Hidden Mastocytosis in Acute Myeloid Leukemia With t(8;21)(q22;q22)

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Key Words: AML with t(8;21); Systemic mastocytosis; Myelomastocytic leukemia; Exon 17 KIT mutations; D816V; N822K; Tryptase; CD25

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ABSTRACT

Objectives: To assess the frequency of systemic mastocytosis (SM) in a large series of acute myeloid leukemia (AML) with t(8;21)(q22;q22).

Methods: We retrospectively characterized 40 bone marrow aspirate smears and biopsy specimens from patients with AML with t(8;21) for the presence of SM. Cases were assessed for mast cell morphology and immunohistochemistry, as well as KIT exon 8 and 17 mutational assessment by reverse transcription polymerase chain reaction.

Results: Four patients met criteria for SM, 1 met criteria for myelomastocytic leukemia, and 8 demonstrated the benign finding of mast cell hyperplasia.

Conclusions: We recommend examining all cases of AML with t(8;21) for the presence of SM via morphology, immunophenotyping, and KIT mutational analysis studies.

Acute myeloid leukemia with t(8;21)(q22;q22); RUNX1-RUNX1T1 [AML with t(8;21)] is an acute leukemia with a recurrent genetic mutation resulting from the fusion of genes RUNX1 (AML1) on chromosome 8 with the RUNX1T1 (ETO) gene on chromosome 21. It is 1 of 2 core binding factor (CBF) acute leukemias (along with AML with inv16(p13.1q22); CBFB-MYH11), and the fusion protein created by the translocation serves as a transcriptional regulator resulting in differentiation toward the neutrophil lineage.1 AML with t(8;21) comprises 5% to 8% of all cases of AML, and its incidence is more common in younger patients. When possessing t(8;21) as the sole

Upon completion of this activity you will be able to:
• list the types of acute myeloid leukemias (AMLs) that are considered core binding factor leukemias and describe their relative association with mutations in the KIT proto-oncogene.
• outline the prognostic role KIT mutations are thought to confer in AML with t(8;21).
• list the morphologic, immunophenotypic, molecular, and other laboratory criteria used to establish the diagnosis of systemic mastocytosis (SM).
• define the difference between type I and type II mast cell morphology as it pertains to the diagnosis of SM.
• list the criteria required for the diagnosis of SM associated with clonal non–mast cell hematologic diseases, including AMLs.

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cytogenetic abnormality, AML with t(8;21) is associated with a good prognosis with higher clinical remission rates, lower rates of relapse, and higher overall survival compared with other AMLs with either normal karyotypes or complex karyotypic abnormalities when treated with high-dose cytarabine.\(^2\,^3\)

\[\text{KIT}\] encodes for a receptor tyrosine kinase present on the cell surface of hematopoietic cells as well as other cell types, including melanocytes and germinal cells. Physiologically, it binds to stem cell factor (KIT ligand), which results in dimerization of the protein, activation of its tyrosine kinase function, and stimulation of various downstream signaling pathways that culminate in DNA transcription.\(^4\,^5\) \text{KIT} is mutated in a variety of neoplasms, including systemic mastocytosis (SM), gastrointestinal stromal tumors, melanoma, and testicular seminoma.\(^6\,\)\(^-\)\(^10\) Most mutations are point mutations that result in a gain of function leading to increased proliferation. Similar to AML with inv(16)(p13;q22), AML with t(8;21) is associated with an increased incidence of \text{KIT} mutations compared with other AMLs. The frequency of \text{KIT} mutations reported in a series of AML with t(8;21) ranges from 19% to 48%.\(^1\)\(^1\)\(^-\)\(^1\)\(^3\) Most \text{KIT} mutations in AML with t(8;21) are located within exon 17 and are most commonly D816V, but mutations within exon 8 have also been reported.\(^1\)

