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Chronic mast cell leukemia (MCL) with *KIT* S476I: a rare entity defined by leukemic expansion of mature mast cells and absence of organ damage

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Abstract

Mast cell leukemia (MCL) is a rare, life-threatening malignancy defined by a substantial increase in neoplastic mast cells (MCs) in bone marrow (BM) smears, drug-resistance and a poor prognosis. In most patients, the survival-time is less than 1 year. However, exceptional cases may present with a less malignant course. We report on a 49-year-old female patient with MCL

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Contributions

P.V. and M.A. designed the study and wrote the paper. S.C.R., L.M. and H.P.H. contributed histomorphological, histopathological and immunohistochemical studies. G.H. and C.M. provided molecular studies and *KIT* sequencing analyses. P.V., J.B., M.S. and M.A. contributed patients and provided patient-derived material. B.P. performed *in vitro* proliferation studies using patient-derived cells and *KIT*-targeting tyrosine kinase inhibitors. G.E. and P.B. performed flow cytometry studies. P.V., P.B., and M.A. contributed cytomorphological studies. All authors approved the final version of the manuscript.

Disclosures

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diagnosed in 2013. In February 2013, first symptoms, including flushing, headache and diarrhoea, were recorded. In addition, mild anemia was detected. The disease was characterized by a massive increase in well-granulated, mature and often spindle-shaped MCs (80%) in BM smears. The serum tryptase level amounted to 332 ng/mL. Like in most other MCL patients, no skin lesions were detected. However, unlike in other patients, tryptase levels remained stable and no other signs or symptoms of MCL-induced organ damage were found. Sequencing studies revealed an isolated S476I point-mutation in *KIT* but no mutation in codon 816. The patient received histamine receptor blockers, but refused cytoreductive therapy. After 9 months, still no progression or organ damage was detected. However, progression with transformation to acute MCL occurred after 12 months. We propose that the chronic type of MCL with stable conditions, absence of organ damage and a mature MC morphology, is recognized as a distinct entity that should be distinguished from the acute variant of MCL.

Keywords

Mast cells; mastocytosis; *KIT*; mast cell leukemia

Introduction

Systemic mastocytosis (SM) is a hematopoietic disease characterized by expansion of tissue mast cells (MCs) in various organ systems, including the bone marrow (BM), liver and spleen [1–4]. Despite recurrent mutations in codon 816 of the *KIT* protooncogene, SM is an extremely heterogeneous disease, ranging from a completely indolent course to life-threatening disease-variants with short survival-times [1–4]. According to the proposed classification of the World Health Organization (WHO) and the EU-US consensus group, patients with advanced SM can be divided into aggressive SM (ASM), SM with an associated clonal hematologic non-MC-lineage disease (SM-AHNMD) and MC leukemia (MCL) [5–7].

MCL is defined by leukemic expansion of immature MCs in various organs, including the BM, liver, and spleen [1–10]. As per definition, the BM smear in MCL contains an excess of immature neoplastic MCs representing at least 20% of all nucleated BM cells [5–7]. In most cases, these MCs are resistant against various targeted drugs and conventional anti-neoplastic drugs [8–13]. In some of these patients, circulating MCs are found and comprise more than 10% of white blood cells [5–7]. In a majority of MCL patients, however, the aleukemic variant of MCL with less than 10% circulating MCs is diagnosed [5–7]. Regardless of the type of MCL, most patients are considered to have a poor prognosis and a short survival-time, lasting for a few months only. More recently, however, a few reports have described patients with MCL who survived more than 12 months or even several years [14,15]. It remains unknown whether these patients are suffering from a less aggressive type of MCL or were diagnosed at an early (pre-malignant) stage of their disease.

We here report on a patient with chronic MCL who presented with a huge burden of neoplastic MCs, but did not develop organ damage within the first 9 months. Most of the

neoplastic MCs were found to be rather mature and well-granulated cells, thereby contrasting the morphology of MCs in the more aggressive variant of MCL.

