

Immunodeficiency and other clinical immunology

Trisomies 9 and 8 detected by fluorescence in situ hybridization in patients with systemic mastocytosis

Michael Lishner, MD,^a Ronit Confino-Cohen, MD,^a
Yoseph A. Mekori, MD,^a Moshe Feigin, MD,^b Yoseph Manor, MD,^a
Arnon Goldberg, MD,^a Mordechai Ravid, MD,^a and Aliza Amiel, PhD^b
Kfar Saba and Tel Aviv, Israel

Background: Systemic mastocytosis is a rare disease characterized by proliferation of mast cells in one or more organs. The origin of the mast cells is still debated, although it has been recently shown that they derive from CD34⁺ hematopoietic progenitors. Some clinical and in vitro studies have suggested a possible link between myeloproliferative disorders and systemic mast cell disease.

Objective: This study was designed to further evaluate the association between systemic mast cell disease and other hematologic disorders by means of conventional cytogenetic analysis and fluorescent in situ hybridization.

Methods: We used cytogenetic analysis and fluorescent in situ hybridization with probes to chromosomes 8 and 9 in six patients with systemic mast cell disease.

Results: Fluorescent in situ hybridization helped to identify five patients with trisomy 9 and one with trisomy 8. In contrast, chromosomal analysis demonstrated an abnormal karyotype (45,XO/46,XY) in only one patient.

Conclusion: The association between myeloproliferative disorders and systemic mast cell disease may be explained by the finding that trisomy 9 and trisomy 8 are common in both disorders. A trisomy was detected in all of the patients in our small group compared with nearly 40% of previously reported patients with myeloproliferative disorders. FISH is more sensitive than conventional cytogenetics in detecting these aberrations. (*J Allergy Clin Immunol* 1996;98:199-204.)

Key words: Systemic mastocytosis, myeloproliferative disorders, fluorescent in situ hybridization, trisomy 9, trisomy 8

Systemic mastocytosis is a rare disease characterized by the proliferation of mast cells in one or more organs.¹ The clinical manifestations range from benign infiltration of the skin or urticaria pigmentosa, to a generalized form, systemic mastocytosis, or even to a mast cell leukemia.² Bone

Abbreviations used

FISH: Fluorescent in situ hybridization
MPDs: Myeloproliferative disorders
SMCD: Systemic mast cell disease

From ^athe Department of Medicine, Sections of Hematology and Allergy-Immunology, Meir Hospital, Kfar Saba, and Sackler School of Medicine, Tel Aviv University, Tel Aviv; and ^bthe Genetic Unit, Meir Hospital, Kfar Saba.

Received for publication Mar. 30, 1995; revised July 26, 1995; accepted for publication Aug. 31, 1995.

Reprint requests: Michael Lishner, MD, Department of Medicine, Meir Hospital, Kfar Saba 44281, Israel.

Copyright © 1996 by Mosby-Year Book, Inc.

0091-6749/96 \$5.00 + 0 1/1/68944

marrow or other extracutaneous biopsy specimens are often required for the diagnosis of the disease.^{1,3}

Mast cell precursors arise from pluripotential bone marrow-derived stem cells and migrate through the blood stream and lymphatics to specific sites within the body where they reach phenotypic maturation under certain microenvironmental influences.⁴ Two principal cytokines that

promote mast cell proliferation, maturation, or both have been identified: IL-3 and stem cell factor, the ligand of the *c-kit* tyrosine kinase receptor.⁵⁻⁷ IL-3 appears to be important for early mast cell proliferation,⁸ whereas stem cell factor, which is produced by stromal cells, maintains mast cell viability and promotes maturation.^{9,10} Recently, the progenitor for human mast cells was identified in bone marrow¹¹ and peripheral blood¹² and characterized as CD34⁺, *c-kit*⁺, Ly⁻, CD14⁻, and CD17⁻ colony-forming cells. Moreover, Nagata et al.¹³ analyzed peripheral blood mononuclear cells of 10 patients with mastocytosis for the presence of a genetic abnormality of *c-kit*. They reported that a subset of patients with mastocytosis had a point mutation in *c-kit* (ASP816val) in contrast to 67 normal subjects who did not have the mutation and suggested that this mutation may have a pathogenetic significance. Thus mast cells are most probably replenished directly from hemopoietic progenitors and constitute a unique cell lineage within the hemopoietic system.