Several large retrospective studies assessed the prognosis of \text{KIT} mutation-positive AML with t(8;21), and most suggest that activating \text{KIT} mutations confer a worse prognosis. Care et al\(^1\)\(^1\) examined 110 CBF leukemias and noted that mutations within exon 8 were associated with an increased relapse rate but not a difference in overall survival. Paschka et al\(^1\)\(^2\) investigated 61 adults with CBF leukemias and noted that exon 17 and exon 8 mutations were associated with an increased relapse risk in both AML with inv(16) and AML with t(8;21), but adverse outcome survival was seen only in AML with inv(16). Cario et al\(^1\)\(^4\) evaluated 67 patients with CBF AML, including 42 patients with AML with t(8;21), and specifically noted an increased risk of relapse and decreased overall survival in AML with t(8;21) bearing the exon 17 \text{KIT} D816V mutation. To our knowledge, only 1 large pediatric series of AML with t(8;21) has been conducted. Pollard et al\(^1\)\(^5\) examined data from 113 pediatric patients with AML with t(8;21) from 4 different oncology group trials and noted no difference in outcomes between the \text{KIT}\(^+\) and \text{KIT}\(^−\) patients in terms of clinical remission rates, relapse rates, and overall survival; this was independent of the type of \text{KIT} mutation present, including codon 816 mutations.

A reported association of AML with t(8;21) is concurrent SM (SM-AML), and several case reports have noted SM in AML with t(8;21).\(^1\)\(^6\)\(^-\)\(^1\)\(^9\) Pullarkat et al\(^2\)\(^0\) examined a multi-institutional series of 10 patients with SM-AML. In 6 patients, SM was prominent after induction chemotherapy, with the mast cells revealing an absence of blasts. Mutations in \text{KIT} (eg, D816V, D816Y) were identified in 7 of 9 cases tested. Of the 10 patients treated with standard chemotherapy regimens, 1 demonstrated complete remission and 6 died of progressive or relapsed leukemia. Mast cells persisted in 2 patients after hematopoietic stem cell transplant, with the mast cells in each case bearing the \text{RUNX1-RUNX1T1} translocation. However, to our knowledge, no large prospective study has established the frequency of SM in AML with t(8;21) and its effect on overall prognosis.

### Materials and Methods

This study was approved by the Institutional Review Board of Stanford University, Stanford, CA, where the study was conducted.

Patients with AML with t(8;21)(q22;q22) identified by conventional karyotype and/or fluorescence in situ hybridization (FISH) studies were identified retrospectively from the Stanford University Department of Pathology database from 1989 through 2011. Eighty-two such patients were identified; 40 had Bouin’s fixed/Formical 4 (Decal Chemical, Tallman, NY) decalcified trephine bone marrow samples available for study [Table I](#TableI). The diagnosis of AML with t(8;21) was confirmed by review of morphology,

<table>
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<tr>
<th>Table I</th>
<th>Study Demographics of Patients With AML With t(8;21)(q22;q22)</th>
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<tbody>
<tr>
<td>Characteristic</td>
<td>Value</td>
</tr>
<tr>
<td>Total No. of patients with AML with available bone marrow samples for study</td>
<td>40</td>
</tr>
<tr>
<td>No. of diagnostic bone marrow specimens</td>
<td>32</td>
</tr>
<tr>
<td>No. (%) of patients with bone marrow tissue available for exon 17 \text{KIT} mutation testing</td>
<td>15 (37.5)</td>
</tr>
<tr>
<td>Age, mean (range), y</td>
<td>38.9 (5-61)</td>
</tr>
<tr>
<td>No. of pediatric patients (age range, y)</td>
<td>7 (5-18)</td>
</tr>
<tr>
<td>Sex ratio, M:F</td>
<td>2:1</td>
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</table>