Case Report and Methods

Case Report

A 49-year-old female patient was referred in June 2013 because of recurrent episodes of flushing, headache and diarrhea. The symptoms had been recorded during the last 5 months (since February 2013) and increased in intensity over time. She had been diagnosed as suffering from Psoriasis vulgaris some years ago. In May 2013, she presented with a rib fracture without known osteoporosis (normal T score) and without visible bone lesions. Physical examination showed no typical skin lesions, but skin flushing with dermographism. No splenomegaly or lymphadenopathy was found. Blood counts revealed mild anemia and leukocytopenia. The hemoglobin concentration was 11.0 g/dL, WBC 2,800/ μ L, and the platelet count was 236,000/ μ L. The differential count was normal, with 58% neutrophils and 2% eosinophil granulocytes. A bone marrow (BM) biopsy and aspiration were performed. Unexpectedly, the BM smear revealed MCL. In fact, large clusters and sheets of rather mature-appearing, well-granulated and in part spindle-shaped MCs were detected. These MCs were found to comprise 80% of all nucleated cells on BM smears. As assessed by flow cytometry, these MCs expressed CD13, CD25, CD30, CD33, CD44, and KIT (CD117), but did not express CD34. No *KIT* mutation at codon 816 was detectable. Histologic and immunohistochemical investigations confirmed the diagnosis of MCL, with an infiltration grade of 75% in tryptase-stained BM sections. The serum tryptase level was 332 ng/mL. Despite the diagnosis MCL and despite extensive information about the severity of the disease the patient refused cytoreductive therapy.

Laboratory Investigations, Staging and Follow-Up

Routine examinations were performed at the time of diagnosis and in the follow up, according to generally accepted recommendations and standards [5,9,16–21]. The patient provided written informed consent before physical examination was performed and BM and blood samples were obtained. A sonographic examination of the abdomen showed a few cystic (non-specific) lesions in the liver, but no hepatomegaly, no ascites, no splenomegaly (12.8 cm in diameter) and no lymphadenopathy. In addition, no hypalbuminemia and no signs of malabsorption were recorded. In the follow-up, serial determinations of laboratory parameters, including blood counts, differential counts, serum chemistry, and the serum tryptase level, were performed.

Examination of Bone Marrow (BM) Smears

BM aspirate smears were obtained from the iliac crest at diagnosis and at the time of progression (March 2014). Wright-Giemsa-stained BM smears were examined for the presence and percentage of MCs, blast cells, and signs of dysplasia. At least 500 nucleated cells were counted to obtain percentage counts. MC types were classified according to published criteria [5,9,19]. BM smears from 6 other patients with (the acute form of) MCL were also examined in this study, and the numbers and percentage counts of MCs were compared among patients.

Histologic and immunophenotypic analyses of the BM

Histologic and immunohistochemical examinations were performed at diagnosis and at the time of progression (March 2014) using formalin-fixed, paraffin-embedded BM biopsy sections. The BM sections were stained with antibodies against CD117 (KIT), tryptase, chymase, CD2, CD25, CD30, CD34 and CD52 (Supplementary Table S1).

Histopathological and immunohistochemical studies were performed according to generally accepted recommendations and standard [2,7,20,21].

Other BM Examinations

Multicolor flow cytometry was performed according to published techniques [17] using fluorochrome-conjugated monoclonal antibodies (mAb) against CD2, CD25, CD30, CD34, CD45, CD52, and CD117. A specification of antibodies used in this study is shown in Supplementary Table S2. Isolated BM cells were also examined by conventional karyotyping and fluorescence in situ hybridization (FISH). BM studies were performed according to generally accepted standards [15,18–21].

Molecular Studies

In all 6 patients examined, BM and blood cells were tested for the presence of codon 816 mutations according to published methods [18,22,23]. In the patient with chronic MCL, all coding exons of the *KIT* gene were analyzed by Sanger sequencing. In addition, cells were subjected to multiplex PCR and conventional PCR to screen for leukemia-related translocations and point mutations, including *BCR/ABL*, *FIP1L1/PDGFR*, *PML/RAR*, *AML1/ETO* and *JAK2 V617F* as well as *NPM1* and *FLT3* mutations.