A clinical association between systemic mast cell disease (SMCD) and other hematologic disorders, especially myeloproliferative disorders (MPDs) has been reported.^{1,14,15} Chromosomal abnormalities involving mainly chromosomes 1, 8, 9, 13, and 20 are detected in up to 40% of patients with polycythemia vera or essential thrombocytosis. In contrast, cytogenetic studies have been reported in only a few patients with mastocytosis in whom a normal karyotype was found.^{16,17} Swolin et al.¹⁶ found that five of 13 patients with mastocytosis had chromosome abnormalities and abnormal in vitro growth patterns or abnormal hematologic values. Three patients with chromosome abnormalities showed an abnormal growth pattern but normal peripheral blood values. Two patients with SMCD had clones with chromosome abnormalities (46,XY/46,XY,-16 +mar and 46,xx/46,xx del (5) [q21 q31]) and some abnormal hematologic values (thrombocytosis and mild eosinophilia). These results were suggestive of an MPD, and an association between SMCD and MPDs was proposed.

To further evaluate the chromosomal abnormalities in mastocytosis, we studied six patients with SMCD with two complementary methods: cytogenetic analysis and fluorescent in situ hybridization (FISH). Because trisomies 9 and 8 are common in MPDs, biotin-labeled α and β satellite DNA probes, for chromosomes 8 and 9, respectively, were used to detect possible abnormalities in these chromosomes by FISH.

METHODS

Patients

Six patients with SMCD who were followed up by physicians in the Allergy-Immunology or Hematology unit at Meir Hospital were evaluated. In five patients the diagnosis was established by bone marrow biopsy: specimens demonstrated paratrabecular and perivascular mast cell infiltrates. Mast cells were also noted focally, away from blood vessels or bony trabeculae resembling granulomas. In the last patient the diagnosis was based on a characteristic clinical presentation and histopathology of a skin lesion. All patients received only symptomatic treatment. Three age- and sex-matched healthy volunteers and three patients with active allergic disorders served as control subjects. The patients and the control subjects gave written informed consent for the study, and the protocol was approved by the hospital human research committee.

Probes

The biotin-labeled α satellite of chromosome 8 (D8Z1) and the biotin-labeled β satellite of chromosome 9 (D9Z5), from Oncor (Gaithersburg, Md.), were used in this study.

Cytogenetic studies

Peripheral blood (2×10^6 cells/ml) was incubated in RPMI-1640 medium (Gibco, Grand Island, N.Y.) with 20% fetal calf serum without any mitogens. Cells were harvested after 3 to 4 days according to standard procedures.^{18,19} At least 20 metaphases were obtained. They were analyzed without knowledge of the FISH results.

Unstained slides for FISH were stored at -20°C until the time of hybridization. FISH was done according to the method of Pinkel et al.²⁰ Fresh or frozen slide spreads were treated with ribonuclease (100 $\mu\text{g/ml}$) at 37°C for 1 hour. The slide preparations were then denatured for 2 minutes in 70% formamide/2 $\times$ standard saline citrate at 70°C and dehydrated in a graded ethanol series. The probe was then applied to air-warmed slides (30 μl mix per slide sealed with a 24×50 mm glass coverslip) and hybridized for 18 hours at 37°C in a moist chamber. After hybridization, the slides were washed in 50% formamide/2 $\times$ standard saline citrate for 20 minutes at 43°C , rinsed twice in 2 $\times$ standard saline citrate at 37°C for 4 minutes each, and placed in 0.05% Tween-40 (Sigma Chemical Co., St. Louis, Mo.). Fluorescence detection was performed by alternating layers of fluorescein isothiocyanate-avidin (5 $\mu\text{g/ml}$) and anti-avidin antibody (Vector Laboratories, Burlingame, Calif.) until two layers of fluorescein isothiocyanate-avidin had been applied. Each layer was incubated for 30 minutes at 37°C under a plastic coverslip in a moist chamber. Slides were washed in 0.05% Tween-40 buffer at room temperature between applications.