AML, acute myeloid leukemia.
histopathology, flow cytometry, and cytogenetic studies. Thirty-two cases represented the initial diagnostic bone marrow specimen, and 8 cases were studies obtained at relapse or with residual leukemic burden. Wright-Giemsa–stained aspirate smears and H&E-stained trephine biopsy specimens were examined for the presence, morphology, and architectural pattern of mast cell involvement. Mast cell morphology was assessed predominantly on Wright-Giemsa–stained aspirate slides and included either spindle-shaped mast cells (atypical type I mast cells) or mast cells with bilobed nuclei, open chromatin, and hypogranular cytoplasm (atypical type II mast cells; promastocytes). The major criterion considered involved by SM if they met either 1 major and 1 minor criterion or 3 minor criteria. The reaction (PCR) products were confirmed electrophoretically by sequencing on an ABI 3130 platform (Applied Biosystems, Carlsbad, CA). Analysis of mast cell mutation status was assessed on frozen bone marrow aspirate samples where available. After isolating RNA from fresh-frozen patient samples stored at –80°C, the sample RNA was converted to complementary DNA (cDNA) using random primers and Moloney murine leukemia virus reverse transcriptase enzyme. Exons 8 and 17 of the c-kit gene were amplified using the cDNA as the template. The polymerase chain reaction (PCR) products were confirmed electrophoretically on an ethidium bromide–stained agarose gel before being analyzed by sequencing on an ABI 3130 platform (Applied Biosystems, Carlsbad, CA).

Cases were classified for the presence of SM using the 2008 World Health Organization classification and considered involved by SM if they met either 1 major and 1 minor criterion or 3 minor criteria. The major criterion was the presence of multifocal dense aggregates comprising at least 15 mast cells per aggregate. The minor criteria included (1) atypical mast cell morphology in greater than 25% of the total mast cells, (2) aberrant expression of CD25 and/or CD2, and (3) the presence of the activating c-kit mutation D816V. Serum tryptase levels could not be used as a diagnostic criterion in cases with an associated non–mast cell hematologic disease, such as AML. The diagnosis of mast cell hyperplasia was made when immunohistochemistry revealed at least a 5% burden of mast cells with the absence of any SM criteria. Cases with less than 5% mast cell burden with normal round mast cell morphology were regarded as negative for mast cell burden or disease. Corresponding clinical information, including incidence of relapse, time to remission, and length of survival, was obtained for all patients where available.

### Results

Forty patients had bone marrow core biopsy specimens and aspirates available for morphologic and immunohistochemical assessment. Fourteen (35%) patients revealed either clustered mast cells or an interstitial increase in mast cells of 5% or greater on tryptase immunohistochemistry. The architectural distribution of mast cells ranged from an interstitial population of mast cells to dense aggregates of mast cells in a paratrabeular location or arranged around vessels or sinusoids. Four cases demonstrated dense multifocal clusters of mast cells, whereas 10 had an interstitial increase in mast cells without clustering.

Four cases (patients 1-4) demonstrated mast cells present in multifocal dense aggregates. Dense aggregates ranged from perisinusoidal, perivascular, and/or paratrabeular in location. The D816V c-kit mutation was identified in 3 of these cases (patients 1-3), although microdissection of mast cells was not performed. Patients 1, 2, and 4 represented the initial diagnostic marrow. Patient 3 represented a bone marrow biopsy specimen obtained 44 days after initiation of therapy that demonstrated persistent leukemic burden after induction chemotherapy; the original diagnosis of AML with t(8;21) was made on a peripheral blood specimen.

Ten of 40 cases (patients 5-14) showed an interstitial pattern of mast cells without clustering, with the percentage of mast cells ranging from 5% to 20% of total nucleated cells (Table 1). Mast cells lacked expression of CD2 and CD25; an N822K exon 17 mutation was identified by reverse transcription PCR. Although this case would appear to be an unmasking of SM after chemotherapy, the lack of sufficient criteria precludes the diagnosis of SM, and a diagnosis of myelomastocytic leukemia (MML) was established. Briefly, MML is a rare disease most commonly associated with some subtypes of myelodysplastic syndrome or AML whereby elevated numbers of immature mast cells are present but criteria for SM are not met. Since this case showed interstitial mast cells with immature morphology, lack of expression of CD25 or CD2, and absence of the D816V mutation, the diagnosis of MML fit most appropriately.

