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Mast cell sarcoma: a rare and potentially under-recognized diagnostic entity with specific therapeutic implications

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Mast cell sarcoma is a rare, aggressive neoplasm composed of cytologically malignant mast cells presenting as a solitary mass. Previous descriptions of mast cell sarcoma have been limited to single case reports, and the pathologic features of this entity are not well known. Here, we report three new cases of mast cell sarcoma and review previously reported cases. Mast cell sarcoma has a characteristic morphology of medium-sized to large epithelioid cells, including bizarre multinucleated cells, and does not closely resemble either normal mast cells or the spindle cells of systemic mastocytosis. One of our three cases arose in a patient with a remote history of infantile cutaneous mastocytosis, an association also noted in one previous case report. None of our three cases were correctly diagnosed as mast cell neoplasms on initial pathological evaluation, suggesting that this entity may be under-recognized. Molecular testing of mast cell sarcoma has not thus far detected the imatinib-resistant *KIT* D816V mutation, suggesting that recognition of these cases may facilitate specific targeted therapy.
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Mast cell neoplasms comprise a clinically and biologically heterogeneous group of disorders.[1] The most common of these are clinically indolent clonal mast cell proliferations predominantly involving the skin (cutaneous mastocytosis) and bone marrow (systemic mastocytosis), whose primary symptomatic manifestations are due to the unique paracrine and systemic effects of mast cell secretory products. Aggressive mast cell neoplasms are rare and are typically variants of systemic mastocytosis that diffusely involve the bone marrow and other anatomic sites, in some cases associated with progression to mast cell leukemia, or the development of a non-mast cell hematologic neoplasm. Although relatively common in dogs,[2] mast cell sarcoma, defined as a malignant mast cell neoplasm presenting as an isolated destructive mass, is exceedingly rare in humans.[1] As only seven cases of mast cell sarcoma have been reported to date in the English language literature,[3–9] each representing a single case report, there is limited information available regarding the diagnostic features, clinical behavior, and genetic aberrations associated with this entity.

Here, we present three new cases of mast cell sarcoma, and place them in context with the previously reported cases. We find that mast cell sarcoma may present in a broad spectrum of anatomic locations and age groups. The cells of mast cell sarcoma are medium to large, often bizarre-appearing epithelioid cells with characteristic morphologic features and a specific immunophenotype. However, because they bear only limited resemblance to normal mast cells, none of our cases were correctly diagnosed on initial pathological evaluation. We note that *KIT* genotyped cases of mast cell sarcoma in our series and prior case reports have not

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demonstrated the imatinib-resistant *KIT* D816V mutation, but rather showed either an absence of *KIT* mutations, or mutations in *KIT* domains associated with imatinib sensitivity in other neoplasms. These findings suggest that accurate diagnosis of mast cell sarcoma may allow for specific targeted therapy for this aggressive malignancy.

Materials and methods

Cases of mast cell sarcoma were retrieved from the consultation files of two of the authors (J.A.F. and J.L.H). Formalin-fixed, paraffin-embedded tissue was stained immunohistochemically using the antibodies listed in Table 1.

Tissue for electron microscopy was extracted from formalin-fixed, paraffin embedded tissue blocks, soaked in 100% xylene overnight, rehydrated in a series of ethanol solutions, rinsed in sodium cacodylate buffer, and fixed for 1.5 h with 2.5% glutaraldehyde, 2.0% paraformaldehyde, and 0.025% calcium chloride, in a 0.1 M sodium cacodylate buffer, pH 7.4. Tissues were further processed in a Leica Lynx automatic tissue processor. Briefly, tissues were post fixed with osmium tetroxide, dehydrated in a series of ethanol solutions, en bloc stained during the 70% ethanol dehydration step for one hour, infiltrated with propylene oxide epoxy mixtures, embedded in pure epoxy, and polymerized over night at 60 °C. Thin sections were stained with lead citrate and examined with an FEI Morgagni transmission electron microscope. Images were captured with an AMT (Advanced Microscopy Techniques) digital CCD camera.

The study protocols were approved by the Institutional Review Board of Partners Healthcare.

Results

Patient 1

Patient 1 was a 12-year-old female who initially presented with a large left middle ear mass. A biopsy of the mass was felt to be consistent with Langerhans cell histiocytosis. The tumor progressed intracranially, despite systemic treatment with vinblastine and prednisone. Two debulking surgeries were performed. Unfortunately, subsequent radiation therapy did not produce a response, and the patient developed local progression involving the skull.

At this time, the pathology was reviewed at a second institution, and an unspecified histiocytic or myeloid neoplasm was favored. She was treated with multiple courses of chemotherapy, including 2CDA/Ara-C, ICE, clofarabine, ALCL 99, idarubicin/velcade/Ara-C, and decitabine over 27 months following initial presentation. Although the tumor showed an initial response, there was subsequent intracranial progression near the left sphenoid and transverse venous sinus.

The pathology was then reviewed at a third institution, and a conclusive diagnosis of a malignant mast cell neoplasm was rendered. Of note, a total of five bone marrow biopsies had been performed in the 20 months following initial diagnosis, all of which were negative for involvement by systemic mastocytosis, even on retrospective review. The patient was treated with radiation and imatinib mesylate, and scheduled for allogeneic hematopoietic stem cell transplantation. Serum tryptase levels have remained markedly elevated (ranging from 122 ng/ml to >200). At last follow-up, the patient is alive with persistent disease 45 months following initial presentation.

