

Prognosis in adult indolent systemic mastocytosis: A long-term study of the Spanish Network on Mastocytosis in a series of 145 patients

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Background: Indolent systemic mastocytosis is a group of rare diseases for which reliable predictors of progression and outcome are still lacking.

Objective: Here we investigate the prognostic impact of the clinical, biological, phenotypic, histopathological, and molecular disease characteristics in adults with indolent systemic mastocytosis, who were followed using conservative therapy.

Methods: A total of 145 consecutive patients were prospectively followed between January 1983 and July 2008; in addition, from 1967 to 1983, 20 patients were retrospectively studied.

Results: Multivariate analysis showed that serum β 2-microglobulin ($P = .003$) together with the presence of mast/stem cell growth factor receptor gene (*KIT*) mutation in mast cells plus myeloid and lymphoid hematopoietic lineages ($P = .02$) was the best combination of independent parameters for predicting disease progression (cumulative probability of disease progression of $1.7\% \pm 1.2\%$ at 5-10 years and of $8.4\% \pm 5.0\%$ at 20-25 years). Regarding overall survival, the best predictive model included age >60 years ($P = .005$) and development of an associated clonal hematological non-mast cell disorder ($P = .03$) with a cumulative probability of death of $2.2\% \pm 1.3\%$ at 5 years and of $11\% \pm 5.9\%$ at 25 years.

Conclusions: Indolent systemic mastocytosis in adults has a low disease progression rate, and the great majority of patients have a normal life expectancy, with the presence of *KIT* mutation in all hematopoietic lineages and increased serum β 2-microglobulin the most powerful independent parameters for predicting transformation into a more aggressive form of the disease. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Mastocytosis, indolent systemic mastocytosis, long-term prognosis

Mastocytosis is a group of heterogeneous diseases characterized by an abnormal expansion and accumulation of mast cells (MCs) in different tissues. In indolent systemic mastocytosis (ISM), the symptoms and signs of the disease are usually related to the release of MC mediators and, to a lesser extent, to the tissue MC burden. The clonal nature of mastocytosis can be established in virtually all cases through demonstration of gain-of-function mutations involving the tyrosine kinase domain of c-kit in lesional skin and/or bone marrow (BM) cells.¹⁻⁶ This and other disease features have been proposed as consensus criteria for the diagnosis and classification of different subtypes of mastocytosis.⁷⁻⁹ However, current knowledge about the prognosis of mastocytosis and the most useful predictors for patient outcome remains rather limited, particularly for the indolent forms of the disease. This is mainly because of the low prevalence of the disease and the lack of prospective studies in large series of patients with a long follow-up. Furthermore, depending on the distribution of different types of mastocytosis, major differences exist on overall survival (OS) rates between distinct series. Accordingly, several studies in which a relatively high percentage of systemic mastocytosis (SM) associated with other clonal non-MC lineage diseases (clonal hematologic non-mast cell disorders [AHNMDs]) were included¹⁰⁻¹² show that prognosis of such cases mainly depends on the associated AHNMD and/or the indolent versus aggressive nature of the mastocytosis;¹³ limited information is provided about the specific disease features that could be useful to predict outcome in ISM.

Here we investigate the prognostic impact of the clinical, biological, phenotypic, histopathological, and molecular characteristics of ISM in 145 consecutive adult patients who were diagnosed and followed using conservative therapy by the Spanish Network on Mastocytosis (REMA). Our results show that patients with ISM have a long life expectancy, and the best combination of independent predictors for disease progression is serum β 2-microglobulin levels and the presence of *D816V KIT* mutation in all hematopoietic lineages.

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Supported by grants from the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (REMA G03/007, FIS050769, FIS060529, FIS061377, and RETICS RD06/0020/0035-FEDER); Comunidad Autónoma de Madrid (GR/SAL/0133/2004), Junta de Castilla y León (Grant SAN196/SA10/07); Junta de Comunidades de Castilla La Mancha (FISCAM 2007/36); and Fundación MMA. A.G.-M. is supported by a grant from FIS/FEDER (CP03/00035) and C.T. from the FCT of Portugal (SFRH/BD/17545/2004).

Disclosure of potential conflict of interest: L. Escribano has written a brochure on tryptase for Phadia Spain. The rest of the authors have declared that they have no conflict of interest.

Received for publication January 9, 2009; revised May 4, 2009; accepted for publication May 5, 2009.

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0091-6749/\$36.00

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doi:10.1016/j.jaci.2009.05.003

Abbreviations used

AHNMD: Clonal hematologic non-mast cell disorder
AML: Acute myeloblastic leukemia
BM: Bone marrow
ISM: Indolent systemic mastocytosis
MC: Mast cell
MDS: Myelodysplastic syndrome
OS: Overall survival
PB: Peripheral blood
PFS: Progression-free survival
REMA: Red Española de Mastocytosis
SM: Systemic mastocytosis
SSM: Smoldering systemic mastocytosis

METHODS**Patients**

A total of 145 consecutive patients with ISM, 63 men (43%) and 82 women (57%) with a median age at diagnosis of 37 years (16–72 years), who gave their informed consent to participate in this study according to the guidelines of the local ethical committees, were analyzed. Patients were prospectively followed at the REMA between January 1983 and July 2008. From 1967 to 1983, a total of 20 patients diagnosed with SM—as assessed by both skin and BM biopsy—were followed up in centers other than the REMA before referral and, thus, retrospectively studied. Patients were diagnosed and classified according to the classification and criteria used at the moment of initial diagnosis: from 1983 to 1991, cases were classified in nonaggressive versus aggressive forms^{14,15}; from 1991 to 2001, classification was made following the criteria established in a US workshop^{16,17}; finally, since 2001, World Health Organization guidelines for both diagnosis and classification,^{7,8} slightly modified with more recent criteria,⁹ were used. These later criteria were also retrospectively applied to all those patients whose diagnosis was made before 2001.