Preparations were counterstained in a propidium iodide antifade solution and analyzed by an Olympus BHS fluorescent microscope (Olympus America, Inc., Suc-

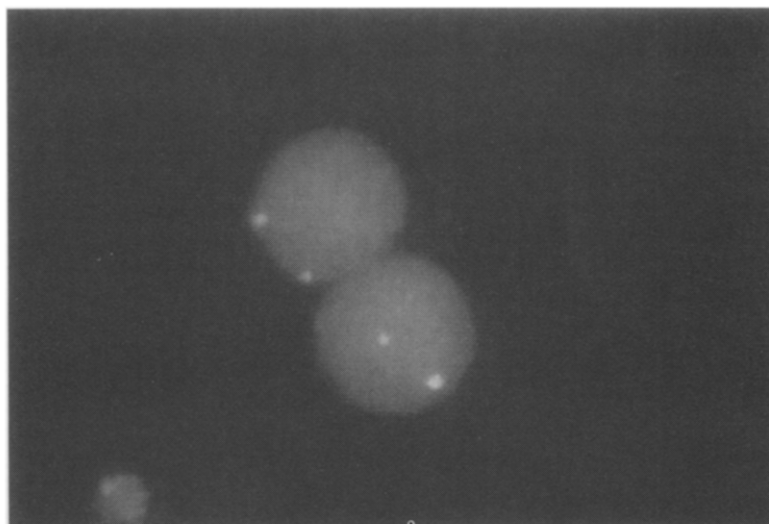


FIG. 1. FISH studies with a chromosome 9-specific β satellite DNA probe. Two copies of chromosome 9 are seen in a nucleus of a normal control subject.

TABLE I. Study population: Demographic characteristics, clinical presentation, and results of imaging and diagnostic procedures

Patient No.	Age (yr)	Sex	Presentation	Skeletal x-ray findings	Bone densitometry (gm/cm ²)*	Diagnostic procedure
1	40	M	Pruritis	Normal	1.34 (100%)	B & BM biopsy
2	64	M	Rash + back pain	Normal	1.057 (88%)	B & BM biopsy
3	60	F	Back pain	no. L1-4	0.567 (60%)	B & BM biopsy
4	60	M	Rash + back pain	no. D10,L4	0.89 (86%)	B & BM biopsy
5	59	F	Back pain	no. D6,7,11	0.789 (73%)	B & BM biopsy
6	19	F	Rash	Normal	ND	ND

B, Bone; BM, bone marrow; ND, not done.

*Values in parentheses indicate percent of normal value.

cess, N.Y.) equipped with fluorescein isothiocyanate-avidin filter (450 to 490 μ m). Two fluorescent spots demonstrated the normal two copies of chromosome 8 or 9 (Fig. 1), whereas three spots indicated a trisomic cell. The slides were scored in blinded fashion. The cutoff level for the presence of trisomy was defined as the highest percentage of three spots among the control specimens (2.5%) plus 2 standard deviations (2.4). Thus a patient with 5% or more cells with trisomy, as demonstrated by FISH, was considered to have the cytogenetic aberration. Similar cutoff levels were previously suggested.²¹⁻²³

RESULTS

The study group comprised six patients, three women and three men, 19 to 64 years old (mean age, 50.3 years). Four patients were first seen with low back pain (two of them also had the typical rash of urticaria pigmentosa), and the other two were first seen with long-standing pruritis. Their

clinical characteristics, laboratory and imaging findings, and results of diagnostic procedures are presented in Table I. All patients had normal blood counts and normal liver function test results. Abdominal computed tomographic scans did not disclose any major abnormality. In two patients osteoporosis and bone fractures were the only findings suggestive of mastocytosis. All patients, except patient 6, who refused the procedure, underwent undecalcified bone marrow biopsy; and specimens were processed as previously described.²⁴ Each specimen demonstrated interstitial perivascular and paratrabeular infiltration of mast cells between normal marrow elements. Granulomas composed of mast cells and lymphoid aggregates, interspersed with mast cells, were also seen. Patients 1 and 6 underwent skin biopsy, and specimens showed typical histopathologic findings of urticaria pigmentosa.