Analysis of drug effects on *in vitro* proliferation of neoplastic cells

In order to determine the effects of various KIT-targeting drugs, *in vitro* drug-testing experiments were performed. Aspirated BM cells were obtained at the time of progression (March 2014) and were layered over Ficoll to obtain mononuclear cells (MNC). These MNC were found to contain >90% MCs. Isolated MCs were incubated with various concentrations (each 0.01–5 μ M) of imatinib, nilotinib, dasatinib (ChemieTek, Indianapolis, IN, USA) or PKC412 (midostaurin) (LC Laboratories, Woburn, MA, USA) at 37°C for 48 hours. Thereafter, uptake of ^3H -thymidine was determined according to a published protocol [24,25]. The study was approved by the local ethics committee of the Medical University of Vienna. The patients provided written informed consent before material was obtained and *in vitro* studies were performed.

Results

Clinical Course

At the time of diagnosis, the patient was hospitalized and observed for the development of signs of an acute leukemia. However, the clinical condition and all laboratory parameters remained stable. The patient was treated with histamine receptor antagonists (desloratidine, ranitidine). Otherwise she refused any cytoreductive drugs. The patient was followed closely and all blood parameters were monitored. During an observation time of 6 months (9 months

after first symptoms had occurred) the clinical condition and all laboratory parameters, including blood counts and serum tryptase levels, remained largely stable, and no C-Findings could be detected. However, in March 2014, the disease progressed. Transfusion-dependent anemia developed, an ultrasound examination revealed a massively enlarged spleen (20 cm in diameter) and the serum tryptase level increased to 1250 ng/ml. The patient was admitted again and at that time agreed to receive induction chemotherapy. However, shortly after the first cycle of chemotherapy (FLAG) the patients' clinical condition deteriorated and she died from septicemia and multi-organ failure in April 2014.

Histology and Cytology of Neoplastic Mast Cells

At the time of diagnosis, the BM smear showed a hypercellular marrow with a huge increase in atypical MCs. As assessed by Wright-Giemsa staining, these MCs were found to be rather mature cells and to comprise 80% of all nucleated BM cells. Most MCs were found to be spindle-shaped cells containing metachromatic granules (Figure 1A). Despite the massive increase in MCs in BM smears, no circulating MCs were detected in the peripheral blood. The BM histology confirmed a massive infiltration of the marrow by atypical, spindle-shaped and rather mature MCs. These MCs formed large clusters and sheets in the BM. As assessed by immunostaining for tryptase and KIT, the MC infiltration grade in the BM was found to be 75% (Figure 2). No morphologic or immunohistochemical signs of an associated hematopoietic non-MC-lineage disease (AHNMD) were found. Following WHO criteria [5–7], the diagnosis of an aleukemic variant of MCL was established. At the time of progression (March 2014), the BM also contained large multifocal clusters of MCs (infiltration grade in tryptase-stained BM sections: 80%). The BM smear also showed a massively elevated MC count (70%). Compared to the initial marrow-sample obtained in 2013, the morphology of MCs appeared to be more immature, including MCs with bi-lobed nuclei and numerous metachromatic blasts (Figure 1B and Table 1).

Comparison of the Morphology of MCs in other Patients with MCL

In an attempt to reveal the cellular background and mechanisms underlying the stable indolent course in our patient with chronic MCL, we compared the morphology of MCs (BM smears) in our patient with MC morphologies detected in other patients with (more aggressive) MCL. In these experiments, we were able to show that in our patient with chronic MCL, MCs exhibited a more mature morphology and more frequently a spindle-shaped form, compared to MC morphologies found in acute MCL (Table 1). We also found that metachromatic blast cells, the most immature stage of MC development, are more frequently detectable in patients with aggressive (acute) MCL compared to our case of chronic MCL (Table 1).

