Case Report

Systemic mastocytosis with associated clonal hematologic non-mast cell lineage disease (SM-AHNMD) involving chronic myelogenous leukemia with complex cytogenetics: A case report and literature review

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Abstract: Systemic mastocytosis with associated clonal hematologic non-mast cell lineage disease (SM-AHNMD) is a subtype of systemic mastocytosis (SM) characterized by neoplastic proliferation of mast cells in association with a hematologic neoplasm defined by the World Health Organization (WHO) criteria. SM-AHNMD is more commonly associated with myeloid neoplasms such as, chronic myelomonocytic leukemia (CMML), myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), myeloproliferative neoplasms (MPN), and occasionally exists with lymphoid neoplasms such as B-cell lymphoproliferative disorders and plasma cell dyscrasia. Although SM-AHNMD is more commonly associated with a myeloid malignancy, an association with chronic myelogenous leukemia (CML) is rarely seen. To date, less than a handful of SM-AHNMD associated with CML cases have been reported. We present another case of SM-AHNMD associated with CML and a review of the literature.

Keywords: chronic myelogenous leukemia, systemic mastocytosis, SM-AHNMD

Introduction

Mastocytosis is a heterogeneous neoplasm characterized by clonal proliferation of mast cells. The current edition of World Health Organization (2008) subclassifies mastocytosis into seven categories: 1. Cutaneous mastocytosis, 2. Indolent systemic mastocytosis, 3. Systemic mastocytosis with associated clonal hematologic non-mast cell lineage disease (SM-AHNMD), 4. Aggressive systemic mastocytosis, 5. Mast cell leukemia, 6. Mast cell sarcoma, 7. Extracutaneous mastocytoma [1]. Systemic mastocytosis (SM) is defined by an abnormal growth and accumulation of clonal mast cells in one or more extracutaneous organs, most commonly in the bone marrow and has variable clinical course and prognosis. Criteria for SM according to WHO (2008) include major criterion of multifocal, dense infiltrates of mast cells ($\geq$15 mast cells in aggregates) detected in sections of bone marrow and/or other extracutaneous organs, and four minor criteria including >25% of the mast cells in the filtrate or aspirate smear are spindle-shaped or have atypical morphology; presence of an activating point mutation at codon 816 of KIT in bone marrow, blood or other extracutaneous organs; mast cells that express CD2 and/or CD25 in addition to normal mast cell markers; and serum total tryptase exceeds 20 ng/mL (unless there is an

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associated clonal myeloid disorder, in which case the parameter is not valid). The diagnosis can be made when the major criterion and one minor criterion or in the absence of the major criterion at least three minor criteria are met [1].

SM-AHNMD is a fairly common subtype of mastocytosis and has been estimated to represent 30-40% of all cases of mastocytosis [2]. The associated clonal hematologic disease must be one of the defined myeloid or lymphoid malignancies based on WHO (2008) criteria and can be diagnosed before, after, or concurrently with SM. Occasionally, the diagnosis in the bone marrow can be challenging since the mast cell infiltrate can be subtle or be obscured by the non-mast cell hematologic disease process [3]. More commonly, the AHNMD component is a myeloid neoplasm such as CMML, MDS, AML, or MPN. Rarely the AHNMD component occurs with B-cell neoplasms [4]. Although the literature seems to suggest the relatively common occurrence of SM-AHNMD with myeloproliferative neoplasms, detailed review shows very rare reported occurrences with chronic myelogenous leukemia (CML). The earliest report of SM-AHNMD associated with CML appears to come from Agis *et al*. in 2005, where CML and SM were diagnosed concurrently [5]. Subsequent large study series of systemic mastocytosis only yielded rare cases of CML while a few more cases were presented in the form of case reports. We report a rare and interesting case of SM-AHNMD involving CML with complex cytogenetics and a review of the literature.