At the moment of referral to the REMA, and every 6 months, a complete clinical and physical work-up together with a routine peripheral blood (PB) count and differential and routine biochemistry were performed. Frozen serum samples from patients were available since 1993, and thus measurement of serum tryptase (CAP, Pharmacia Diagnostics, Sweden) was performed in all cases since 1993 on a 6-month basis. Furthermore, abdominal ultrasonography and/or computed tomography scan and bone densitometry x-ray of dorsal and lumbar vertebra and pelvis were also systematically performed when available at the hospital. The presence of either osteopenia or osteoporosis was defined following well established criteria^{18–20}; furthermore, the presence of patchy or diffuse bone sclerosis as assessed by x-ray of bones, computed tomography scan, and/or magnetic resonance imaging (when available at the hospital) was also recorded. Imaging studies were repeated every 2 years, except for cases with osteoporosis, in which follow-up studies were performed on a yearly basis. Skin biopsy samples were obtained in all cases showing cutaneous lesions, and skin sections were stained on a routine basis for hematoxylin-eosin and Giemsa, as well as for tryptase and/or c-kit using conventional immunocytochemical stains. In biopsies obtained before immunostaining for tryptase and c-kit was available, new sections from paraffin-embedded samples were cut and such stainings performed when they became standard practice. All samples were analyzed and reviewed by 3 independent experts (L.E., M.G.-C., and C.B.), strictly following the recently reported standards and recommendations.⁹ Diagnostic BM aspirate and biopsy were performed in 20 cases before referral to the REMA and at referral in all the remaining patients (n = 125); furthermore, in those patients whose BM study was performed before flow cytometry immunophenotypical analysis was available in 1992, a new BM study was performed. BM MC morphology was analyzed in all BM samples following previously defined criteria²¹; patient BM smears were stained by toluidine blue and May-Grünwald-Giemsa and analyzed by 3 independent observers (L.E., L.S.-M. and R.N.), in parallel with a total of 855 BM control samples, 57 of them corresponding to healthy subjects (data not shown). In addition, hematoxylin-eosin and Giemsa as well as tryptase and c-kit-stained patient BM sections were also studied and

evaluated by 3 independent observers (L.E., M.G.-C., and C.B.) following previously established criteria,^{22,23} in parallel with a group of 48 BM control samples. In 145 cases, immunophenotypical analysis of CD25 and/or CD2 expression on BM MC (and when present also in PB MC) was performed by using a multiparameter 4-color direct immunofluorescence technique according to consensus procedures and criteria previously defined by our group.^{24,25} From the 145 BM samples analyzed, 73 were studied in parallel in 2 different laboratories of the REMA for external quality control purposes. Detection of somatic activating codon Asp816 → Val *KIT* mutation (exon 17 of the *KIT* gene) or other *KIT* mutations was performed in genomic DNA^{6,26} from fluorescence-activated cell sorting–purified BM MCs (n = 95 cases); in addition, in 74 of these cases, *KIT* mutations were also analyzed, in other BM hematopoietic cell compartments (maturing neutrophil granulocytes, eosinophils, monocytes, lymphocytes, CD34⁺ cells, and nucleated red cells), by using highly purified, fluorescence-activated cell sorted cell populations as described elsewhere^{6,26} (purity of each cell fraction > 97%). Information about *KIT* mutation was prospectively available since 2002.

Treatment of patients included (1) careful counseling of patients and care providers; (2) avoidance of factors triggering acute mediator release and preventive measures in risk procedures such as general anesthesia and x-ray studies using contrast media, among others; (3) treatment of acute MC-mediator release including a training for self-administration of epinephrine in patients at risk for anaphylaxis; (4) treatment of chronic MC mediator release; and (5) availability of a 24-hour emergency system.

Treatment was carefully selected in each patient on the basis of the intensity and/or severity of clinical symptoms related to release of MC mediators. Different drugs (alone or in distinct combinations) were used following previously described criteria.^{27,28} Basically, therapy consisted of (1) MC stabilizers such as oral disodium cromolyn, (2) scheduled or at demand sedating H1 antihistamines (dexchlorpheniramine, hydroxyzine, and doxepin), and (3) scheduled or on-demand nonsedating H1 antihistamines depending on their availability throughout this study (cetirizine, levocetirizine, fexofenadine, ebastine, and desloratadine, among others), sometimes combined with a sedating antihistamine in highly symptomatic cases. In addition, other therapies were used for specific clinical situations; in cases with abdominal cramping and diarrhea unresponsive to disodium cromolyn alone, nonsteroidal anti-inflammatory agents (ie, ibuprofen or aspirin) were used and, in nonresponders, short cycles of low doses of prednisone (0.3 mg/kg/d) with progressively decreasing doses were used. In stress-induced anaphylaxis, a psychiatric work-up was performed, and adequate sedation and/or antidepressant therapy were used. For patients with osteoporosis requiring therapy, calcium supplements, vitamin D, and oral bisphosphonates (alendronate or risedronate) were used, since they were available. Cyto-reductive therapy in the absence of disease progression was used in 8 patients: (1) in 3 cases with severe osteoporosis and pathological fractures, IFN- α -2b alone (n = 1) or hydroxyurea followed by IFN- α -2b was used; (2) in 3 cases with severe MC mediator–related symptoms refractory to highly intensive antimediator therapy, either IFN- α -2b (n = 2) or hydroxyurea (n = 1) was used; and (3) in 1 patient who developed giant axillary mastocytomas, hydroxyurea was employed with a rapid response (Table I). In addition, in 1 woman with advanced dilated cardiomyopathy, a heart transplant was performed in June 2006,²⁹ 12 years after the diagnosis of ISM, and she is still under immunosuppressive therapy with cyclosporine, mycophenolate mofetil, and deflazacort.