Cytogenetic and Molecular Results

Conventional karyotyping and FISH revealed a normal karyotype (46,XX). In initial sequencing studies no *KIT* point mutation at codon 816, the most frequent codon affected in SM [22,23,27–29] could be detected. In a next step, we sequenced all exons of *KIT* including hot-spot regions where point mutations are expected to occur (exons 5, 9, 10 and 17) [4,29,30]. In these studies, the heterozygous point mutation *KIT*c.1427G>T, pS476I was detected in neoplastic cells (Supplementary Figure S1). We also screened for additional

mutations (hot spot regions) in several other leukemia-related genes (apart from *KIT*) by multiplex-PCR. However, no additional molecular lesions were detected.

Phenotype of Neoplastic MCs

As assessed by immunocytochemistry, MCs were found to stain positive for tryptase, KIT (Figure 2) and CD25 (Table 2). In addition, most neoplastic MCs were found to express CD30 (Figure 2) and CD52 (Table 2). However, the levels of CD30 were relatively low when compared to that found in MCs in other cases of (more aggressive) MCL. We were also unable to detect chymase in neoplastic MCs. Moreover, most of the neoplastic MCs were found to stain negative for Ki-67 (Figure 2) which contrasts the situation in acute MCL [15,31]. Table 2 shows a summary of markers expressed in the cytoplasm of neoplastic MCs in our patient with chronic MCL. As assessed by multicolor flow cytometry, the KIT+ MCs were found to co-express CD13, CD25, CD43, CD52 and CD123 on their surface. In addition, these MCs expressed low levels of CD30. By contrast, MCs did not stain positive for CD2, CD34, CD133 or CD184 (Table 3).

Response of Neoplastic MCs to Tyrosine Kinase Inhibitors (TKI)

The *KIT* mutation S476I may provide growth-promoting, pro-oncogenic effects in neoplastic cells *in vitro* [30,32]. Therefore, we were interested to learn whether KIT-targeting drugs can counteract growth of neoplastic MCs in our patient. As assessed by ³H-thymidine uptake experiments, neoplastic MCs obtained from the patient at diagnosis were found to respond to the KIT-targeting TKIs PKC412 (midostaurin) and nilotinib, but did not respond to dasatinib (Figure 3).

Discussion

MCL is a life-threatening condition defined by leukemic expansion of MCs in the BM and other organs, drug resistance and a poor survival [5–9,12–15]. In most patients described to date, the survival time was less than one year. However, in exceptional cases, a more chronic form of the disease has been reported [14,15]. We here describe a young female patient in whom chronic MCL was diagnosed. The disease appeared to be stable over 9 months without development of relevant organ damage. Unlike in other cases of MCL, MCs in this case showed a rather mature morphology. We propose to separate this chronic form of MCL from the more aggressive and sometimes even acute form(s) of MCL. The tentative term proposed for this novel indolent entity of MCL is: chronic MCL [15].

It remains unclear whether patients with this indolent form of MCL are suffering from an occult MCL pre-phase resembling an indolent SM or are just diagnosed in an early stage of their disease. In fact, in some of these patients, chronic MCL may develop from a so-called well-differentiated form of SM (WDSM) [15]. However, in our patient, most MCs were spindle-shaped and mature, whereas in patients with WDSM or WDSM-derived MCL, MCs are round and well granulated cells. Another possibility could be that chronic MCL develops from smouldering SM (SSM), a variant of SM defined by slow progression and a huge burden of MCs, features that may also be detected in chronic MCL. However, unlike in chronic MCL, the BM smear in SSM always contains less than 20% MCs [1,5,15].

All in all, it remains unknown how frequently SM can develop into MCL and what subtypes of SM are at a particular risk of transformation. Investigations of larger collections of patients, preferably in a multi-center registry with long-term follow-up, are clearly warranted. The European Competence Network on Mastocytosis (ECNM) has recently launched such a registry and will collect these cases [33].