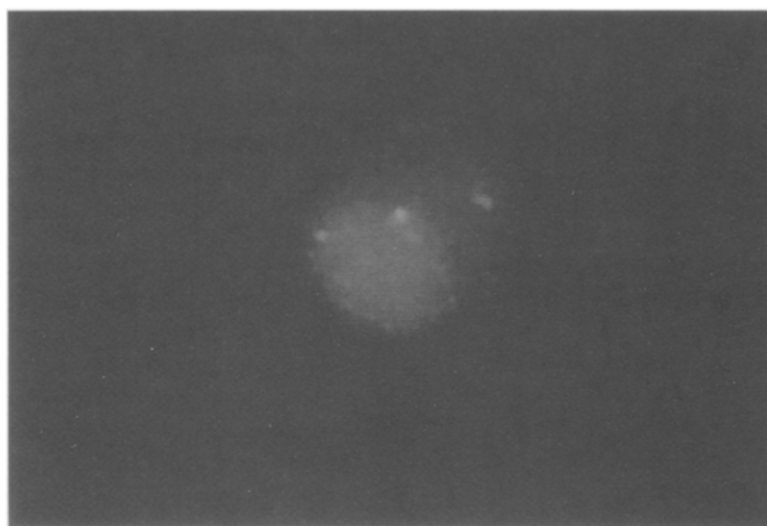


FIG. 2. FISH studies with a chromosome 9-specific β -satellite DNA probe. Three copies of the chromosome (three fluorescent signals) are seen in an interphase nucleus of a patient with systemic mastocytosis (no. 1).

TABLE II. Cytogenetic analysis and FISH in patients with SMCD

Patient No.	Cytogenetics	FISH 8 (%)	FISH 9 (%)
1	45,XO/46,XY	0 (83)	24 (105)
2	46 XX	2 (100)	18 (91)
3	46 XY	0 (73)	13 (86)
4	46 XY	2 (60)	12 (60)
5	46 XX	17 (30)	0 (35)
6	46 XX	0 (85)	12 (82)

Number of cells studied is indicated in parentheses.

The results of the FISH studies, which were performed on peripheral blood mononuclear cells in every patient, are presented in Table II. FISH enabled us to detect trisomy 9 in five patients (Fig. 2) and trisomy 8 in one patient. No patient had more than one chromosomal aberration. Cytogenetic analysis yielded a normal karyotype in all patients, except patient 1 who had 45,XO/46,XY. All patients with active allergic diseases had trisomies in the range of control specimens (<2.5% of the cells).

DISCUSSION

Trisomy 9 was found in five of six patients with SMCD as determined by FISH, and trisomy 8 was found in the remaining patient. In contrast, conventional cytogenetic analysis demonstrated an abnormality (45,XO/46,XY) in only one patient. Thus FISH emerges as a far more sensitive tool for the study of chromosomal

aberrations in SMCD than the classical cytogenetic method.

Trisomies 8 and 9 are commonly found in patients with MPDs, especially in patients with polycythemia vera^{25,26} and also in those with other myeloid malignancies.²⁷⁻²⁹ An association between MPDs and SMCD, which possibly derive from a common stem cell, has previously been suggested.^{1,30,31} Travis et al.¹⁵ reported that hematologic, mainly myeloproliferative, syndromes occur in a relatively high percentage of patients with SMCD. Lewis et al.³² described a 29-year-old woman who had urticaria pigmentosa, which subsequently progressed through systemic mastocytosis to a Philadelphia chromosome-negative, chronic myeloid leukemia with t(8;17). A possible link between MPDs and SMCD was also suggested by in vitro studies in which long-term culture techniques and immunophenotypic staining were used. Horton et al.³³ showed that human mast cells grown in culture expressed "panhematopoietic" cell surface markers. Denburg et al.³⁴ used methylcellulose cultures to demonstrate the existence of basophil-mast cell precursors in peripheral blood from patients with systemic mastocytosis and chronic myeloid leukemia. Swolin et al.¹⁶ used cytogenetic studies and in vitro growth for bone marrow granulocytic-macrophage stem cells in 13 patients with mastocytosis. They showed an abnormal growth pattern and chromosome abnormalities, which were also common in patients with MPDs. Moreover, transition to acute nonlymphocytic leukemia, similar to the terminal blastic phase of an MPD,