Case Report

Clinical History

The patient is a 77-year-old man with a complex past medical history including gastroesophageal reflux disease, hyperlipidemia, obstructive sleep apnea, depression, lung cancer status post surgery in 1998, with recurrence in 2002, and prostate cancer status post radical prostatectomy in 2002. The patient has no significant past family history and splits his time between New Mexico in the summer and Arizona in the winter. In November 2009, he was diagnosed with chronic myelogenous leukemia (CML), chronic phase, in New Mexico, and was subsequently started on imatinib (400 mg) and followed exclusively with RT-PCR for response. At the end of 2010, based on clinical evidence of disease progression, he was switched to dasatinib (100 mg), which achieved a transient response but also caused side effects including skin rash, fatigue, and shortness of breath. He was maintained on dasatinib until May 2011 when he was found to have a rise in BCR-ABL transcript level and was referred to our institution for consideration of clinical trial for ponatinib. While under the care at our institution, he was deemed ineligible for the ponatinib trial and was continued on dasatinib. He underwent bone marrow biopsies in June 2011 and February 2012 as a part of his care, both of which showed persistent molecular evidence of CML. His CBC during this time was only remarkable for mild anemia and thrombocytopenia.

He returned to Arizona in February 2012 and established care at Mayo Clinic in Scottsdale. He was maintained on dasatinib until a BCR-ABL PCR level of 5% (IS) which prompted a switch to ponatinib. He tolerated ponatinib well with only an initial side effect of skin rash. A bone marrow biopsy was performed at Mayo Clinic in May 2013 which showed persistent CML and new finding of systemic mastocytosis, which prompted a new diagnosis of SM-AHNMD. He was subsequently taken off of ponatinib in October 2013 due to temporary market withdrawal and placed back on dasatinib. A tyrosine kinase inhibitor resistance testing was proposed but not performed due to cost. In January of 2014, he returned to our institution for care and a bone marrow biopsy was performed to assess disease status. He also had an elevated serum tryptase level of 31 ng/mL at that time. Shortly after the bone marrow biopsy, the patient returned to New Mexico and has not been seen at our institution since.

Table 1: Bone marrow biopsies with corresponding cytogenetics and molecular results.

Biopsy Date	Cytogenetics	BCR-ABL1% (IS)
June 2011	46,XY,del(20)(q11.2)[3]/46,XY,t(2;17;7)(p23;q23;q11.2), t(9;22)(q34;q11.2)[3]/46,XY,t(2;17;7),del(20)[2]/46,XY,t(2;17;7)[1]/ 46,XY[11]	5.18%
February 2012	46,XY,t(2;17;7)(p23;q23;q11.2)[6]/46,XY,del(20)(q11.2)[3]/ 46,XY,sl,t(9;22)(q34;q11.2)[1]/46,XY[11]	1.16%
May 2013 (outside institution)	46,XY,t(2;17;7)(p23;q21;q11.2)[7]/46,sl,del(11)(q21q23), del20(q11.2q13.3)[2]/46,sl,t(4;11)(q21;q23)[1]/46,XY, del(20)(q11.2q13.3)[1]/46,XY[9]	4.8%
	BCR-ABL1 FISH: Negative	
January 2014	46,XY,t(2;17;7)(p23;q23;q11.2)[8]/46,XY,del(20)(q11.2)[3]/46,XY, t(2;17;7),del(11)(q14q23),del(20)[3]/46,XY[7]	0.19%
	MDS FISH panel: Negative	

Pathology

Bone Marrow Biopsy (June 2011)

The bone marrow biopsy showed a normocellular marrow (30%) with trilineage hematopoiesis and no morphologic evidence of residual CML; however, cytogenetic studies showed a complex karyotype including t(9;22) and BCR-ABL by PCR was 5.18% (IS), consistent with persistent disease (Table 1).

Bone Marrow Biopsy (February 2012)

The bone marrow biopsy showed similar findings as the biopsy from June 2011 but appears slightly hypercellular with trilineage hematopoiesis and no morphologic evidence of residual CML. Similarly, cytogenetic studies showed a complex karyotype including t(9;22) and BCR-ABL was 1.16% (IS), consistent with persistent disease (Table 1).

Bone Marrow Biopsy (May 2013)

The bone marrow biopsy performed at an outside institution showed normocellular marrow (30%) with trilineage hematopoiesis; however, there were rare hypolobate megakaryocytes suspicious for persistent CML. Although cytogenetics was negative for t(9;22), it showed a similar complex karyotype as prior biopsies but with additional abnormalities of deletion 11q and t(4;11) (Table 1). FISH analysis for BCR-ABL confirmed the absence of t(9;22). BCR-ABL by PCR showed evidence of persistent disease at 4.8% (IS). Additionally, atypical mast cells were present by morphology and immunohistochemistry (tryptase+, CD117+, CD25+, and CD2-), and KIT D816V mutation analysis was positive. There was also no significant marrow fibrosis. Based on the overall findings, the diagnosis of SM-AHNMD was made.