In chronic leukemias, the morphology usually differs markedly from acute leukemias originating from the same lineage. This is seen in acute and chronic myeloid leukemia and acute and chronic lymphoid leukemia. In the case of MCL, a similar morphologic distinction may be possible. In fact, in our case of chronic MCL, most of the neoplastic MCs appeared to be mature cells. Many of these MCs were even spindle-shaped and well granulated cells, otherwise typically seen in indolent SM. These observations suggest that chronic MCL may be a condition that is defined by the predominance of morphologically mature MCs. To confirm this hypothesis, we compared MC-morphologies in our patient with that found in aggressive (acute) MCL with rapid organ damage. As expected, the morphology of MCs in the 5 control cases differed from the MC morphology in our patient with chronic MCL in several aspects. First, most of the MCs recorded in patients with acute MCL were found to be immature MCs or even metachromatic blast cells. Only a very few mature MCs were seen in BM smears in these control cases. Interestingly, the MC morphology also changed in our patient at the time of progression. In fact when comparing to the initial BM samples, MCs at the time of progression were more immature with bi- or poly-lobed nuclei. In addition, numerous metachromatic blasts were found. All in all, the clinical course and variant of MCL seems to correlate with MC-morphologies which confirms observations made in (most) other leukemia models in the past. This hypothesis was further supported by staining results obtained with an antibody against the proliferation-marker Ki67. It has been described that MCs in aggressive MCL frequently express Ki67, whereas in indolent SM, MCs usually stain negative [15,31]. In our patient, most MCs also stained negative for Ki67, confirming the low proliferation rate and indolent clinical course with absence of organ damage.

In MCL, MCs usually exhibit a similar phenotype compared to MCs in indolent SM [34–36]. However, there are number of subtle phenotypic differences to consider: first, MCs in MCL usually express only low amounts or lack CD2 [34,36]. Second, MCs in MCL usually express substantial amounts of CD52 and CD123, whereas CD52 and CD123 are expressed less abundantly on MCs in indolent SM [36,37]. In the present study we found that MCs in our patient with chronic MCL express CD52 and CD123, but lack CD2. This phenotype resembles the phenotype of leukemic MCs, suggesting that the type of SM but not the clinical course may be associated with MC phenotypes. This observation is also in line with the hypothesis that low expression of CD2 facilitates a more diffuse (leukemic) spread of MCs in various organs [38]. In other words, phenotyping of MCs cannot differentiate between acute and chronic MCL but may explain the leukemic spread of clonal cells.

Another aberrant marker detectable in neoplastic MCs is CD30 [26,39,40]. This antigen is expressed abundantly in the cytoplasm of MCs in advanced SM, including MCL [26,27]. In our patient, CD30 was also expressed in the cytoplasm of neoplastic MCs, but the levels of CD30 were rather low, similar to the pattern found in patients with indolent SM. Moreover,

we were able to detect low levels of CD30 on the surface of neoplastic MCs, thereby confirming recently published data [39].

In about 50–80% of all patients with MCL, a *KIT* mutation can be detected [4,26–30]. In a majority of all patients with SM, MCs express the recurrent *KIT* mutation D816V [26–30]. However, not all patients with MCL exhibit a *KIT* mutation at codon 816. Rather, in these patients, other mutations in *KIT* have also been described. In our patient with chronic MCL, the rare *KIT* mutation S476I was detected by sequencing analyses. Interestingly, this mutation has previously been described to promote the growth of Ba/F3 cells and to be expressed in neoplastic cells in patients with pediatric (indolent) mastocytosis and patients with gastrointestinal stroma cell tumors [32]. However, to the best of our knowledge, this is the first case of MCL in whom neoplastic MCs are demonstrated to express *KITS476I*. In light of the indolent nature of pediatric mastocytosis, this finding is remarkable. In fact, despite the transforming nature of the mutation, it is considered a relatively harmless defect. In fact, progression of SM has not been reported for carriers of this mutation so far. One explanation for the unusual finding in our patient could be that an unrecognized (and maybe long-lasting) pre-phase of indolent mastocytosis with *KITS476I* preceded the evolution of MCL. However, even upon repeated consultation, the patient was not aware of a phase of mastocytosis in early childhood. In any case, additional lesions and hits may have been responsible for progression to chronic MCL and from chronic MCL into acute MCL. Indeed, several molecular lesions have recently been associated with a more aggressive course of SM [41,42]. However, although we screened for several known mutations we were unable to detect another driver lesion responsible for disease evolution. Next generation sequencing is currently ongoing and may reveal new lesions, at least at the time of progression. We also were unable to detect chromosomal abnormalities in this patient. Alternatively, germ line-based genetic factors, epigenetic modifications or a certain comorbidity was responsible for disease evolution.