also occurs in SMCD.³⁵⁻³⁷ Finally, human interferon- α has been shown to be effective in disease control in patients with MPDs, as well as in a single patient with systemic mastocytosis.³⁸⁻⁴⁰ Our findings that patients with SMCD commonly carry trisomies 9 and 8, chromosomal abnormalities frequently found in patients with MPDs, lend further support to the possible association between these disorders.

It is commonly believed that MPDs have a clonal origin in a multipotent stem cell that gives rise to granulocytes, erythrocytes, megakaryocytes, monocytes, and macrophage cells.⁴¹ It has been recently shown that mast cells are replenished from CD34⁺ multipotent hematopoietic progenitors in the presence of mast cell growth factors.^{11, 12} The presence of similar chromosomal aberrations in these stem cell diseases suggests that SMCD is a hematopoietic stem cell disorder similar to MPDs. However, it is not clear whether these chromosome abnormalities represent primary or secondary events in the malignant transformation. Further study is needed to identify genes on these chromosomes, which may have pathogenetic significance when present in triplicates.

The normal karyotype found in these patients, despite the presence of trisomy 8 or 9, emphasizes the limitations of cytogenetic evaluation. It has been previously demonstrated in different diseases that FISH is more sensitive than cytogenetic analysis for the detection of numeric chromosomal abnormalities. We have previously shown that in chronic lymphocytic leukemia, FISH detected trisomy 12 in 60% of the patients with a normal karyotype.⁴² We have also used FISH to identify trisomies 8 or 9 in seven of 14 patients with polycythemia vera, none of whom showed these abnormalities on conventional cytogenetic analysis.²² Similarly, Jankins et al.²³ reported that some patients with myelodysplastic syndromes, MPDs, and acute myeloid leukemia with normal karyotype or with clonal karyotype but without trisomy 8 had an occult trisomy 8 demonstrated by FISH. The percentage of interphase cells containing three spots was not high. Because we used two different probes, however, each of these served as a control for the other one. In no case were both trisomies 8 and 9 present. This indicates that the finding of a trisomy reflects a true biologic phenomenon.

The advantage of FISH becomes critically important in diseases in which neoplastic cells fail to proliferate despite mitogenic activation,⁴³ whereas the existing metaphases represent normal cells and

therefore show a normal karyotype.⁴⁴ FISH, however, enables evaluation of both metaphase and interphase cells^{42, 45} and also certain abnormalities in malignant cells that cannot be detected by conventional cytogenetics. Because only specific probes can be used when FISH is performed, at present it remains a tool complementary to classic karyotype analysis.

In conclusion, we have demonstrated in this study that FISH is a sensitive method for the detection of trisomies 8 and 9 in patients with systemic mastocytosis. This pattern of involvement suggests that certain genes on these chromosomes have an influence on the initiation or progression of the disease. Our finding may also suggest a connection between mast cell and myeloid disorders. Clearly, larger studies will be necessary to verify the pathogenetic and clinical importance of these abnormalities in SMCD.