The differential diagnosis of an increased interstitial mast cell infiltrate also includes mast cell hyperplasia, a benign finding in which mast cells lack expression of CD2 or CD25,
Table 2
Clinical and Pathologic Findings of Mast Cell Disease in Cases of AML With t(8;21)(q22;q22)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, y/Sex</th>
<th>Cytogenetics/Karyotype</th>
<th>KIT Exon 17 Mutation Status</th>
<th>Mast Cell Morphology, Histology, and Immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>48/F</td>
<td>46,XX,t(8;21)(q22;q22)[20]</td>
<td>D816V</td>
<td>Interstitial and clustered mast cells with spindle morphology comprising 15% of marrow; CD25+</td>
</tr>
<tr>
<td>Patient 2</td>
<td>38/F</td>
<td>45,X,X,t(8;21)(q22;q22)[17]/46,XX[5]</td>
<td>D816V</td>
<td>Interstitial and clustered mast cells with spindle morphology comprising 25% of marrow; CD25+</td>
</tr>
<tr>
<td>Patient 3</td>
<td>71/F</td>
<td>46,XX,t(8;21)(q22;q22)[17]/46,XX[5]</td>
<td>D816V</td>
<td>Interstitial and clustered mast cells with spindle morphology comprising 20% of marrow; CD25+</td>
</tr>
<tr>
<td>Patient 4</td>
<td>16/F</td>
<td>46,XX,t(8;21)(q22;q22)[20]</td>
<td>ND</td>
<td>Interstitial and clustered mast cells with predominantly normal morphology if present; CD25+</td>
</tr>
<tr>
<td>Patient 5</td>
<td>64/M</td>
<td>46,XY,t(8;21)(q22;q22)[21]</td>
<td>N822K</td>
<td>Interstitial mast cells with normal morphology comprising 5% to 15% of marrow; CD25+</td>
</tr>
<tr>
<td>Patient 6</td>
<td>39/M</td>
<td>46,XY,der(8)t(8;21)(q22;q22),der (21)t(8;21)(q22;q22)?add(8q24)[946], idem,t(12;20)(q24.1 or q24.3);q11.2 or q13.1[11]</td>
<td>NT</td>
<td>Interstitial mast cells with spindle morphology comprising 10% of marrow; CD25+</td>
</tr>
<tr>
<td>Patients 7-14</td>
<td>Age range 12-81 y</td>
<td>Variable; all included t(8;21)(q22;q22)</td>
<td>N822K (patient 7), ND (patients 8-9), NT (patients 10-14)</td>
<td>Fewer than 5% mast cells; round mast cell morphology if present</td>
</tr>
<tr>
<td>Patients 15-40</td>
<td>Age range 4-67 y</td>
<td>Variable; all included t(8;21)(q22;q22)</td>
<td>N822K (patient 15), D816V (patient 16), ND (patients 17-22), NT (patients 23-40)</td>
<td>Fewer than 5% mast cells; round mast cell morphology if present</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukemia; CMV, cytomegalovirus; CNS, central nervous system; CR, clinical remission; GVHD, graft-vs-host disease; HSCT, hematopoietic stem cell transplant; MML, myelomastocytic leukemia; ND, KIT mutation not detected; NT, not tested (tissue not available for KIT mutation assessment); SM, systemic mastocytosis; VZV, varicella-zoster virus.

* Represents a recurrent/residual involved marrow sample (original not available for review).

* Karyotype of a recurrent/residual involved marrow sample.

are of normal round morphology with appropriate granulation obscuring the nucleus, and lack the D816V KIT mutation. Eight (20%) of 40 cases (patients 7-14) demonstrated mast cell hyperplasia, with the degree of mast cell infiltrate ranging from 5% to 15% of total nucleated cells. In addition, 1 (patient 6) showed an increased population of CD25-positive mast cells in an interstitial pattern; mast cells were spindle shaped and comprised approximately 10% of bone marrow elements by immunohistochemistry. No tissue was available to assess for a KIT mutation (Image 2 and Table 1). As this case meets only 2 minor criteria for SM and because expression of CD25 is not associated with mast cell hyperplasia,25,26 the mast cell findings are atypical and cannot be characterized further.