With regard to therapy of chronic MCL, no guidelines are available. This is mostly due to the fact that only a very few patients have been described so far, and almost nothing is known about the clinical course, survival and response to cytoreductive treatment. From our patient it seems clear that at least in a subset of these patients, malignant progression to acute MCL will occur. In our patient, we offered cytoreductive therapy early because of the relatively high burden of neoplastic MCs and relatively young age of the patient. However, the patient refused cytoreductive therapy at first presentation. Nevertheless, after having obtained approval from the patient, we explored therapeutic options by *in vitro* drug testing. Based on literature-data, *KITS476I*+ cells may respond to several different KIT TKI, including imatinib [32]. In our patient, imatinib was found to suppress the growth of neoplastic MCs at relatively high concentrations, whereas the cells responded nicely to PKC412 (midostaurin) and nilotinib. This result is best explained by additional mutations or pro-oncogenic pathways that were accumulated by neoplastic MCs (during disease evolution) and conferred resistance against imatinib. Because of the unclear situation and potential resistance, we decided to administer poly-chemotherapy with the aim to prepare the patient for allogeneic stem cell transplantation.

In summary, we present a case of chronic MCL, in whom the clinical course remained stable during an observation period of several months and no organ damage could be detected. Unlike in other cases with MCL, the morphology of MCs in this case appeared to be mature, many MCs presenting as well granulated, mature and spindle-shaped, MCs. Thus, the morphology of MCs may be key to diagnose chronic MCL. Because of the unique clinical course and different prognosis, we recommend defining these cases as a separate subset of MCL, called chronic MCL [15].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A **B** **Figure 1. Morphology of neoplastic mast cells on bone marrow (BM) smears**

BM smears were obtained at the time of diagnosis (A) and at the time of malignant progression (B). BM smears were stained with Wright-Giemsa. At the time of diagnosis a massive infiltration (80%) by mature, well-granulated and mostly spindle-shaped mast cells was found. At the time of progression, 70% of all nucleated BM cells appeared to be mast cells, and these cells were in part more immature, including promastocytes and metachromatic blasts. Original magnification: x60.

Figure 2. Immunohistochemical characterization of neoplastic mast cells (MCs)

Diagnostic bone marrow sections were stained with antibodies against KIT (CD117) (A,C,D), CD30 (B) and Ki-67 (C,D) using the indirect immunoperoxidase staining technique (see text). Note weak expression of CD30 in neoplastic MCs. Double staining experiments (C,D) were performed using a brown color reactions for the anti-KIT antibody and red color reaction for anti-Ki-67 antibody. In our patient with chronic MCL, about 3% of all neoplastic MCs were found to stain positive for the proliferation-associated antigen Ki-67. Original magnification: x40. By contrast, in a case of acute MCL (D), about 30% of all nucleated bone marrow cells (and about 20% of all MCs) were found to stain positive for Ki-67.

Figure 3. Effects of KIT-targeting drugs on neoplastic mast cells (MCs)

Primary neoplastic MCs were obtained from bone marrow aspirates and layered over Ficoll to remove granulocytic cells. The purity of MCs amounted to >90% and cellular viability was >80%. Cells were incubated in control medium (Co) or in various concentrations of imatinib, PKC412 (midostaurin), nilotinib or dasatinib, as indicated, at 37°C for 48 hours. Then, ^3H -thymidine uptake was measured. Results are expressed as percent of control (Co) and represent the mean $\pm$ S.D. of triplicates.