Histologically, all biopsies and excision specimens showed similar morphologic findings (Figure 1). There was a dense proliferation of morphologically heterogeneous, medium-sized to large cells, with well-defined cell borders, clear to palely eosinophilic cytoplasm, and irregular nuclei. A prominent, patchy infiltrate of eosinophils was also present. Scattered very large epithelioid cells were also present; these often showed bizarre features, including multilobated nuclei and multinucleation. There was occasional emperipolesis of eosinophils within the large cells. Mitotic activity

Table 1 Antibodies used for immunohistochemistry

Antigen	*Clone*	*Antigen retrieval*	*Dilution*	*Source*
KIT	YR 145	Ventana CC1, 30 min	Ready to use	Cell Marque, Rocklin, CA
KIT	Polyclonal	None	1:200	Dako, Carpinteria, CA
Mast cell tryptase	G3	Ventana CC1, 30 min	Ready to use	Ventana, Tucson, AZ
Mast cell tryptase	AA1	Trypsin	1:500	Dako, Carpinteria, CA
Chymase	CC1	EDTA + steamer	1:1000	Abcam, Cambridge, MA
CD2	AB75	Ventana CC1, 30 min	1:50	Leica, Buffalo Grove, IL
CD4	4B12	Ventana CC1, 30 min	Ready to use	Leica, Buffalo Grove, IL
CD25	4C9	EDTA + steamer	1:200	Novocastra, Newcastle upon Tyne, UK
CD30	BerH2	Ventana CC1, 30 min	Ready to use	Ventana, Tucson, AZ
CD43	DFT-1	Ventana CC1, 30 min	1:40	Biogenex, Fremont, CA
CD68	KP-1	Ventana CC1, 30 min	Ready to use	Ventana, Tucson, AZ
MITF	D5	Ventana CC1, 60 min	1:30	Lab Vision/Neomarkers, Fremont, CA
Ki-67	30-9	Ventana CC1, 30 min	Ready to use	Ventana, Tucson, AZ

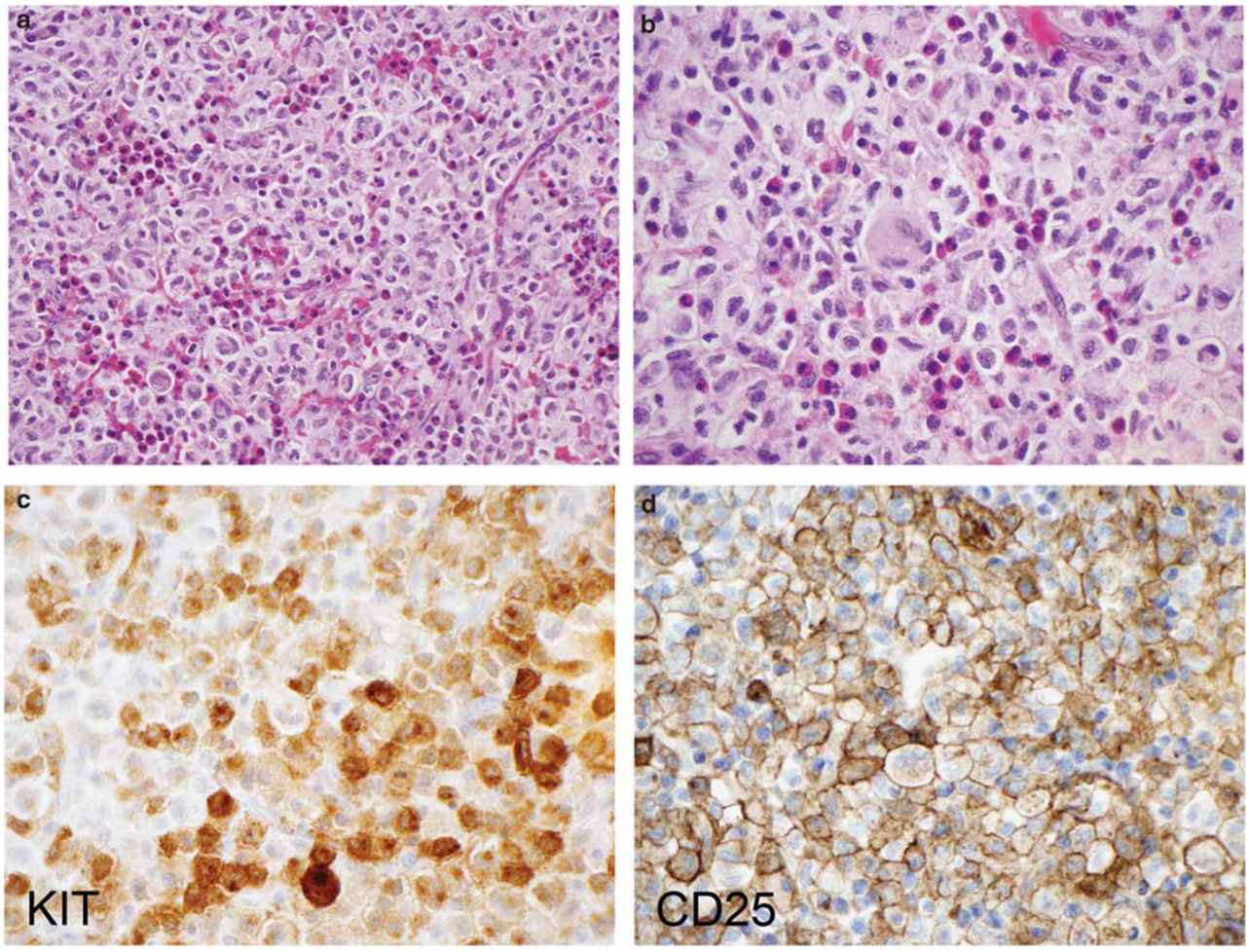

Figure 1 Representative histological images from the intracranial tumor in case 1. Note the dense infiltration of eosinophils, tumor cells with well-defined cell borders and lobulated nuclei, and scattered multinucleated tumor cells (**a**–**b**). The tumor cells show variable expression of KIT (**c**), and strong membranous CD25 (**d**). Mast cell tryptase was also positive (not shown).

varied from 2 per 10 high power fields in the initial specimen to 18 per 10 high power fields in a recurrence.

Sequencing of the *KIT* gene in tumor tissue showed no evidence of a mutation. Immunohistochemical findings for all three cases are summarized in Table 2.

Patient 2

Patient 2 was a 19 year-old male who presented with a progressively enlarging submucosal nodule on the right inner aspect of his lower lip. His history was notable for childhood-onset cutaneous mastocytosis, which had lasted from 1 month to approximately 2 years of age, including blistering lesions on the chest and upper trunk. The lesions were reportedly treated with clobetasol ointment, and healed without scarring or residual hyperpigmentation. The patient reported having a tan-colored lesion on his right lip for his entire childhood, which was stable until a few months before presentation. The patient denied urticaria or flushing, but did report recurrent periods of nausea, vomiting and diarrhea as well as a 50-pound weight loss. An excisional biopsy of the lesion was performed.