Bone Marrow Biopsy (January 2014)

The bone marrow biopsy showed apparent hypercellular marrow (estimated 50-60%) but no morphologic evidence of residual CML. Cytogenetics results continued to demonstrate a complex karyotype similar to previously reported (Table 1). BCR-ABL was again positive at 0.19% (IS). A panel of immunohistochemical stains was performed to assess for atypical mast cells and showed mildly increased number of phenotypically abnormal mast cells (tryptase+, CD117+, CD25+, and CD2-) consis-

tent with mastocytosis and previously diagnosed SM-AHNMD (Figure 1). Significant marrow fibrosis was not identified.

Based on the findings in bone marrow biopsies from May 2013 and January 2014, a retrospective examination of the bone marrow biopsies from June 2011 and February 2012 was performed and rare morphologically atypical mast cells were identified in the aspirate smears (Figure 2). However, the morphologic findings did not fully meet WHO criteria for SM and further follow-up studies were not performed.

Discussion and Literature Review

SM-AHNMD is fairly common subtype of mastocytosis. The diagnosis requires fulfillment of criteria for SM and a non-mast cell hematologic disorder. The AHNMD can be a myeloid or lymphoid neoplasm. In most cases, a myeloid malignancy, such as CMML, MDS, AML, or MPN, is diagnosed. Interestingly, association with CML has rarely been reported. Our case fulfills the three minor WHO criteria for SM-AHNMD but also demonstrates an unusual complex cytogenetics. Several interesting aspects of the case will be discussed, as well as a review of previously reported cases.

The diagnosis of SM-AHNMD should be considered when examining myeloid neoplasms in particular; however, given the rarity of association with CML, a systematic approach of SM assessment in cases of CML is probably not necessary. In other cases, particularly CMML and AML, a general evaluation for mast cells on the aspirate smear should be performed routinely. In the event where morphologically atypical mast cells are identified, immunophenotypic analysis by flow cytometry or immunohistochemistry should be considered. If phenotypically abnormal mast cells are present (CD25+ and/or CD2+), molecular analysis for KIT D816V mutation should be performed. As seen in our case, atypical mast cells are not always readily identifiable on H&E sections of the core biopsy, but immunohistochemistry can provide increased sensitivity in detection of mast cells as well as immunophenotypic abnormalities.

Our patient's diagnosis of SM-AHNMD was made in May 2013; however, retrospective review of the previous bone marrow biopsies showed rare atypical, elongated mast cells in the aspirate smears. The finding raises the possibility of early development of SM that was undetected. Unfortunately, we were not able to perform additional follow-up studies such as PCR or immunohistochemistry to confirm this possibility.

Multiple chromosome analyses were performed on this patient. The first two analyses (performed in June 2011 and Feb 2012) detected multiple abnormal cell lines with a number of chromosomal abnormalities including a t(9;22) resulting in the BCR-ABL1 gene fusion, a deletion within the long arm of chromosome 20, and a three-way translocation involving chromosomes 2, 17 and 7. The t(9;22) is consistent with the diagnosis of CML. 20q deletion is a common secondary abnormality to t(9;22). The three-way translocation represents a rare chromosomal abnormality that results from exchanging segments involving 3 different chromosomes. Some three-way translocations may represent rare variants of known characterized translocations in hematological malignancies. However, this particular three-way translocation has not been reported as a recurrent abnormality and the genes involved in this translocation are not known. Therefore, the clinical significance of this three-way translocation is uncertain. Due to the lack of the original test result at diagnosis, it is not clear whether this three-way translocation arose as a secondary abnormality to t(9;22) or as a concurrent primary abnormality. Interestingly, the three-way translocation maintained the similar level over time, irrespective of the therapy. The third and fourth chromosome analyses (performed in May 2013 and Jan 2014) both identified the same three-way translocation and 20q deletion as seen in the previous tests and a novel deletion within the long arm of chromosome 11 as a newly emerging clonal abnormality.

