erythrocytic and megakaryocytic series in the marrow of chronic granulocytic leukemia, but not in the lymphocytic series.[14,20,21] The chromosome disappears

Table 2.—White Cell and Differential Counts

Date	cu. mm. W.B.C.	Segmented Neutrophils	Band Neutrophils	Metamye-locytes	Myelo-cytes	Lympho-cytes	Prolym-phocytes and Lympho-blasts	Mono-cytes %
12/14/68	111,500	6.5	4.5	1	0.5	39	47	0.5
1/6/69*	6000	50	6	2	2	32	8	0
1/27†	10,000	24	3	1	1	59	10	2
2/14†	4100	49	1	0	1	49	0	0
2/25†	17,750	30	35	5	1	19	10	0
2/28*	7300	58	6	1	1	30	3	1
3/7*	12,500	41	10	2	0	38	5	4
3/16	13,300	0	1	0	0	23	76	0

*Blood chromosome cultures.
†Ph^1 chromosomes in marrow aspirate.

from the blood when remission of CGL is obtained by therapy but persists in the marrow in such cases. The absence of the Ph^1 chromosome has been cited as "the most satisfying evidence of the divergence of myeloid and lymphoproliferative elements".[22] Nowell,[23] however, refers to a patient he studied with chronic granulocytic leukemia who developed generalized lymphosarcoma. The Ph^1 chromosome was found in both marrow and lymph node cells, even though smears of node aspirate showed only lymphoid elements. Nowell suggested that, in his patient, a pluripotent neoplastic stem cell differentiated to leukemic myeloid cells in the marrow and to sarcomatous cells in the nodes. The findings in our case suggest that a lymphocytic stem cell affected by the chromosomal change eventuated in a clone of leukemic cells. This explanation would seem more probable than the occurrence of a pluripotent neoplastic stem cell and would be more consistent with the 49–64 per cent Ph^1 marrow chromosome counts.

The fact that our patient had been a radiologist for 12 years and often performed fluoroscopy, leads one to speculate on the possible role of radiation as a contributing factor in his disease and in the Ph^1 chromosome anomaly. Goh[24] has reported three men, who were accidentally exposed to radiation (68–339 R) and who were found to have a chromosome similar to the Ph^1 in 2.5 per cent of the metaphases from bone marrow and peripheral blood cultures. Perhaps, repeated exposure to X-ray irradiation caused chromosome damage resulting in the occurrence of the Ph^1 anomaly in lymphocytic leukemia in our patient.

In contrast to chronic granulocytic leukemia, the usual chromosomal aberrations in acute leukemia are nonspecific. Many patients have normal chromosomes, whereas others are characterized by clones of aneuploid cells and structural aberrations.[8] Marrow chromosome counts on our patient showed 15 per cent aneuploidy and 5 per cent structural defects (Table 1). The chromosomal aberrations were not of the type which would suggest an irradiation effect. There was chromosome breakage in a few cells but this was almost entirely of the chromatid type. No ring or dicentric forms were seen.[25]

One must consider whether the Ph^1 chromosome was a constitutional or

congenital anomaly in our patient. The absence of the Philadelphia chromosome in the 72-hour chromosome culture of his venous blood, stimulated by PHA, and presumably consisting of metaphases of normal lymphocytes, rules against this possibility.

ACKNOWLEDGMENTS

We are indebted to Peter C. Nowell, M.D., who reviewed the chromosome slides on this patient and confirmed the presence of the Ph¹ chromosome. He also contributed comments and a reference, and subsequently, read the completed manuscript.

REFERENCES

1. Nowll, P. C., and Hungerford, D. A.: A minute chromosome in human chronic granulocytic leukemia. Science 132:1497, 1960.

2. Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A.: Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell Res. 20:613, 1960.

3. Tjio, J. H., and Whang, J.: Chromosome preparations of bone marrow cells without prior in vitro culture or in vivo colchicine administration. Stain Techn. 37: 17, 1962.