End points

The primary end points of the study were disease progression and OS as defined by transformation into a more aggressive subtype of mastocytosis according to the World Health Organization criteria^{7,8} (aggressive SM, SM-AHNMD, or MC leukemia) and death, respectively.

In addition, patients who developed biological changes in the absence of disease progression were recorded. Biological changes were defined by at least 1 of the following (previously absent) events: (1) sustained increase of serum tryptase levels above 200 ng/mL, which could not be explained by other conditions such as acute myeloblastic leukemia (AML), myelodysplastic syndrome (MDS), a subset of hypereosinophilic

TABLE I. Cytoreductive therapy in ISM in the absence of disease progression

Case no./sex	Time from diagnosis (mo)	Indication for therapy	Type of therapy	Duration of therapy (mo)	Response	Adverse event(s)	Time from end of therapy (mo)
1/M	238	OF	IFN- α -2b*	13	Yes	No	8
2/M	104	MRS	IFN- α -2b†	47	Yes	No	99
3/M	1	MRS	IFN- α -2b*	13	No	D	39
4/M	215	OF	IFN- α -2b*	12	Yes	No	106
5/M	39	BP	HU‡	2	No	LT	8
6/F	178	OF	HU‡	3	No	No	17
6/F	184	OF	IFN- α -2b*	4	Yes	D	10
7/F	416	Giant axillary mastocytomas	HU‡	12	Yes	No	19
8/F	49	MRS	HU‡	7	No	No	20

BP, Severe bone pain secondary to patchy bone sclerosis; D, depression; F, female; HU, hydroxyurea; LT, liver toxicity; M, male; MRS, severe mast cell mediator-related symptoms refractory to intensive antimediator therapy; OF, osteoporosis with pathological bone fractures.

*1.5 million U 3 times/wk.

†2 million U/d.

‡Dose adjusted in each case on the basis of peripheral blood cell counts.

syndromes, or diffuse bone sclerosis²⁷⁻³¹; (2) development of osteoporosis, diffuse or patchy bone sclerosis, or presence of a pathological bone fracture associated to severe osteoporosis; (3) appearance of organomegalies, as detected by imaging techniques, in the absence of an impaired organ function, not attributable to other pathological conditions, or skin mastocytomas; and (4) development of eosinophilia ($>0.5 \times 10^9/L$) and/or monocytosis ($>1 \times 10^9/L$) not attributable to pathological conditions other than mastocytosis.

Statistical analyses

The Mann-Whitney *U* and χ^2 tests were used to assess the statistical significance of differences observed between groups for continuous and categorical variables, respectively.

Overall survival and progression-free survival (PFS) curves were estimated according to the Kaplan-Meier method and survival curves compared with the log-rank test. A multivariate Cox proportional hazards model was constructed with those variables showing independent predictive value; inclusion in the final model was determined by a backward stepwise process. *P* values $\leq .05$ were considered to be associated with statistical significance. For all statistical analyses, SPSS 15.0 (SPSS, Chicago, Ill) was used.

RESULTS

Overall, the 145 patients included in this study were followed for 1526 person-years (median, 9 years; range, 2 months to 40 years) since confirmation of diagnosis by BM studies. The most relevant clinical, biological, morphological, immunophenotypic, and molecular characteristics of the patients analyzed are summarized in Table II.

Disease progression

Among the 145 patients analyzed, 5 (3%) showed progression to a more aggressive form of the disease (Table III) after a median follow-up from diagnosis of 147 months (range, 61-329 months): 1 patient developed an ASM 27 years after diagnosis; 3 patients developed an AHNMD corresponding to an MDS, AML, and polycythemia vera, 19, 12, and 5 years after diagnosis of mastocytosis, respectively; and the other patient with ISM showed evolution to MCL after 5 years. The cumulative probability of disease progression in ISM was $1.7\% \pm 1.2\%$ at 5 and 10 years, $3.8\% \pm 2.4\%$ at 15 years, and $8.4\% \pm 5.0\%$ at 20 and 25 years. In the

univariate analysis, the following variables showed an adverse impact on PFS: age >60 years at diagnosis ($P = .03$), presence of 1 or more cytopenias ($P = .02$), increased serum $\beta 2$ -microglobulin ($P = .003$), and presence of D816V *KIT* mutation in all hematopoietic lineages ($P = .02$; Table IV; Fig 1, A-C). Multivariate analysis showed that serum $\beta 2$ -microglobulin levels ($P = .003$) together with the presence of D816V *KIT* mutation in all hematopoietic lineages ($P = .02$) was the most powerful combination of independent prognostic factors to predict PFS.

Biological progression

Thirty-nine of 145 patients (27%) showed biological changes suggesting biological progression with a median time from diagnosis of 86 months (range, 6-382 months). The median age at biological progression was 47 years (range, 25-76 years). Biological progression consisted of an increase in serum tryptase—in the absence of diffuse bone sclerosis—above 200 ng/mL in 4 patients (10%), appearance of monocytosis in 1 case (3%) and eosinophilia in 2 patients (5%); development of osteoporosis, diffuse bone sclerosis, and patchy bone sclerosis were found in 22 (56%), 4 (10%), and 5 (13%) patients, respectively; 4 cases (10%) had pathological fractures related to severe osteoporosis; 6 (15%) and 8 (21%) patients developed hepatomegaly and splenomegaly, respectively. Finally, 1 patient (3%) developed cutaneous mastocytomas. In 11 of the 39 patients who showed biological progression (28%), 2 or more biological progression events coincided.