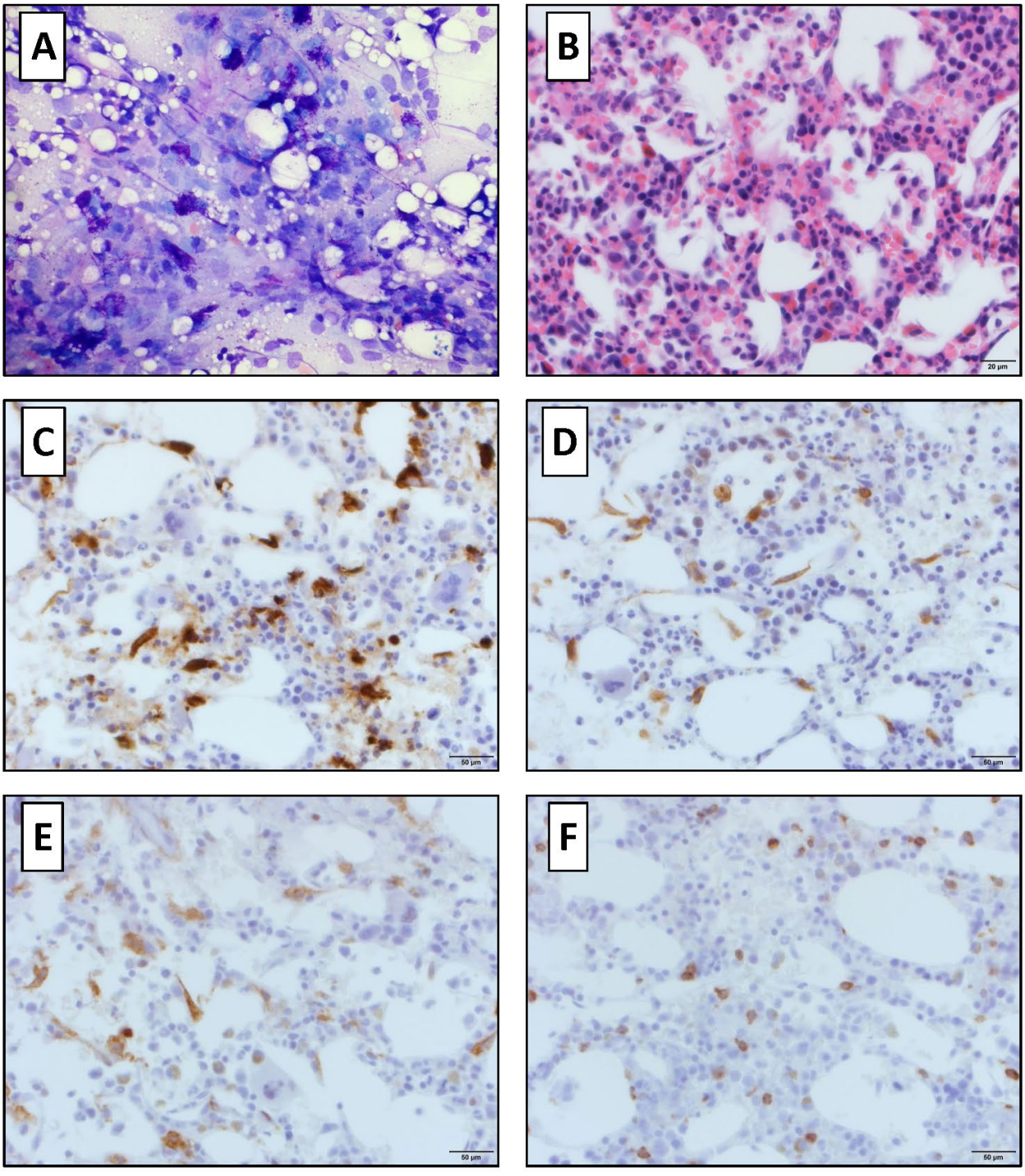

Figure 1: Bone marrow biopsy from January 2014.
Morphologically atypical mast cells are increased on the aspirate smear (A) but are not readily seen in the core biopsy (B). Immunohistochemistry demonstrated increased atypical mast cells that express mast cell tryptase (C), CD117 (D), CD25 (E), and negative for CD2 (F). All images are at 400x magnification.