Histologically, the excision reveled a discrete, densely cellular mass extending from the deep dermis into subcutaneous tissues, with destructive invasion of minor salivary glands and skeletal muscle. The tumor was composed of large, pleomorphic epithelioid cells with abundant finely granular eosinophilic cytoplasm (Figure 2a). Some cells showed a more uniformly eosinophilic cytoplasm, while other cells also contained large clear vacuoles especially at the periphery, and some contained densely eosinophilic perinuclear aggregates imparting a vaguely rhabdoid appearance. Scattered very large cells with bizarre nuclear features were present, including forms with bilobed, horseshoe-shape, and multilobulated nuclei. The nuclei of most cells showed round to moderately irregular contours and coarse chromatin with

Table 2 Summary of immunohistochemistry

	Case 1	*Case 2*	*Case 3*
Strongly positive	Mast cell tryptase, CD68, CD25, CD43, CD33	Mast cell tryptase, KIT, chymase, CD68, CD2, CD25, CD43, MITF	Mast cell tryptase, KIT
Variably positive	KIT	CD30, CD4	
Negative	Chymase, CD30, CD2, CD3, CD8, CD34, MPO, CD1a, S100, CD163	CD45, CD34, MPO, ALK, tyrosinase, CD8, CD20, CD3, CD5, chromogranin, NSE, S100, PLAP, HMB-45, RCC, desmin, CD163, SMA, p63, lysozyme[a], keratin cocktail[a], EMA[a]	CD45, lysozyme, CD3, CD20, CD138, kappa & lambda immunoglobulin light chain, CD61, CK cocktail, CK7, CK20, EMA, ER, TTF1, synaptophysin, GCDFP15, keratin AE1/AE3[a]

[a]Very focal or equivocal staining seen for these markers.

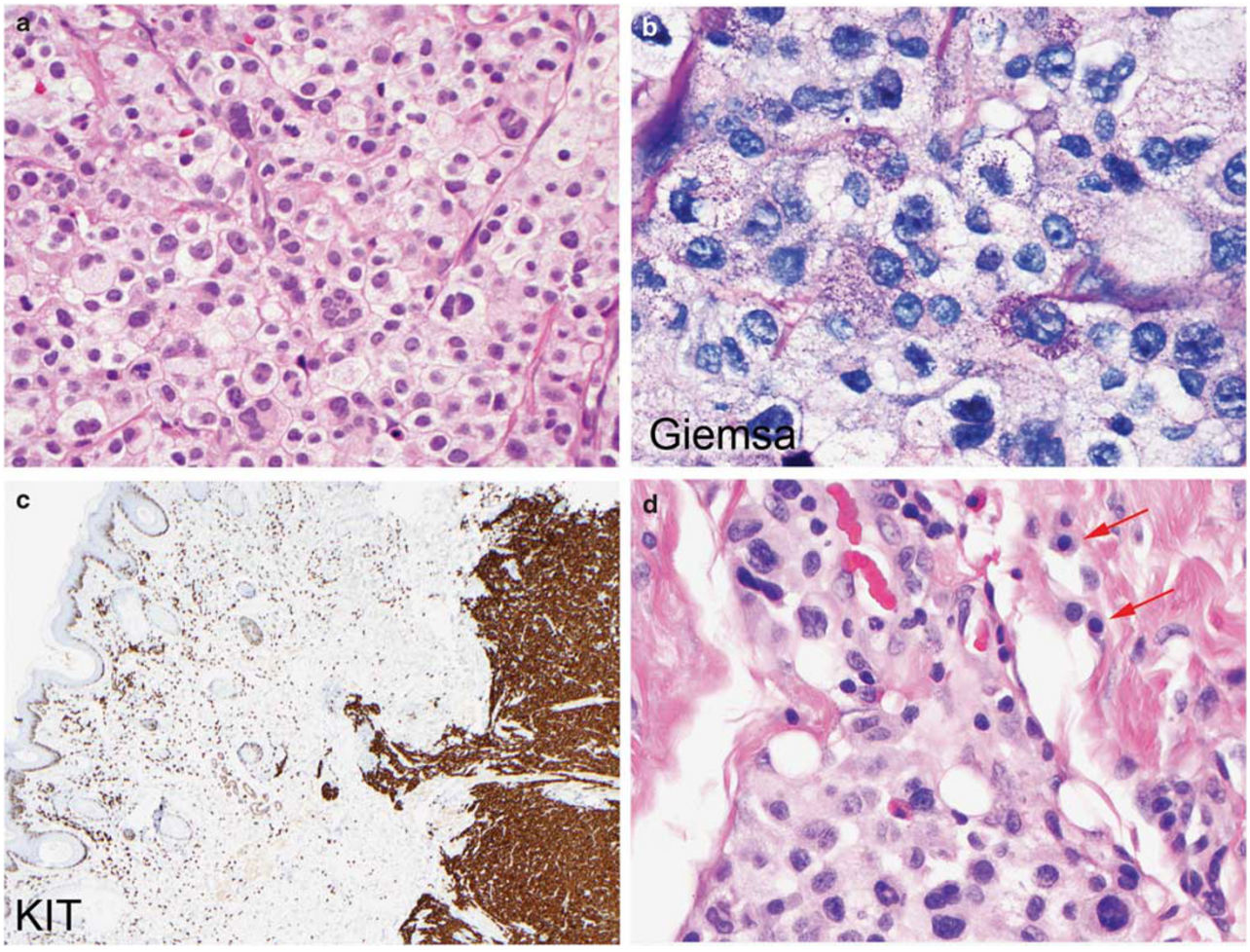

Figure 2 Representative histological images from the lip tumor in case 2. Tumor cells show well-defined borders, marked anisocytosis, and round to multilobulated nuclei (**a**). Giemsa stain reveals scant, fine metachromatic granules in many cells, as well as scattered prominently granulated cells (**b**). The sarcoma was strongly KIT positive, and largely involved the deep dermis and subcutaneous tissue. However, there was also a diffusely increased population of morphologically typical, KIT-positive mast cells within the superficial dermis, consistent with residual urticaria pigmentosa (UP) (**c**). The highly pleomorphic mast cells of the sarcomatous component contrast with the benign-appearing mast cells of the UP component (arrows) in this field from the tumor edge (**d**).

numerous basophilic chromocenters, and usually solitary eosinophilic nucleoli. Mitoses, including atypical forms, were readily identified, up to 16 per 10 high-power fields. Scattered karyorrhectic tumor cells were also present. Morphologically normal eosinophils were scattered throughout the tumor. Large cells were absent from the superficial dermis, but this area was involved by an infiltrate of numerous morphologically typical interstitial mast cells, consistent with the findings of urticaria pigmentosa. The excisional margin was involved by tumor cells.