In turn, 2 patients with ISM developed a smoldering systemic mastocytosis (SSM) 224 and 382 months after diagnosis, respectively. Interestingly, both patients still remain alive without evidence of progression to a more aggressive category of the disease 53 and 66 months after development of SSM.

Survival

Until the moment of closing this study, 7 patients (5%) had died after a median follow-up of 8 years (range, 2 months to 27 years). Among these patients, death was directly related to the MC disorder in only 2 cases; 1 corresponded to a woman with D816V

TABLE II. Clinical and laboratory characteristics of patients (n = 145)

Variable	No. of cases (%)	Variable	No. of cases (%)
Male	63/145 (43)	Eosinophilia ($>0.5 \times 10^9/L$)	10/145 (7)
Age at diagnosis >60 y	8/145 (6)	Monocytosis ($>1.0 \times 10^9/L$)	1/145 (1)
Pruritus	119/145 (82)	Serum tryptase >20 ug/L	101/145 (71)
Flushing	81/145 (56)	Serum tryptase >200 ug/L	7/145 (5)
Anaphylaxis	33/145 (23)	Serum tryptase >200 ug/L [‡]	2/145 (1)
Peptic symptoms	29/145 (20)	Serum LDH >240 IU/L	46/145 (33)
Abdominal cramping	43/145 (30)	SAP >140 IU/L	7/145 (5)
Diarrhea	51/145 (35)	SAP >140 IU/L [§]	2/145 (2)
Neuropsychiatric symptoms*	34/145 (23)	β 2-Microglobulin >2.5 mg/L	12/145 (9)
Skin lesions	131/145 (90)	Serum cholesterol <120 mg/dL	3/145 (2)
Hepatomegaly [†]	17/145 (12)	Serum vitamin B12 >1500 pg/mL	1/145 (1)
Splenomegaly [†]	12/145 (8)	Serum ferritin >200 ng/mL	11/145 (8)
Lymph node enlargement [†]	5/145 (3)	Abnormal BM MC morphology	131/145 (94)
Osteoporosis	26/145 (18)	Compact BM MC aggregates¶	119/145 (84)
Patchy bone sclerosis	6/145 (4)	Aberrant MC immunophenotype #	133/145 (98)
Diffuse bone sclerosis	9/145 (6)	Percentage of BM MC $\geq 1\%$ #	4/145 (3)
Osteolysis	3/145 (2)	Promastocytes**	1/145 (1)
Bone fracture	5/145 (3)	Circulating PB MC ^{††}	2/145 (1)
Anemia (Hb <12 g/dL)	1/145 (1)	$>30\%$ BM MC infiltrate¶	5/145 (3)
Leukopenia ($<3.5 \times 10^9/L$)	1/145 (1)	D816V <i>KIT</i> mutation in MC	91/95 (96)
Platelets $<100 \times 10^9/L$	1/145 (1)	D816V <i>KIT</i> mutation in myeloid plus lymphoid cell lineages	7/74 (9.5)

LDH, Lactate dehydrogenase; SAP, Serum alkaline phosphatase.

Clinical and laboratory data in the table correspond to the first study available for each case.

*Decreased attention span, difficulty in concentrating, forgetfulness, irritability, depression, poor motivation, confusion, anger, anxiety, and sleepiness.

[†]As assessed by abdominal ultrasonography and/or computed tomography scan.

[‡]In the absence of diffuse bone sclerosis, because development of diffuse bone sclerosis is associated with a progressive increase in serum tryptase not related to MC burden.³¹

[§]In the absence of bone lesions.

||For details, see text.

¶In tryptase and c-kit-stained BM sections.

#As assessed by flow cytometry.

**Mast cells with bilobed or polylobed nuclei.²¹

^{††}The detection limit of $<1 \times 10^{-5}$ ($<0.001\%$).

KIT mutation in all hematopoietic lineages who developed a chemorefractory CD117⁺ AML and died 12 years after diagnosis of ISM; the second patient developed an MDS with CD117⁺D816V[−] blast cells and died 27 years after diagnosis of ISM (8 years after developing MDS; Table V). The remaining 5 patients died of causes apparently not related to mastocytosis 2 months and 1, 5, 8, and 21 years after diagnosis of ISM. The median age at death was of 71 years (range, 37-80 years; Table V).

In the univariate analysis, the following variables showed an influence on OS: age at diagnosis >60 years ($P < .001$), increased serum alkaline phosphatase ($P = .009$), increased serum vitamin B12 ($P = .001$), and development of an AHNMD ($P = .01$; Table VI). Multivariate analysis showed that the combination of independent variables with the highest predictive value for a shorter OS included age at diagnosis >60 years ($P = .005$), increased serum alkaline phosphatase ($P = .03$), and development of an AHNMD ($P = .03$; Table VI; Fig 1, D-G). The cumulative probability of death in patients with ISM was of $2.2\% \pm 1.3\%$ at 5 years, $3.4\% \pm 1.7\%$ at 10 years, $5.5\% \pm 2.7\%$ at 15 and 20 years, and $11\% \pm 5.9\%$ at 25 years.

DISCUSSION

In the current study, an in-depth analysis of prognosis and predictors of outcome is performed in a cohort of 145 adults with ISM and a long follow-up by using homogeneous criteria for patient diagnosis and conservative management at the REMA. In

contrast with most previously published series, which include relatively high percentages of poor prognosis forms of mastocytosis such as SM-AHNMD¹⁰⁻¹² or ASM,¹³ here only ISM adult cases were analyzed, allowing for the first time evaluation of long-term outcome of these patients after a median follow-up of 9 years.