REFERENCES

1. Travis WD, Li C-Y, Yam LT, Bergstralh EJ, Swee RG. Systemic mast cell disease: analysis of 58 cases and literature review. *Medicine* 1988;67:345-68.
2. Metcalfe DD. Mastocytosis. In: Wyngaarden JB, Smith LH, Bennet JC, eds. *Cecil textbook of medicine*. Philadelphia: Saunders, 1992:1483-5.
3. Horny HP, Parwaresch MR, Lennert K. Bone marrow findings in systemic mastocytosis. *Hum Pathol* 1985;16:808-14.
4. Kirshenbaum AS, Rottem M, Metcalfe DD, Goff JP. The origin and development of murine and human mast cells. In: *The mast cell in health and disease*. Kaliner MA, Metcalfe DD, eds. New York: Marcel Dekker, 1993:203-17.
5. Razin E, Ihle JN, Seldin D, et al. Interleukin-3: a differentiation and growth factor for the mouse mast cell that contains chondroitin sulfate E proteoglycan. *J Immunol* 1984;132:1479-86.
6. Anderson DM, Lyman SD, Baird A, et al. Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. *Cell* 1990;63:235-43.
7. Nocka K, Buck J, Levi J, Besmer P. Candidate ligand for the c-kit transmembrane kinase receptor. KL, a fibroblast derived growth factor stimulates mast cell and erythroid progenitors. *EMBO J* 1990;9:3287-91.
8. Kirshenbaum AS, Goff JP, Dreskin SC, Irani AM, Schwartz LB, Metcalfe DD. IL-3-dependent growth of basophil-like cells and mast like cells from human bone marrow. *J Immunol* 1989;142:2424-9.
9. Mekori YA, Oh CK, Metcalfe DD. IL-3 dependent murine mast cells undergo apoptosis on removal of IL-3. Prevention of apoptosis by C-kit ligand. *J Immunol* 1993;151:3775-84.
10. Tsai M, Takashi T, Thompson H, et al. Induction of mast cell proliferation, maturation, and heparin synthesis by the rat c-kit ligand, stem cell factor. *Proc Natl Acad Sci USA* 1991;88:6382-6.
11. Kirshenbaum AS, Kessler SW, Goff JP, Metcalfe DD. Demonstration of the origin of human mast cells from