The initial immunohistochemical workup did not include stains for mast cell-specific antigens. A diagnosis of ALK-negative anaplastic large cell lymphoma was favored by the submitting pathologist. On referral, additional immunostains (Figure 3, Table 2) confirmed a diagnosis of mast cell sarcoma. A Giemsa stain (Figure 2b) revealed the presence of distinctive metachromatic purple granules which were highly variable in density from cell to cell.

Electron microscopy performed on formalin-fixed tissue retrieved from paraffin demonstrated numerous vacuoles in most cells. Many of the vacuoles were empty (degranulated), but scattered cells had characteristic mast cell features (Figure 4),[10] containing intracytoplasmic membrane-bound granules with electron-dense material or finely granular material, as well as some granules with a suggestion of lamellar structures. *KIT* gene mutation screening revealed the presence of a deletion mutation in exon 8 (D419del).

Subsequently a serum tryptase level was found to be elevated at 18 ng/ml. A bone marrow biopsy showed a mildly hypocellular marrow with maturing trilineage hematopoiesis and 2% mast cells, including occasional spindled forms and small clusters of mast cells (<15 cells per aggregate). A small subset of the mast cells was positive for CD25 by immunohistochemistry and flow cytometry. Overall, although the W.H.O. criteria were not fulfilled, the findings were suspicious for limited involvement by systemic mastocytosis.

The patient was started on imatinib, 400 mg daily. Re-excision of the surgical site seven months after the initial excision showed morphologically normal, but quantitatively increased mast cells, consistent with residual urticaria pigmentosa; there was no evidence of residual sarcoma. CT scans of the head, neck, chest, abdomen, and pelvis showed no suspicious lesions at eight months post excision, and serum tryptase levels were within the normal range at nine and twelve months post-excision. At 19 months post-excision, an enlarged cervical lymph node was detected, and biopsy demonstrated recurrent mast cell sarcoma.

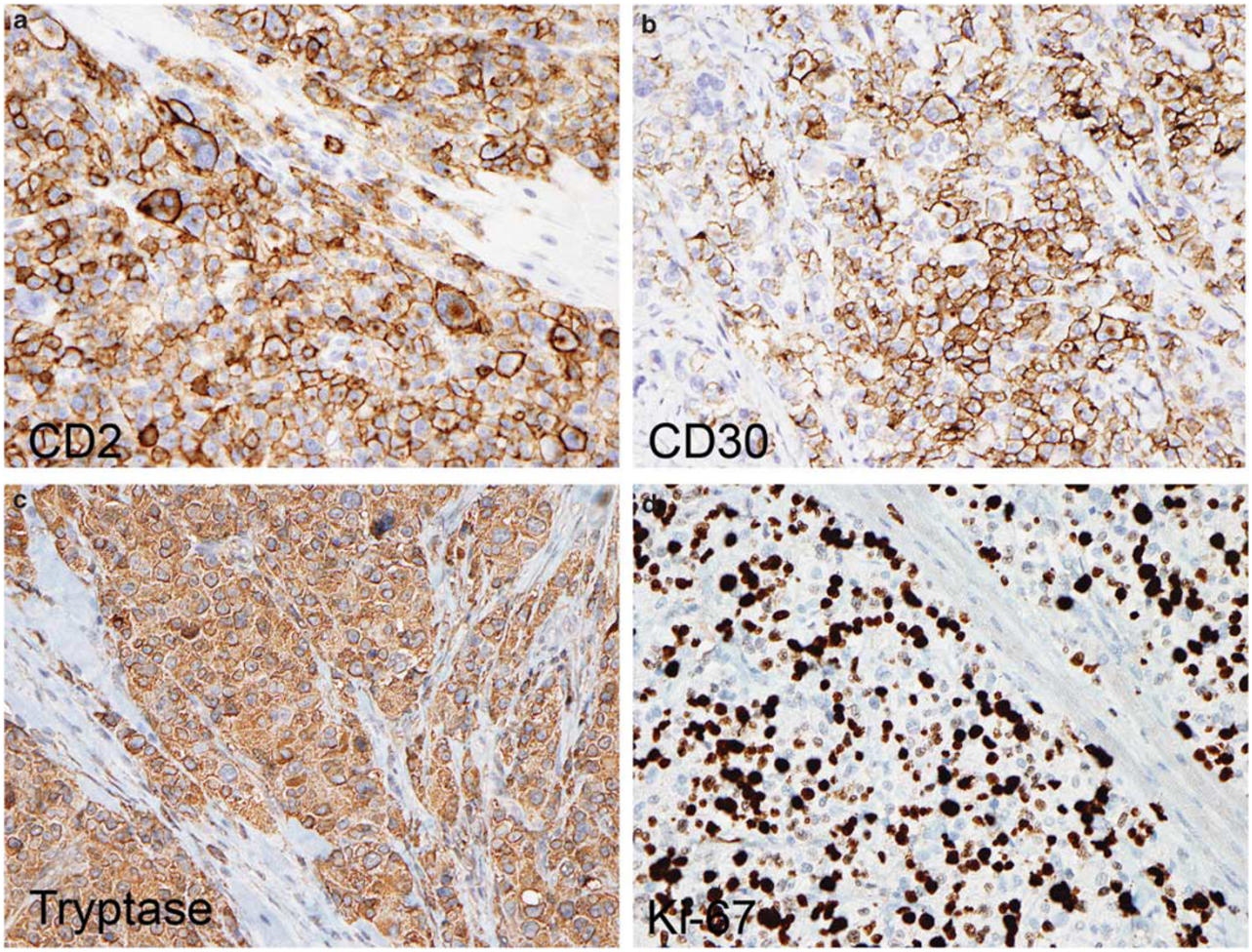

Figure 3 Immunohistochemistry in case 2 showed strong tumor cell expression of CD2 (**a**), CD30 (**b**), and mast cell tryptase (**c**). The Ki-67 staining index was approximately 50% (**d**).