From the prognostic point of view, our results show that despite the relatively high frequency of signs of biological progression, transformation of ISM to a more aggressive form of the disease was an infrequent event restricted to a minority of ISM cases. Of note, among patients with ISM, serum β 2-microglobulin together with the presence of D816V *KIT* mutation in all hematopoietic lineages represented the best combination of independent parameters for predicting disease progression. To the best of our knowledge, this is the first report in which predictors of progression are identified for adult ISM. Despite this, a provisional subvariant of ISM considered to have a more aggressive behavior, SSM, has been defined recently.⁷⁻⁹ Interestingly, we found evolution of ISM to SSM to be rather infrequent and, despite previous reports,^{7,8} to have no clear impact on the prognosis of the disease, at least with the follow-up time reported here. Thus, our results indicate that in ISM, more attention should be paid to the presence of D816V *KIT* mutation in all hematopoietic lineages and serum β 2-microglobulin. Interestingly, from 1995 to 2008, a total of 18 patients with ASM were referred to the REMA (data not shown), 13 of them with skin lesions; 9 of these 13 cases (70%) evolved from an ISM to ASM after a period of between 4 and 44 years

TABLE III. Demographics and disease-related parameters at diagnosis and at progression in those 5 patients who showed disease progression

Variable	Patient no.									
	1		2		3		4		5	
	Diagnosis	Progression	Diagnosis	Progression	Diagnosis	Progression	Diagnosis	Progression	Diagnosis	Progression
Age	56	62	33	61	64	70	51	70	25	37
Palpable hepatomegaly	No	Yes	Yes	Yes	No	Yes	No	No	No	No
Palpable splenomegaly	No	Yes	No	Yes	No	No	No	No	No	No
Lymphadenopathy*†	No	Yes	No	Yes	No	No	No	No	No	No
Bone lesion	No	DBS	O	O	No	No	O	O	No	No
Cytopenia(s)	No	Yes	No	No	No	No	Yes	Yes	No	Yes
Serum tryptase (ug/L)	52	1500	104	197	140	159	67	50.8	188	157
Serum β 2-M (ng/mL)	1.8	5.8	2.8	4.5	9.5	5.5	2.0	2.6	3.3	3.9
Vitamin B12 (pg/mL)	713	2,700	272	408	855	622	250	323	331	311
Promastocytes‡	Yes	Yes	No	Yes	No	No	No	No	No	Yes
>30% BM MC infiltrate	No	Yes	No	No	No	No	No	No	Yes	No
Multilineal D816V <i>KIT</i> mutation	M, L		M, L		M		No		M, L, blasts	
Time from diagnosis to progression (y)	5		27		5		19		12	
Biologic progression	Yes		Yes		No		No		No	
Type of disease progression	ISM to ASM		ISM to ASM		SM-AHNMD (PV)		SM-AHNMD (MDS)		SM-AHNMD (AML)	
Therapy after progression	IFN- α -2b, HU		IFN- α -2b, HU		HU		None		CT	
Death	No		No		No		Yes		Yes	
Time from progression (mo)	14		2		61		94		4	

ASM, Aggressive systemic mastocytosis; β 2-M, β 2-microglobulin; CT, chemotherapy; DBS, diffuse bone sclerosis; HU, hydroxyurea; L, lymphoid lineage; M, myeloid lineage; MDS, myelodysplastic syndrome; O, osteoporosis; PV, polycythemia vera.

* ≥ 2 cm as assessed by computed tomography scan and/or abdominal ultrasonography.

†Progressive abdominal lymphadenopathy (>4 cm).

‡Mast cells with bilobed or polylobed nuclei.²¹

TABLE IV. Univariate and multivariate analysis of prognostic factors associated with disease progression

Variable	Disease progression				
	Univariate analysis			Multivariate analysis	
	Percentage of cases	RR (CI)	P value	HR (CI)	P value
Age at diagnosis >60 y	Yes/no	20/5.7	13.18 (1.16-149.6)/1.00	.03	NS
Serum β 2-M*	(ng/mL)	NA	1.65 (1.19-2.29)/1.00	.003	1.90 (1.24-2.92)/1.00
Multilineal D816V <i>KIT</i> mutation†	Yes/no	60/5.8	8.01 (1.31-49.0)/1.00	.02	13.3 (1.42-123.8)/1.00

NA, Not applicable; NS, not statistically significant ($P \geq .05$); RR, relative risk.

*Serum β 2-microglobulin (ng/mL); serum β 2-microglobulin did not show an impact on overall survival).

†In all hematopoietic lineages.

since the onset of skin lesions. Of note, in 8 of 8 cases studied, the *KIT* mutation was detected in BM MCs as well as all myeloid and lymphoid hematopoietic cell lineages, further supporting the stem cell origin of the disease^{4,32} and the relevance of the study of *KIT* mutations in all hematopoietic cell lineages^{6,26} as a predictor of outcome in ISM. Further studies investigating the potential association between multilineage involvement of myeloid and lymphoid cells^{4,6} and the positivity for the *KIT* mutation in PB leukocytes^{4,6} are required to determine whether the complex *KIT* mutational analyses of multiple cell populations performed in our study could be avoided.