- CD34⁺ bone marrow progenitor cells. *J Immunol* 1991;146:1410-5.
12. Agis H, Wellheim M, Sperr WR, et al. Monocytes do not make mast cells when cultured in the presence of SCF. *J Immunol* 1993;151:4221-7.
 13. Nagata H, Worobec A, Oh C, Suzuki Y, Metcalfe DD. Identification of a point mutation of the proto-oncogene *c-kit* in patients with mastocytosis [Abstract]. *J Allergy Clin Immunol* 1995;95:176.
 14. Cooper AJ, Winkelmann RK, Wiltsie JC. Hematologic malignancies occurring in patients with urticaria pigmentosa. *J Am Acad Dermatol* 1982;7:125-9.
 15. Travis WD, Chin Yang Li, Lung TY, Bergstrale EJ, Sweet RG. Significance of systemic mast cell disease with associated hematologic disorders. *Cancer* 1988;6:965-72.
 16. Swolin B, Rodger S, Roupe G. Cytogenetic studies and in vitro colony growth in patients with mastocytosis. *Blood* 1987;70:1928-32.
 17. Yam LT, Yam CF, Li CY. Eosinophilia in systemic mastocytosis. *Am J Clin Pathol* 1980;73:48-54.
 18. Rege-Cambrin G, Mecucci C, Tricot G, et al. A chromosomal profile of polycythemia vera. *Cancer Genet Cytogenet* 1987;25:233-45.
 19. Diez-Martin JL, Graham DL, Pettitt RM, Dewald GW. Chromosome studies in 104 patients with polycythemia vera. *Mayo Clin Proc* 1991;66:287-99.
 20. Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high sensitivity fluorescence hybridization. *Proc Natl Acad Sci USA* 1986;83:2934-8.
 21. Kibbelaar RE, Mulder JWR, Dreef EJ, et al. Detection of monosomy 7 and trisomy 8 in myeloid neoplasia: a comparison of banding and fluorescent in situ hybridization. *Blood* 1993;82:904-13.
 22. Amiel A, Gaber E, Manor Y, et al. Fluorescence in situ hybridization for the detection of trisomy 8 and 9 in polycythemia vera patients. *Cancer Genet Cytogenet* 1995;79:153-6.
 23. Jankins RB, LaBeau M, Kraker WJ, et al. Fluorescent in situ hybridization: a sensitive method for trisomy 8 detection in bone marrow specimens. *Blood* 1992;79:3307-15.
 24. Lidor C, Frisch B, Gazit D, Gepstein R, Hallel T, Mekori YA. Osteoporosis as the sole presentation of bone marrow mastocytosis. *J Bone Miner Res* 1990;5:871-6.
 25. Westin J, Whalstrom J, Swoling B. Chromosome studies in untreated polycythemia vera. *Scand J Hematol* 1976;17:183-96.
 26. Swolin B, Weinfeld A, Westin J. A prospective long term cytogenetic study in polycythemia vera in relation to treatment and clinical course. *Blood* 1988;72:386-95.
 27. Bagby GC. The preleukemic syndrome. *Blood* 1988;2:194-205.
 28. Hagenmeijer A. Chromosome abnormalities in CML. In: Goldman JM, ed. *Bailliere's clinical hematology. Chronic myeloid leukemia*. London: Bailliere Tindall, 1987:963-81.
 29. Smith RE, Chelmowski MK, Szabo EJ. Myelofibrosis: a concise review of clinical and pathologic features and treatment. *Am J Hematol* 1988;29:174-80.
 30. Parker RI. Hematologic aspects of mastocytosis. II. Management of hematologic disorders in association with systemic mast cell disease. *J Invest Dermatol* 1991;96:52S-54S.
 31. Horny HP, Ruck M, Wehrman M, Kaiserling E. Blood findings in generalized mastocytosis: evidence of simultaneous occurrence of myeloproliferative disorders. *Br J Haematol* 1990;76:186-93.
 32. Lewis TP, Welborn JL, Meyers FJ, Levy NB, Roschak T. Mast cell disease followed by leukemia with clonal evolution. *Cancer Res* 1987;11:769-73.
 33. Horton MA, O'Brien HAW. Characterization of human mast cells in long term cultures. *Blood* 1983;62:1251-4.
 34. Denburg JA, Richardson M, Telizyn S, Bienenstock J. Basophil/mast cell precursors in human peripheral blood. *Blood* 1983;61:775-80.
 35. Lennert K, Parwaresch MR. Mast cells and mast cell neoplasia: a review. *Histopathology* 1979;3:349-63.
 36. Sagher F, Liban E, Ungar H, et al. Urticaria pigmentosa with bone involvement, mast cell aggregates in bone and myelosclerosis found at autopsy in a case dying of monocytic leukemia. *J Invest Dermatol* 1956;27:355-68.
 37. Cains LJ. Systemic mastocytosis. *Aust J Dermatol* 1972;13:111-3.
 38. Gisslinger H, Linkesch W, Fritz E, Ludwig H, Chott A, Radaszkiewicz TH. Long term interferon therapy for thrombocytosis in myeloproliferative diseases. *Lancet* 1989;1:634-7.
 39. Kloke O, Niederle N, Qiu JY, et al. Impact of interferon alpha induced cytogenetic improvement on survival in chronic myelogenous leukemia. *Br J Haematol* 1993;83:399-403.
 40. Kluin-Nelemans HC, Jansen JP, Breukelman H, et al. Response to interferon alpha-2b in a patient with systemic mastocytosis. *N Engl J Med* 1992;326:619-23.
 41. Strife A, Clarkson B. Biology of chronic myelogenous leukemia: Is discordant maturation the primary defect? *Semin Hematol* 1988;25:1-19.
 42. Lalkin A, Lishner M, Gaber M, et al. In situ hybridization: a simple and sensitive method for the detection of trisomy 12 in chronic lymphocytic leukemia. *Can Genet Cytogenet* 1993;70:21-4.
 43. Autio K, Elonen E, Teerenhovi L, Knuutila S. Cytogenetic and immunologic characterization of mitotic cells in chronic lymphocytic leukemia. *Eur J Hematol* 1990;39:289-294.
 44. Bird ML, Ueshina Y, Rowley JD, Haren JM, Vardiman JW. Chromosomal abnormalities in B-cell chronic lymphocytic leukemia and their clinical correlations. *Leukemia* 1989;3:182-91.
 45. Nederlof PM, Van der Flier S, Raap AK, et al. Detection of chromosome aberrations in interphase tumor nuclei by non-radioactive in situ hybridization. *Cancer Genet Cytogenet* 1989;42:87-98.