Table 1

Mast cell (MC) morphologies in patients with mast cell leukemia (MCL)

Variable	data recorded in Patients with							
	c-MCL at diagnosis	c-MCL at progression*	MCL #1	MCL #2	MCL #3	MCL #4	MCL #5	MCL #6
% MCs of all BM leukocytes	80	90	40	90	30	85	20	60
% metachromatic blasts of all MCs	01	03	06	13	17	72	32	37
% atypical MCs Type II	06	05	34	45	49	20	26	61
% atypical MCs Type I (immature)	10	15	21	27	20	08	32	02
% atypical MCs Type I (mature)	75	65	27	12	09	00	08	00
% mature round typical MCs	08	12	12	03	03	00	02	00
% Ki-67+ MCs	03	10	n.t.	n.t.	n.t.	n.t.	n.t.	20
<i>KIT</i> mutation	S476I	S476I	D820G	KIT Dup 501-502	wt	wt	D816V	D816H
age	49	50	44	66	36	25	69	55
overall survival from diagnosis (months)	alive	alive	06	10	12	16	13	05

c-MCL, chronic MCL, BM, bone marrow; wt, wild type.

* progression to acute MCL

Table 2

Immunophenotype of neoplastic mast cells (MCs) in bone marrow (BM) sections obtained from the patient with chronic MC leukemia (MCL)

Antigen/Marker	CD	Expression detected in			
		MC in patients with			
		The patient's BM MCs*	ISM**	ASM**	MCL**
LFA-2	CD2	-	+/-	-/+	-
IL-2RA	CD25	+/-	+	+	+/-
Ki-1	CD30	+/-	-/+	+	+
HPCA-1	CD34	-	-	-	-
Campath-1	CD52	+	-	+/-	+
KIT	CD117	+	+	+	+
Chymase	n.c.	-	+/-	-	-
Tryptase	n.c.	+	+	+	+
Ki-67	n.c.	***	-	-	+/-

Data were obtained by indirect immunohistochemistry using antibodies directed against various leukocyte (differentiation) antigens. MCs, mast cells; BM, bone marrow; ISM, indolent SM; ASM, aggressive SM; IL-2RA, interleukin-2 receptor alpha chain; HPCA-1, human progenitor cell antigen-1; n.c., not yet clustered.

* Data were collected at the time of diagnosis.

** Data refer to the published literature and own unpublished observations

*** As assessed by KIT/Ki-67 double-staining experiments, about 3% of all mast cells were found to stain positive for Ki-67 in this patient at diagnosis. By contrast, at the time of progression, about 10% of all mast cells were found to react with an antibody directed against Ki-67.

Table 3

Cell surface phenotype of neoplastic mast cells (MCs) determined by antibody-staining and flow cytometry

CD	Antigen	expression on neoplastic MCs	
		at diagnosis	at the time of progression
CD2	LFA-2	–	–
CD13	Aminopeptidase N	++	+/-
CD15	Lewis X antigen	–	–
CD25	IL-2RA	+	+
CD26	DPPVI	–	–
CD30	Ki-1 antigen	+/-	+
CD33	Siglec-3	n.t.	++
CD34	HPCA-1	–	–
CD43	Leukosialin	++	+
CD44	Pgp-1	n.t.	+
CD51	VNRA	–	–
CD52	Campath-1	+	+
CD56	NCAM	–	–
CD61	VNRB	–	–
CD87	uPAR	n.t.	+
CD88	C5aR	n.t.	–
CD117	KIT	+	++
CD123	IL-3RA	+	–
CD133	AC133	–	–
CD183	CXCR4	–	–

Expression of cell surface antigens on bone marrow (BM) mast cells (MCs) was analyzed by multicolor flow cytometry using fluorochrome-conjugated monoclonal antibodies. n.t., not tested.