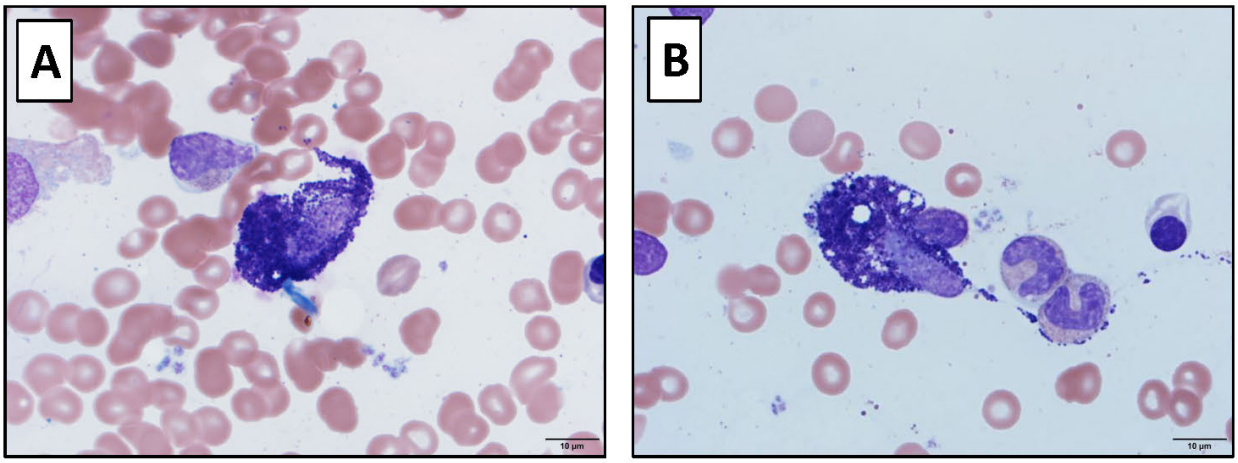

Figure 2: Retrospective examination of bone marrow biopsies from June 2011 (A) and February 2012 (B). Both biopsies show rare morphologically atypical mast cells with elongated nuclei. Images are at 1000x (oil) magnification. (Bone marrow biopsy from May 2013 performed at an outside institution was not available for photography but reported similar findings.)

However, t(9;22) was not present in these two latter tests. The absence of t(9;22) and the presence of the novel 11q deletion suggest the existence of a persistent hematologic malignancy independent of the Ph-positive CML and could possibly represent clonal evolution. Despite the complex cytogenetic findings, the patient's performance and disease status appear to be stable. It would be interesting to compare the results from these cytogenetic studies to the diagnostic sample, however, the diagnostic sample results were not available and could not be obtained.

In comparison to the previously reported cases of SM-AHNMD associated with CML, our case showed similar findings (Table 2). In the case reported by Agis *et al*., a 43-year-old female was diagnosed with CML and SM concurrently [5]. The mast cells demonstrated atypical morphology and aberrant CD25 expression by immunohistochemistry. KIT D816V mutation was also positive. Interestingly, additional molecular analysis of the KIT mutation was carried out on microdissected bone marrow cells in attempt to identify the mutation in non-mast cells. Results showed presence of KIT mutation in the mast cells and not in the non-mast cells. This finding is suggestive of two separate neoplastic clones in that patient. Similar results have also been described in SM-AHNMD associated with AML, but not in SM-AHNMD associated with CMML, where the KIT mutation is also detectable in non-mast cells [6]. In a second case of SM-AHNMD associated with CML, described by Hussein *et al*., a single case was identified from 81 total cases of CML [7]. The patient also had concurrent diagnosis of CML and SM. Similar to our case, the mast cell infiltrate was difficult to identify by H&E but was readily visible by immunohistochemistry and showed aberrant expression of CD25. Surprisingly, the KIT D816V mutation was negative in microdissected mast cells. In addition, the CML component of the disease revealed the typical t(9;22) by cytogenetics, and an additional finding of trisomy 17 was also identified in the same clone. Since KIT mutation was negative in this case, the authors looked for t(9;22) and trisomy 17 by FISH in microdissected mast cells. The result showed normal signal patterns in the mast cells thus suggesting that the mast cells are not clonally related to the CML cells. In our case, we were not able to carry

Table 2: Cases of reported CML with mastocytosis and pertinent findings.