4. Forrester, R. H., and Louro, J. M.: Philadelphia chromosome abnormality in agnogenic myeloid metaplasia. Ann. Intern. Med. 64:622, 1966.

5. Khan, M. H., and Martin, H.: Multiple chromosomal aberrations in a case of malignant myelosclerosis. Acta Haemat. (Basel) 39:299, 1968.

6. Heath, C. W., and Maloney, W. C.: The Philadelphia chromosome in an unusual case of myeloproliferative disease. Blood 26:471, 1965.

7. Kiossoglou, K. A., Mitus, W. J., and Dameshek, W.: Cytogenetic studies in the chronic myeloproliferative syndrome. Blood 28:241, 1966.

8. —, —, and —: Chromosomal aberrations in acute leukemia. Blood 26:610, 1965.

9. —, —, and —: Two Ph¹ chromosomes in acute granulocytic leukemia. Lancet 2: 665, 1965.

10. Khan, M. H., and Martin, H.: Myeloblastenleukamie mit Philadelphia-Chromosom. Klin. Wschr. 45:821, 1967.

11. Luers, T., Struck, E., and Albrecht, M.: Strukturanomalien der Chromosomen bei einer akuten Paramyeloblasten-leukamie. Med. Welt 17:948, 1963.

12. Kemp, N. H., Stafford, J. L., and Tanner, R.: Chromosome studies during early and terminal chronic myeloid leukemia. Brit. Med. J. 1:1010, 1964.

13. Dougan, L., Woodliff, H. J., and Onesti, P.: Cytogenetic studies in megakaryocytic myelosis. Med. J. Aust. 1:62, 1967.

14. Tough, I. M., Jacobs, P. A., Court Brown, W. M., Baikie, A. G., and Williamson, E. R. D.: Cytogenetic studies on bone marrow in chronic myeloid leukemia. Lancet 1:844, 1963.

15. Kauer, G. L., and Engle, R. L.: Eosinophilic leukemia with Ph¹-positive cells. Lancet 2:1340, 1964.

16. Gruenwald, H., Kiossoglou, K. A., Mitus, W. J., and Dameshek, W.: Philadelphia chromosome in eosinophilic leukemia. Amer. J. Med. 39:1003, 1965.

17. Elves, M. W., and Israels, M. C. G.: Cytogenetic studies in unusual forms of chronic myeloid leukemia. Acta Haemat. (Basel) 38:129, 1967.

18. Court Brown, W. M.: Human Population Cytogenetics. Amsterdam, North Holland Publishing Co., 1967, p. 85.

19. Wiener, L.: A family with a high incidence leukemia and unique Ph¹ chromosome findings. Proceedings of the American Society of Hematology, Eighth Annual Meeting, Philadelphia, 1965. Blood 26:871, 1965.

20. Whang, J., Frei, E., Tjio, J. H., Carbone, P. P., and Brecher, G.: The distribution of the Philadelphia chromosome in patients with chronic myelogenous leukemia. Blood 22:664, 1963.

21. Rastrick, J. M., Fitzgerald, P. H., and Gunz, F. W.: Direct evidence for presence of Ph¹ chromosome in erythroid cells. Brit. Med. J. 1:96, 1968.

22. Lawler, S. D.: Chromosomal aberrations in hematological disorders. In: Jaffé, E. R. (Ed.) Plenary Session Papers, Proceedings of the XII Congress of the Inter-

national Society of Hematology, New York, 1968, p. 40.

23. Nowell, P. C.: Chromosome abnormalities in human leukemia and lymphoma. In: Zarafonetis, C. J. D. (Ed.): Proceedings International Conference on Leukemia-Lymphoma, Philadelphia, Lea and Febiger, 1968, p. 50.

24. Goh, K.: Smaller G chromosome in irradiated man. Lancet 1:659, 1966.

25. Bender, M. A., and Gooch, P. C.: Persistent chromosome aberrations in irradiated subjects. Radiat. Res. 18:389, 1963.