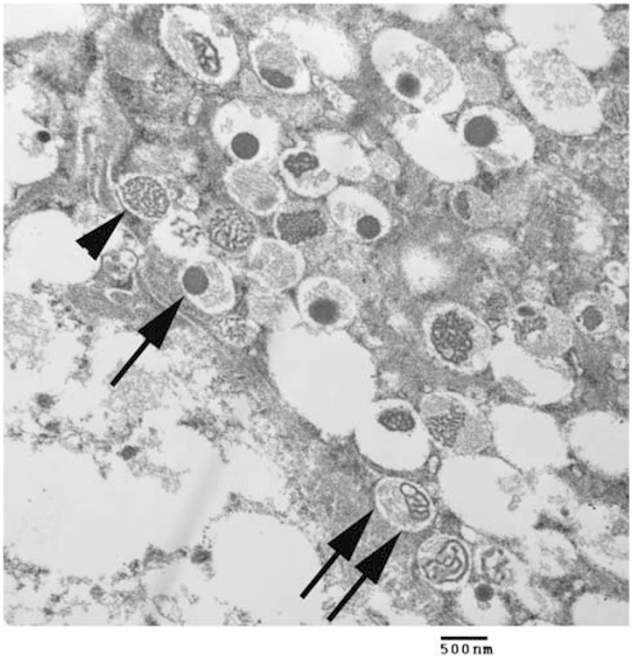

Figure 4 Electron microscopic examination of case 2 demonstrated that the neoplastic cells contained numerous intracytoplasmic membrane-bound granules with electron-dense material (single arrow), particulate content (arrowhead), and a suggestion of lamellar structures (double arrows).

Patient 3

Patient 3 was a 77 year-old woman who presented with a six-month history of progressively worsening pain in her right hip and groin. She had a history of nephrolithiasis and cystectomy for chronic cystitis, as well as chronic anemia. Her family history was significant for ovarian cancer, an unspecified brain tumor, and melanoma. A pelvic CT scan initially showed no identifiable lesion at the site of the pain (Figure 5a). Six months later, an MRI scan demonstrated a pelvic mass, which was now clearly visible by CT scan as a destructive lesion arising within the right supra-acetabular pelvis, and extending into the adjacent soft tissues (Figure 5b). A bone scan showed markedly increased signal at the site of the right pelvic mass, but no evidence of disease at other sites (Figure 5c). A core needle biopsy was performed, but no definitive diagnosis was made on initial pathology evaluation, and she was treated with 30 Gy of external beam radiation to the mass as a palliative measure. Subsequently, pathological consultation and additional immunohistochemistry yielded a conclusive diagnosis of mast cell sarcoma.

Histologically, the tumor was composed of sheets of highly pleomorphic epithelioid cells, ranging from medium-sized cells with round nuclei to large cells with multiple or lobulated nuclei (Figure 5d). The cytoplasm was eosinophilic, granular, and variably vacuolated. Morphologically unremarkable eosinophils were scattered throughout the tumor. A Giemsa stain showed a fine scattering of metachromatic purple granules in some tumor cells, while others showed similar granules in dense perinuclear clumps. Stains for KIT and mast cell tryptase were strongly positive.

Following establishment of the diagnosis, the patient was found to have an elevated serum tryptase at 28.2 ng/ml. The patient declined a bone marrow biopsy. She was briefly treated with pamidronate. A CT scan showed a slight interval decrease in the right pelvic mass, from 8.5×6 cm to 7.6×5 cm, two months after completion of radiotherapy. She subsequently developed a urinary tract infection resistant to multiple antibiotics, declined further therapy, and died under hospice care four months after diagnosis.

Discussion

Mast cell sarcoma is defined in the 2008 WHO classification as a variant of mastocytosis that presents as a unifocal mast cell tumor with destructive growth and high-grade cytology. These cases do not fulfill the major criterion for systemic mastocytosis, which requires multifocal, dense infiltrates of mast cells in the bone marrow or other extracutaneous organs. By these criteria, the entire published literature on this entity to date consists of seven case reports, the clinical and histological characteristics of which are compared to the current three cases in Table 3.

The age at presentation for these cases ranges from 4 to 77 years, similar to the broad age distribution of systemic mastocytosis, but not cutaneous mastocytosis, which typically presents in infancy. The sites of presentation for mast cell sarcoma are also diverse, with two cases presenting in squamous mucosal sites (lip and subglottis), two in the gastrointestinal tract, two involving the cranial bones and meninges, two in the extracranial skeleton, one in the uterus, and one in the skin. This distribution may be related to the normal tissue distribution of mast cells in mucosal sites and bone marrow.

Despite their diverse sites of presentation, the three cases described in our study showed similar histological features, which were distinct from typical cases of systemic mastocytosis, but were shared by many of the previously reported cases of mast cell sarcoma. The majority of tumor cells in our cases were medium-sized to large epithelioid cells with abundant cytoplasm and well-defined cell borders. Cells with prominent nuclear irregularities and bilobation, as well as bizarre multinucleated tumor giant cells were prominent in our cases, and were reported in most of the published cases as well. Thus, the cells of mast cell sarcoma do not closely resemble either normal tissue mast cells or the spindled mast cells typical of most systemic mastocytosis cases, but are similar to the cytologically defined 'atypical type II mast cells' or 'promastocytes' reported in some cases of aggressive systemic