Previous reports have suggested the occurrence of a frequent association between mastocytosis and other hematologic malignancies,^{33,34} and it has been speculated that patients with SM are at a high risk (10% to 30%) of developing myeloid malignancies.³⁵ In contrast, our experience suggests that such evolution should be considered an exceptional event in ISM. In our series, only 4 ISM cases developed a myeloid malignancy (an AML, an MDS, a polycythemia vera, and a MCL); of note, in those 2 cases developing AML and MCL, the D816V *KIT* mutation, apart from being present in MC, was also positive in highly purified CD34⁺ hematopoietic precursors, myeloid and lymphoid

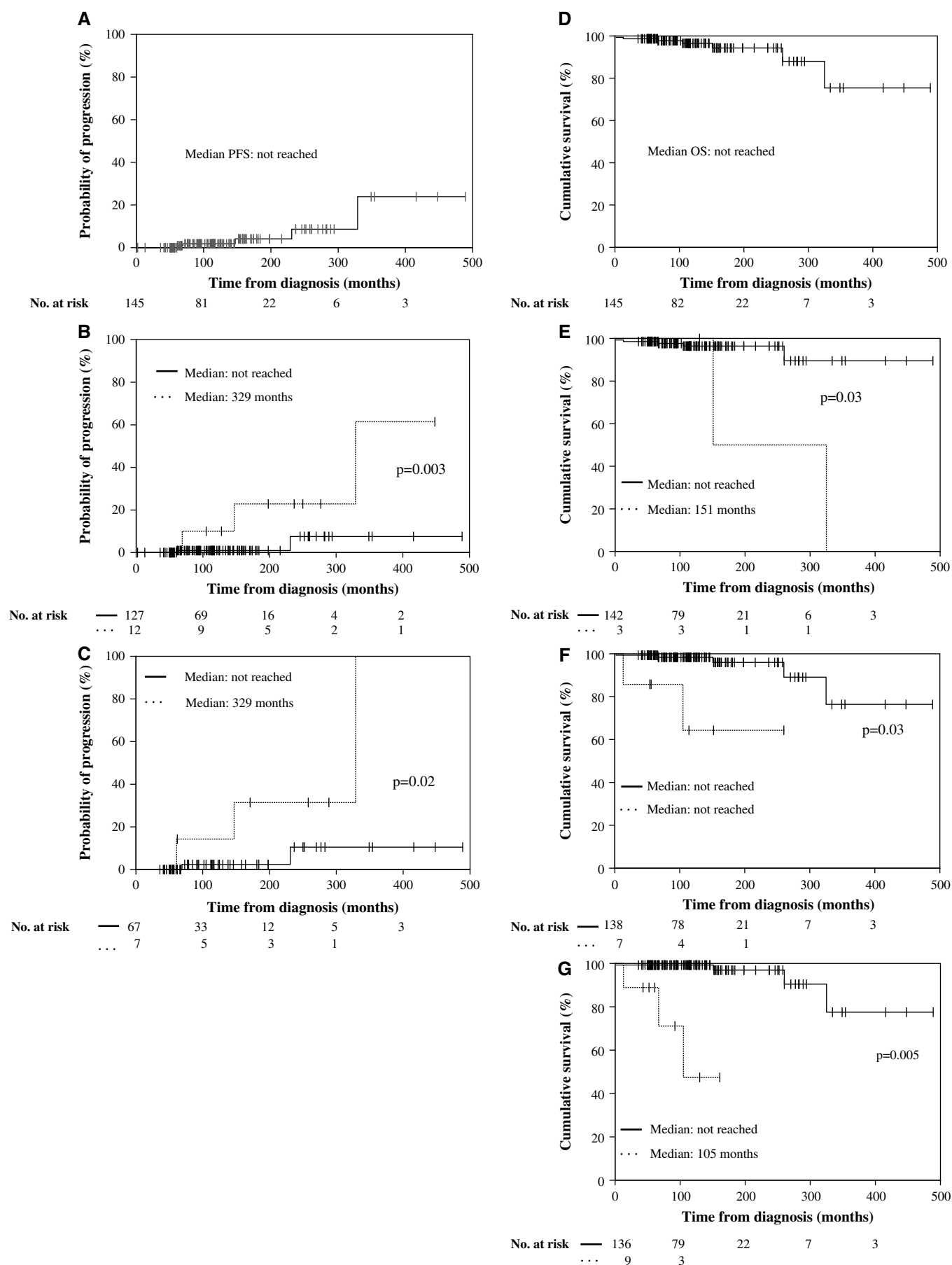


FIG 1. Progression-free survival (PFS) and overall survival (OS) in mastocytosis (n=145).

TABLE V. Causes of death for individual patients

Patient characteristics	Patient no.						
	1	2	3	4	5	6	7
Sex	F	M	M	M	F	M	M
Age at diagnosis (y)	51	69	72	71	25	40	51
Disease progression	No	No	No	No	Yes	No	Yes
Cause of death	Breast cancer	Prostatic cancer	AMI	Septic shock	AML	AMI	MDS
Age at death	51	71	78	80	37	62	78
Time from diagnosis (mo)	2	13	67	105	151	260	325

AMI, Acute myocardial infarction; F, female; M, male.

TABLE VI. Univariate and multivariate analysis of prognostic factors associated with OS

Variable	OS					
	Univariate analysis			Univariate analysis		
	Percentage of deaths	RR (CI)	P value	HR (CI)	P value	
Age at diagnosis >60 y	Yes/no	43/4	33.4 (5.40-206.5)/1.00	<.001	43.8 (3.08-621.6)/1.00	.005
Increased SAP*	Yes/no	29/4	9.73 (1.77-53.4)/1.00	.009	10.9 (1.24-95.1)/1.00	.03
Vitamin B12 >1500 pg/mL	Yes/no	17/0	53.2 (4.75-595.0)/1.00	.001		NS
Progression to AHNMD	Yes/no	29/1	8.48 (1.50-47.8)/1.00	.01	8.96 (1.12-71.8)/1.00	.03

NS, Not statistically significant ($P \geq .05$); RR, relative risk.

*Serum alkaline phosphatase >140 IU/L.

cells, suggesting the occurrence of D816V *KIT* mutations in all hematopoietic lineages in both patients, as sporadically mentioned in the literature for individual ISM cases evolving to a more aggressive subtype of the disease^{6,36-40}; however, to establish the impact of presence of D816V *KIT* mutation in all hematopoietic lineages as a predictor of outcome in ISM, longer follow-up of cases accomplishing this characteristic is mandatory.