Case	Reference	*KIT* D816V	Mast cell immunophenotype	Other findings
1	Agis *et al*. 2005	Positive	MCT+, CD117+, CD25+	Treated with imatinib resulted in complete cytogenetic remission of CML
2	Horny *et al*. 2006	Positive	MCT+, CD117+, CD25+	n/a
3	Hussein *et al*. 2011	Negative	MCT+, CD117+, CD25+	t(9;22) and trisomy 7 found in same clone
4*	Cairoli *et al*. 2004	Negative#	n/a	Treated with imatinib resulted in remission of CML and absence of *KIT* mutation
5*	Vigil *et al*. 2011	Negative	n/a	Treated with dasatinib+Ara-C for 11 months and achieved remission
6	Current case	Positive	MCT+, CD117+, CD25+, CD2-	Complex cytogenetics including t(2;17;7) and t(9;22) in same clone

* Cases that did not meet the WHO criteria for SM-AHNMD but nevertheless showed an increased number of mast cells along with CML.
# Positive for variant *KIT* D816Y mutation.
MCT: mast cell tryptase; n/a: not available.

out similar investigative steps to further elucidate the clonal relationship of mast cells and non-mast cells. A possible avenue would be to sort for mast cells and compare KIT mutation and t(9;22) status in mast cells and non-mast cells. However, given the relative low number of mast cells present in the bone marrow, the process yield is expected to be low. A third case of SM-AHNMD associated with CML reported by Horny *et al*. had limited data but did show CD25 positive spindled mast cells along with KIT D816V mutation in a patient with chronic phase CML [8].

Two additional cases that did not fulfill the criteria for SM-AHNMD but are worthy of discussion include a 36-year-old male with CML and subsequently found to have bone marrow mast cell infiltrates five years later, which prompted a KIT mutation analysis that showed a D816Y variant of the mutation [9]. The mast cell infiltrate, however, was of normal morphology. Based on these findings, a diagnosis of SM-AHNMD was not reached. Interestingly, levels of BCR-ABL1 and the KIT mutation were monitored post-treatment with imatinib, which showed a decrease of BCR-ABL1 and an absence of the KIT mutation. The authors suggested that this could represent a single clone and it is likely that the KIT mutation added growth advantage to the CML cells and may account for the transient bone marrow mastocytosis. A second case published by Vigil *et al.*, described an unusual case of CML, blast phase, presenting as various forms of mast cell neoplasms [10]. A diagnosis of SM was considered in the case but was ultimately not made due to a lack of KIT D816V mutation and a normal tryptase level. An interesting aspect of the case was the complete response of mast cell lesions and CML in response to dasatinib therapy, suggesting that the variable cell line involvement was a manifestation of the underlying CML.

It seems that literature evidence exists both for and against the idea that CML with associated SM arise from a single pleuripotent stem cell. Not exclusive to CML associated SM-AHNMD, two theories on the pathogenesis of SM-AHNMD in general has been proposed. The first involves an activating KIT mutation that occurs along with other genetic abnor-

malities in a myeloid stem cell, and could result in a proliferative advantage to the mutated stem cells and lead to mast cell differentiation and proliferation [4]. Evidence for this idea has been shown through FISH analysis for t(8;21) in a case of AML with t(8;21) and SM-AHNMD, where the t(8;21) fusion transcript was detected in both mast cells and leukemic blasts [11]. A second proposed mechanism suggests a transformation of a subclone of the myeloid progenitor cells through an acquired KIT mutation leading to the development of SM [4]. Evidence for this possible mechanism is illustrated by the case discussed earlier reported by Vigil *et al*. Given the extremely small number of reported cases of CML associated SM-AHNMD, it is difficult to determine the disease pathogenesis and more cases are required for larger studies. In our case, the patient's CML and SM appear to be managed and controlled by dasatinib since 2013, and there was no significant disease progression from the 2013 biopsy to the 2014 biopsy, in fact, the BCR-ABL1 had decreased significantly. One can argue that this is suggestive of a subclone transformation from a single myeloid progenitor clone that gained mast cell differentiation and KIT mutation.

In summary, this case represents another rare and interesting case of SM-AHNMD associated with CML. In contrast to other types of SM-AHNMD, where the two disease processes usually have different therapeutic targets, the association with CML offers a common therapeutic option of single agent TKIs such as dasatinib or imatinib. The response appears to be effective in treating both components of the disease and does not usually pose a significant treatment dilemma.

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