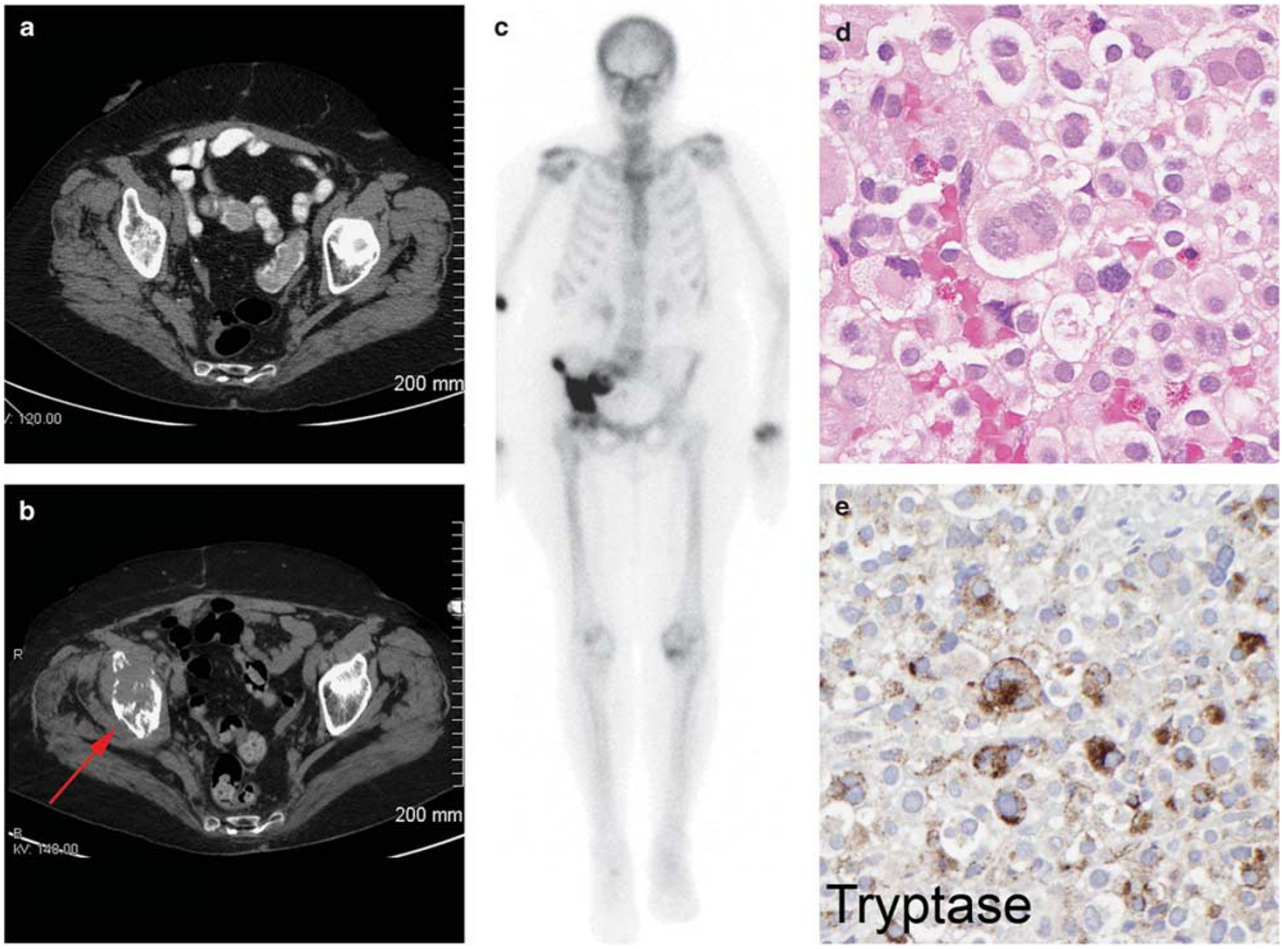

Figure 5 Pelvic CT scans of patient 3 at the time of diagnostic biopsy (**b**), and 6 months prior (**a**) demonstrate rapid growth of a destructive mass arising above the right acetabulum. A bone scan demonstrated abnormal signal localized to the right pelvis (**c**). The tumor cells showed histological features similar to those of the other two cases (**d**), and were variably positive for mast cell tryptase (**e**).

mastocytosis.[11] Neoplastic mast cells with lobulated nuclei have been described in histologic sections from cases of aggressive systemic mastocytosis,[1] including at least one case which progressed to include sarcoma-like mass lesions.[12] 'Metachromatic blasts' with a high nuclear-to-cytoplasmic ratio have been reported in association with mast cell leukemia, but were not a feature of our three cases, although several of the previously reported cases of mast cell sarcoma did progress to mast cell leukemia and showed a more blast-like morphology at that time. An important morphologic clue linking these cases to systemic mastocytosis was the presence of infiltrating eosinophils, although this finding is certainly shared with other entities, as highlighted by the initial misdiagnosis of case 1 as Langerhans cell histiocytosis.

It is not surprising that none of the three cases of mast cell sarcoma were correctly diagnosed as mast cell neoplasms at the time of initial pathological evaluation, given their lack of morphological resemblance to normal mast cells, or to more familiar mast cell neoplasms. All three cases were initially evaluated with broad immunohistochemical panels that did not include specific mast cell markers. Case 2 highlights the potential immunophenotypic pitfalls of this entity, as this tumor was positive for antigens commonly associated with histiocytic or myeloid sarcoma (CD68, CD117, weak lysozyme), anaplastic large cell lymphoma (CD2, CD4, CD25, CD30, CD43), and melanoma (MITF). MITF is a transcription factor that is known to be expressed in normal mast cells,[13] but is not often used as a marker for this lineage in diagnostic practice.

Diagnostic considerations suggested by referring pathologists in our cases included poorly differentiated carcinoma, melanoma, ALK-negative anaplastic large cell lymphoma, Langerhans cell histiocytosis, and other myeloid or histiocytic neoplasms. Like our mast cell sarcoma cases, Langerhans cell histiocytosis typically shows tumor cells with abundant pink cytoplasm, and a dense infiltrate of eosinophils. However, the characteristic longitudinal nuclear grooves of Langerhans cell histiocytosis were not seen in our mast cell sarcoma cases, and essentially all cases of Langerhans cell histiocytosis should show immunohistochemical expression of S100, CD1a, and langerin. The well-