In a retrospective study in which 58 proved cases of SM were analyzed, Travis et al¹¹ found only 46.5% to remain alive after a median follow-up of 7.6 years. In another study from the same institution in 40 SM cases (24 ISM and 16 ASM), 8 patients (20%) died (7 patients with ASM died of MS-related complications and 1 of an unknown cause), none corresponding to ISM cases.¹³ Among the patients studied here, only a minority ($n = 7/145$) of patients with ISM had died after a significantly longer follow-up than previously reported^{11,13}; moreover, in only 2 of these cases, death was directly related to mastocytosis because of development of an AML and MDS, respectively.

The specific subtype of mastocytosis, anemia, increased serum alkaline phosphatase, and the degree of BM involvement (as assessed by histopathology) have been previously identified as the most informative parameters for predicting OS of mastocytosis as a whole.^{11-13,41} Nevertheless, no specific information about the predictors of outcome in ISM is currently available. Here, we confirm the association of these parameters with OS of patients with ISM and show for the first time that from them, only age at diagnosis >60 years, increased serum alkaline phosphatase, and development of an AHNMD had independent predictive value for OS.

In summary, our results clearly show that patients with ISM treated with conservative therapy have both a low disease progression rate and long life expectancy, which should prevent

their recruitment to trials with cytoreductive or novel targeted drugs without appropriate selection of patients, on the basis of definitive signs of aggressiveness of disease; an exception could be those few patients with uncontrolled mediator-related symptoms and a clearly increased MC burden who may benefit in the future from well studied MC-targeting drugs with no potential mutagenicity. Because of the impact of the presence of D816V *KIT* mutations in all hematopoietic lineages on disease progression, careful follow-up is mandatory, particularly in patients with long disease duration.

Clinical implications: Patients with indolent systemic mastocytosis treated with conservative therapy have a normal life expectancy. The presence of *KIT* mutations in all hematopoietic lineages is strongly associated with disease progression and indicates the need for periodic follow-up.

REFERENCES

- Nagata H, Worobec AS, Oh CK, Chowdhury BA, Tannenbaum S, Suzuki Y, et al. Identification of a point mutation in the catalytic domain of the protooncogene *c-kit* in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. *Proc Natl Acad Sci U S A* 1995;92:10560-4.
- Longley BJ, Tyrrell L, Lu SZ, Ma YS, Langley K, Ding TG, et al. Somatic *c-KIT* activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. *Nat Genet* 1996;12:312-4.
- Longley BJ Jr, Metcalfe DD, Tharp M, Wang XM, Tyrrell L, Lu SZ, et al. Activating and dominant inactivating *c-KIT* catalytic domain mutations in distinct clinical forms of human mastocytosis. *Proc Natl Acad Sci U S A* 1999;96:1609-14.
- Yavuz AS, Lipsky PE, Yavuz S, Metcalfe DD, Akin C. Evidence for the involvement of a hematopoietic progenitor cell in systemic mastocytosis from single-cell analysis of mutations in the *c-kit* gene. *Blood* 2002;100:661-5.
- Pardanani A, Reeder T, Li CY, Tefferi A. Eosinophils are derived from the neoplastic clone in patients with systemic mastocytosis and eosinophilia. *Leuk Res* 2003;27:883-5.