Table 3 Summary of reported mast cell sarcoma cases

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*	*Case 7*	*Case 8*	*Case 9*	*Case 10*
Source	*This series*	*This series*	*This series*	*Auquit-Auckbur et al*[9]	*Bugalia et al*[7]	*Ma et al*[8]	*Brcic et al*[6]	*Guenther et al,*[22] *Chott et al*[5]	*Kojima et al*[4]	*Horny et al*[3]
Age at diagnosis	12	19	77	39	'elderly'	39	4	8	32	74
Sex	F	M	F	M	M	F	M	F	F	F
Site	Left ear, intracranial	Inner lip (arising in residual UP)	Right pelvis	Localized CM of ankle as neonate. Mastocytoma excised same site age 35. Recurred as MCS	Small intestine	Uterus (3×3.2 cm mass), miliary peritoneal nodules	Tibia	Intracranial: 2×4 cm subdural & cranial left temperopariatal mass	Ascending colon, 2 yrs later had widespread abdomen infiltration	Subglottic, recurrence skin and larynx, progression to MCL
Bone marrow	Uninvolved $\times 5$	Suspicious $\times 1$ (small clusters normal mast cells, subset CD25+ flow)	NA	Normal following initial MCS excision	Uninvolved	Uninvolved	Initially normal blood counts, unclear if BM was ever negative	Not reported, but patient had thrombocytopenia, anemia, 22% eos on recurrence	Not done	Negative 2 yrs post dx., densely positive 4 yrs post dx (progression to MCL)
Serum Tryptase	>200 ng/ml	18 ng/ml (post excision)	28.2 ng/ml (post XRT)	Normal following initial MCS excision	NA	NA	'High' shortly after presentation	<1 mcg/l, repeatedly. Histamine not elevated	NA	24 h urine histamine 1510 & 860 mcg (nl 60 mcg)
Sequencing	Negative for mutations in *KIT*	*KIT* exon 8 mutation: deletion D419	NA	Negative for mutations in *KIT* (exons 8, 9, 11, 13, and 17) and *PDGFRA* (exons 11 and 17)	*KIT* exon 17 mutation: N822K	Negative for mutations in *KIT*	NA	Negative for *KIT* D816V mutation by RT-PCR and RFLP	NA	NA
FISH	NA	NA	NA	NA	NA	FIP1L1-PDGFRA neg	NA	NA	NA	NA
Comments	See text	See text	See text	Local radiotherapy applied to excision site. Bone and lymph node metastases appeared within months of excision. Imatinib and combination imatinib plus cytotoxic therapy attempted.	Ascites. Tx imatinib	Ascites, peripheral eosinophilia. Tx imatinib	Initial tumorous mass, 10 month later progression to aleukemic MCL	Headache $\times 1$ year, Tx surgery, IFNa2b, XRT, Pred&Ara-c (response), AML-BFM-93 protocol, local recurrence, low counts & eos	Tentative initial dx of malignant lymphoma, unclassifiable (1980)	Initial Bx 'Wegener's-like'. Tx cortisone x 2 yrs, chemo bleomycin (no response), XRT—minimal response, 'modified DeVita' chemo—response. Progressed to MCL
Outcome	Alive with disease 3 years 9 months post presentation	Alive with disease 19 months post presentation	Died 4 months post presentation	Died 2 years (26 months) post MCS presentation	Alive 9 months post presentation	Alive 3.3 y post presentation	Died 10 months post presentation	Died 58 weeks (approx 1 year) post presentation	Died 3 years post presentation	Died 4 yrs post presentation

Table 3 (Continued)

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*	*Case 7*	*Case 8*	*Case 9*	*Case 10*
Source	*This series*	*This series*	*This series*	*Auquit-Auckbur et al*[9]	*Bugalia et al*[7]	*Ma et al*[8]	*Brcic et al*[6]	*Guenther et al,*[22] *Chott et al*[5]	*Kojima et al*[4]	*Horny et al*[3]
Histology	Medium to very large oval to pleomorphic cells with lobated and multiple nuclei. Many eosinophils	Large to very large pleomorphic cells with lobated and multiple nuclei. Eosinophils	Large to very large pleomorphic cells with lobated and multiple nuclei. Eosinophils	Well-defined lesion with pleomorphic tumor cells, multinucleated cells, atypical mitoses	Round to oval lobated nuclei	Bilobed to multi-lobed nuclei, eosinophils	Large cells, not spindled; oval, polygonal, some bilobed and multilobated nuclei	Medium-sized cells; oval or lobated nuclei; mono or multinucleated giant cells; no spindle cells; many eosinophils	Medium to large cells, oval, indented, lobated nuclei, some bilobation and mitoses. Many eosinophils	Larynx: slightly pleomorphic medium-sized cells, irregular or indented nuclei
Mitoses	2–18/10 HPF	16/10 HPF	25/10 HPF	NA	NA	NA	NA	NA	'a small number'	NA
Metachromatic granules	NA	Giemsa +	Giemsa +	Giemsa +	Tol blue +	Tol blue +, Giemsa −	Tol blue +, Giemsa +	Giemsa + (minority of cells)	Tol blue −, Giemsa −	Tol blue +, Giemsa +
MC tryptase	+	+	+	+	+	+	+	+	+	NA
Chymase	−	+	NA	NA	NA	NA	NA	NA	NA	NA
KIT	+	+	+	+	+	+	+	+	NA	NA
CD68	+	+	NA	+	+	+	+	+ (CD68R-macrosialin)	+	NA
CD2	−	+	NA	+	NA	+ (IHC and flow)	variable +	−	NA	NA
CD25	+	+	NA	NA	NA	+ (IHC and flow)	NA	NA	NA	NA
CD30	−	+ (partial)	NA	NA	−	−	NA	NA	−	NA
CD43	+	+	NA	+	NA	NA	+	NA	NA	NA
CD13	NA	NA	NA	NA	NA	+ (flow)	NA	+ (cytology IF)	NA	NA
CD33	+	NA	NA	NA	NA	+ (flow)	NA	NA	NA	NA
CD34	−	−	NA	−	NA	− (flow)	−	−	NA	NA
CD45	NA	−	−	NA	+	NA	+	+	+	NA
Ki-67	5–7%	50%	NA	20%	NA	3%	NA	40%	NA	NA
EM	NA	Vacuoles, membrane-bound cytoplasmic granules with electron-dense material and particulate content	NA	NA	NA	NA	NA	NA	NA	Amorphous and granular granules, no lamellae

defined cytoplasmic borders seen in mast cell sarcoma also contrasts with the indistinct borders seen in most cases of Langerhans cell histiocytosis and histiocytic sarcoma. Like our mast cell sarcoma cases, anaplastic large cell lymphoma typically demonstrates sheet-like growth of large epithelioid cells which often show bilobed or multilobated nuclei. Abundant eosinophils have been reported in anaplastic large cell lymphoma, but are relatively uncommon,[14] and should prompt consideration of other diagnoses. Variable KIT expression may occasionally be seen in T cell lymphomas, but the uniformly strong expression seen in two of our three mast cell sarcoma cases would be highly unusual, and expression of CD68 should not be seen. Because no single immunohistochemical marker is specific for ALK-negative anaplastic large cell lymphoma, it may be prudent to exclude mast cell sarcoma before making this diagnosis in cases lacking expression of CD3, CD5, EMA, or cytotoxic granule markers, or when PCR studies fail to demonstrate a clonal T cell receptor rearrangement.

Both our cases and those from the literature support mast cell tryptase as a highly specific stain for confirming the lineage of mast cell sarcoma, once the diagnosis has been considered. Giemsa stain was also positive, albeit in a subset of cells; metachromatic granules were not universally detected in mast cell sarcoma among the previously reported cases. While it has recently been reported that CD30 immunohistochemistry may be helpful in distinguishing between indolent and aggressive cases of systemic mastocytosis,[15] this marker may be less helpful in mast cell sarcoma, as it was only positive in 1 of 2 evaluated cases from our series, and was negative in all three of the prior mast cell sarcoma cases for which it was reported.

KIT sequencing results were only available for two of our cases, due to tissue exhaustion of the very small biopsy from case 3, and the patient's decision to decline a repeat biopsy. However, among the five cases of mast cell sarcoma screened for the presence of a *KIT* mutation in our series and the literature, it is striking to note that none demonstrated the canonical D816V mutation of the *KIT* kinase domain, despite the fact that this mutation has been reported in >95% of cases of adult systemic mastocytosis.[1] Two mast cell sarcoma cases showed alternate *KIT* mutations, while no *KIT* mutation was detected in the sequenced exons from the other 3 cases. While the sensitivity of direct sequencing of bone marrow aspirates from typical cases of systemic mastocytosis may be compromised due to a dilution by normal bone marrow cellular elements, depending on the molecular method employed, this seems unlikely to explain the lack of detected mutations in these three cases, given the high tumor cell content of mast cell sarcoma. This observation is not merely of academic interest, as mast cell neoplasms with the D816V mutation are resistant to therapy with imatinib and some other clinically available *KIT* tyrosine kinase inhibitors.[16] In contrast, cases of mastocytosis that lacked *KIT* D816V mutations have been effectively treated with tyrosine kinase inhibitors, including a case of pediatric cutaneous mastocytosis that responded to imatinib and had a mutation identical to that found in our mast cell sarcoma case 2.[17] In the case of uterine mast cell sarcoma reported by Ma *et al.*, which lacked a detectable *KIT* mutation, the patient achieved a durable clinical response with imatinib therapy, and in our case 2, which contained a known imatinib-sensitive *KIT* mutation, initiation of imatinib therapy coincided with a decrease of the patient's serum tryptase to the normal range and a tumor-free interval of well over a year, although recurrent disease was subsequently detected in a regional lymph node.

There is limited evidence for efficacy of other therapies in mast cell sarcoma. Radiation therapy failed to produce a response in our Case 1, and produced a minimal radiologic response in our Case 3; this is consistent with the limited efficacy noted in other case reports. Radiation therapy has been shown to be ineffective at reducing mast cell numbers or activation *in vitro*,[18] suggesting that this treatment modality may not be adequate to control the expansion of mast cell sarcomas. In our patient 1, the use of cytotoxic chemotherapy regimens appropriate for myeloid or lymphoid neoplasia produced only transient responses, consistent with other literature reports.

Our case 2 is of particular interest in that the tumor occurred in a patient who had recovered from infantile cutaneous mastocytosis 17 years previously, and apparently arose in a residual lesion of urticaria pigmentosa in the mucosa of the lip, a clinical evolution similar to that of the case recently reported by Auquit-Auckbur *et al*.[9] Interestingly, case 2 showed urticaria pigmentosa and mast cell sarcoma in two different regions of the same biopsy. These two cases appear to represent malignant transformation of a previously quiescent mast cell clone, implying that at least some cases of mast cell sarcoma may be biologically more closely related to pediatric cutaneous mastocytosis than to adult systemic mastocytosis. This hypothesis is supported by the fact that the exon 8 *KIT* mutation seen in case 2 is more characteristic of pediatric cutaneous mastocytosis than adult systemic mastocytosis.[19] There are at least two prior reports of cutaneous mastocytomas with atypical cytological features,[20,21] including occasional neoplastic mast cells with bilobed nuclei, but the cases of cutaneous mast cell sarcoma differed from these in showing frankly malignant cytomorphology, with a high degree of tumor cell anaplasia, numerous mitoses (including atypical forms), and a relatively high Ki-67 index.

In summary, mast cell sarcoma is an aggressive mast cell neoplasm with clinical, histological, and

genetic features distinct from other forms of mastocytosis, and which may rarely arise due to late malignant transformation of cutaneous mastocytosis. Although this diagnosis is readily confirmed with specific immunohistochemical markers, its rarity and anaplastic morphology present a diagnostic challenge. Given the specific therapeutic possibilities available to patients with this entity, including KIT tyrosine kinase inhibitors and therapies optimized for other aggressive myeloid neoplasms, it is essential for pathologists to accurately diagnose this rare tumor type.

Author's note added in proof

Following acceptance of this manuscript, two additional cases of mast cell sarcoma were described in a report by Georgin-Lavialle *et al.* (Georgin-Lavialle *et al.* Mast cell sarcoma: a rare and aggressive entity-report of two cases and review of the literature. J Clin Oncol 2012 Nov 5. (E-pub ahead of print) PMID: 23129735). One tumor arose in a patient with a longstanding history of familial indolent mastocytosis with urticaria pigmentosa. The second case was initially misdiagnosed as anaplastic large cell lymphoma (due to CD30 expression), and later showed a transient partial response to steroids and dasatinib. Both cases lacked the KIT D816V mutation; the latter case harbored a KIT exon 11 mutation (p. Val560Gly). Both tumors showed an aggressive clinical course; the patients died 6 months and 13 months after initial diagnosis.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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