6. Garcia-Montero AC, Jara-Acevedo M, Teodosio C, Sanchez ML, Nunez R, Prados A, et al. KIT mutation in mast cells and other bone marrow haematopoietic cell lineages in systemic mast cell disorders: a prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. *Blood* 2006;108:2366-72.
7. Valent P, Horny H-P, Escribano L, Longley BJ, Li CY, Schwartz LB, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. *Leuk Res* 2001;25:603-25.
8. Valent P, Horny H-P, Li CY, Longley BJ, Metcalfe DD, Parwaresch MR, et al. Mastocytosis (mast cell disease). In: Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. *World Health Organization (WHO) classification of tumours: pathology & genetics: tumours of haematopoietic and lymphoid tissues*. Lyon, France: IARC Press; 2001. p. 291-302.
9. Valent P, Akin C, Escribano L, Fodinger M, Hartmann K, Brockow K, et al. Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. *Eur J Clin Invest* 2007;37:435-53.
10. Horny HP, Parwaresch MR, Lennert AK. Bone marrow findings in systemic mastocytosis. *Hum Pathol* 1985;16:808-14.
11. Travis WD, Li CY, Bergstralh EJ, Yam LT, Sweet RG. Systemic mast cell disease: analysis of 58 cases and literature review. *Medicine* 1988;67:345-68.
12. Lawrence JB, Friedman BS, Travis WD, Chinchilli VM, Metcalfe DD, Gralnick HR. Hematologic manifestations of systemic mast cell disease: a prospective study of laboratory and morphologic features and their relation to prognosis. *Am J Med* 1991;91:612-24.
13. Pardanani A, Baek JY, Li CY, Butterfield JH, Tefferi A. Systemic mast cell disease without associated hematologic disorder: a combined retrospective and prospective study. *Mayo Clin Proc* 2002;77:1169-75.
14. Lennert K, Parwaresch MR. Mast cell and mast cell neoplasia: a review. *Histopathology* 1979;3:349-65.
15. Parwaresch MR, Horny HP, Lennert K. Tissue mast cells in health and disease. *Pathol Res Pract* 1985;179:439-61.
16. Metcalfe DD. Classification and diagnosis of mastocytosis: current status. *J Invest Dermatol* 1991;96:2S-4S.
17. Metcalfe DD. Clinical advances in mastocytosis: an interdisciplinary roundtable discussion. *J Invest Dermatol* 1991;96(suppl):1S-6S.
18. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: report of a WHO Study Group. *World Health Organ Tech Rep Ser* 1994;843:1-129.
19. Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. *WHO Study Group. Osteoporos Int* 1994;4:368-81.
20. Miller PD. Guidelines for the diagnosis of osteoporosis: T-scores vs fractures. *Rev Endocr Metab Disord* 2006;7:75-89.
21. Sperr W, Escribano L, Jordan JH, Scherthaner GH, Kundi M, Horny H-P, et al. Morphologic properties of neoplastic mast cells: delineation of stages of maturation and implication for cytological grading of mastocytosis. *Leuk Res* 2001;25:529-36.
22. Horny HP, Sillaber C, Menke D, Kaiserling E, Wehrmann M, Stehberger B, et al. Diagnostic value of immunostaining for tryptase in patients with mastocytosis. *Am J Surg Pathol* 1998;22:1132-40.
23. Horny HP, Valent P. Diagnosis of mastocytosis: general histopathological aspects, morphological criteria, and immunohistochemical findings. *Leuk Res* 2001;25:543-51.
24. Escribano L, Orfao A, Diaz-Agustin B, Villarrubia J, Cervero C, Lopez A, et al. Indolent systemic mast cell disease in adults: immunophenotypic characterization of bone marrow mast cells and its diagnostic implications. *Blood* 1998;91:2731-6.
25. Escribano L, Diaz-Agustin B, López A, López RN, García-Montero A, Almeida J, et al. Immunophenotypic analysis of mast cells in mastocytosis: when and how to do it. *Proposals of the Spanish Network on Mastocytosis (REMA)*. *Cytometry* 2004;58B:1-8.
26. Sotlar K, Escribano L, Landt O, Möhrle S, Herrero S, Torrelo A, et al. One-step detection of c-kit point mutations using PNA-mediated PCR-clamping and hybridization probes. *Am J Pathol* 2003;162:737-46.
27. Sperr WR, Jordan JH, Baghestanian M, Kiener HP, Samorapoompichit P, Semper H, et al. Expression of mast cell tryptase by myeloblasts in a group of patients with acute myeloid leukemia. *Blood* 2001;98:2200-9.
28. Sperr WR, Stehberger B, Wimalaz F, Baghestanian M, Schwartz LB, Kundi M, et al. Serum tryptase measurements in patients with myelodysplastic syndromes. *Leuk Lymphoma* 2002;43:1097-105.
29. Sperr WR, Hauswirth AW, Valent P. Tryptase a novel biochemical marker of acute myeloid leukemia. *Leuk Lymphoma* 2002;43:2257-61.
30. Klion AD, Noel P, Akin C, Law MA, Gilliland DG, Cools J, et al. Elevated serum tryptase levels identify a subset of patients with a myeloproliferative variant of idiopathic hypereosinophilic syndrome associated with tissue fibrosis, poor prognosis, and imatinib responsiveness. *Blood* 2003;101:4660-6.
31. Kushnir-Sukhov NM, Brittain E, Reynolds JC, Akin C, Metcalfe DD. Elevated tryptase levels are associated with greater bone density in a cohort of patients with mastocytosis. *Int Arch Allergy Immunol* 2006;139:265-70.
32. Kocabas CN, Yavuz AS, Lipsky PE, Metcalfe DD, Akin C. Analysis of the lineage relationship between mast cells and basophils using the *c-kit* D816V mutation as a biologic signature. *J Allergy Clin Immunol* 2005;115:1155-61.
33. Travis WD, Li CY, Yam LT, Bergstralh EJ, Sweet RG. Significance of systemic mast cell disease with associated hematologic disorders. *Cancer* 1988;62:965-72.
34. Travis WD, Li C-Y, Bergstralh EJ. Solid and hematologic malignancies in 60 patients with systemic mast cell disease. *Arch Pathol Lab Med* 1989;113:365-8.
35. Sperr WR, Horny HP, Lechner K, Valent P. Clinical and biologic diversity of leukemias occurring in patients with mastocytosis. *Leuk Lymphoma* 2000;37:473-86.
36. Akin C, Fumo G, Yavuz AS, Lipsky PE, Neckers L, Metcalfe DD. A novel form of mastocytosis associated with a transmembrane c-kit mutation and response to imatinib. *Blood* 2004;103:3222-5.
37. Escribano L, García-Montero A, Núñez-López R, López-Jiménez J, Almeida J, Prados A, et al. Systemic mastocytosis associated with acute myeloid leukemia: case report and implications for disease pathogenesis. *J Allergy Clin Immunol* 2004;114:28-33.
38. Tang X, Boxer M, Drummond A, Ogston P, Hodgins M, Burden AD. A germline mutation in *KIT* in familial diffuse cutaneous mastocytosis - art. no. e88. *J Med Genet* 2004;41:1189-93.
39. Hartmann K, Wardelmann E, Ma YS, Merkelbach-Bruse S, Preussner LM, Woolery C, et al. Novel germline mutation of *KIT* associated with familial gastrointestinal stromal tumors and mastocytosis. *Gastroenterology* 2005;129:1042-6.
40. Zhang LY, Smith ML, Schultheis B, Fitzgibbon J, Lister TA, Melo JV, et al. A novel K5091 mutation of *KIT* identified in familial mastocytosis: in vitro and in vivo responsiveness to imatinib therapy. *Leuk Res* 2006;30:373-8.
41. Horan RF, Austen KF. Systemic mastocytosis: retrospective review of a decade's clinical experience at the Brigham and Women's Hospital. *J Invest Dermatol* 1991;96:5S-13S.