Clinical data are summarized in Table 2. Twenty-three of 26 patients for whom treatment data were available received treatment with standard induction therapy using cytarabine plus an anthracycline (usually idarubicin or daunorubicin). Two of 5 patients with mast cell disease had sufficient clinical follow-up data, with both patients presumed to have died of relapsed leukemia; patient 5 had MML23 and possessed the N822K KIT mutation, and patient 3 had SM with a D816V KIT mutation. Of the remaining 3 patients, 2 (patients 1-2) were lost to follow-up, and 1 (patient 4) attained clinical remission. Five of 8 patients with mast cell hyperplasia had clinical data available for review; all achieved clinical remission, although 1 (patient 8) died 39 months after bone marrow transplantation of graft-vs-host disease and cytomegalovirus and varicella-zoster virus viremia. Ten of 26 patients with no evidence of mast cell disease had clinical data available for review; all 10 achieved clinical remission, although 1 (patient 24) died 42 months after bone marrow transplantation of graft-vs-host disease and pulmonary emboli. The original leukemic diagnostic bone marrow of the patient with atypical mast cell hyperplasia (patient 6) was not available for review. This patient maintained clinical remission for 86 weeks, relapsed, and later achieved a second remission after salvage chemotherapy. When the patient relapsed a second time, an interstitial pattern of spindle-shaped mast cells was recognized. Cytogenetic studies confirmed the t(8;21) translocation along with a complex karyotype, and the patient died 3 weeks later.
**Table 2**

<table>
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<tr>
<th>Mast Cell Diagnosis</th>
<th>Clinical Outcome</th>
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<tr>
<td>SM</td>
<td>Attained CR with no history of relapsed leukemia noted.</td>
</tr>
<tr>
<td>MML</td>
<td>Attained CR for 47 weeks; subsequent relapse treated with salvage chemotherapy followed by HSCT; relapsed 15 weeks after HSCT; transferred to hospice and presumed deceased.</td>
</tr>
<tr>
<td>Atypical mast cell infiltrate</td>
<td>Attained CR for 86 weeks; subsequent relapse treated with salvage chemotherapy followed by HSCT; relapsed 3 weeks after HSCT and died of AML.</td>
</tr>
<tr>
<td>Mast cell hyperplasia</td>
<td>Data available from 5 patients; all 5 achieved CR. One patient (8) died of VZV and CMV viremia and GVHD without evidence of leukemia at autopsy 39 months after HSCT.</td>
</tr>
<tr>
<td>Negative for mast cell burden</td>
<td>Data available from 10 patients; all 10 achieved CR. One patient (24) died of pulmonary emboli and GVHD without evidence of leukemia at autopsy 42 months after HSCT.</td>
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</table>

Fifteen patients had frozen material available for KIT mutational analysis by PCR. Of the 15 cases assessed for the presence of a mutation in KIT, 4 were positive for the D816V mutation and 3 showed an N822K mutation in exon 17. Three of the 4 (patients 1-3) positive for the D816V mutation also showed SM. The fourth D816V-positive case (patient 16) revealed no mast cell burden by immunohistochemistry or morphology, and this patient is currently in his second clinical remission after initial relapse and subsequent allogeneic stem cell transplant. N822K mutation-positive status corresponded to 1 case of MML (patient 5), 1 case of mast cell hyperplasia (patient 7), and 1 case without SM (patient 15). Patient 5 is presumed to have died of MML, while patients 7 and 15 are currently in clinical remission without history of relapse. No mutations within exon 8 were identified.

Although several patients in this study with SM, MML, or atypical mast cell infiltrates subsequently died of acute leukemia, our sample size of 40 patients is too small to draw conclusions in differences in overall survival. Other comparisons between patients with and without SM were attempted. No difference in percentage of blasts at presentation, average duration to a morphologically or cytogenetically negative bone marrow study after induction, or incidence of relapse was noted between the patients with and without SM, even when adult and pediatric patients were evaluated separately (data not shown). None of the patients with SM were reported to have clinical findings typically associated with aggressive SM, including hepatomegaly, ascites, osteolytic lesions, splenomegaly, or evidence of malabsorption, but such findings were not evaluated for prospectively.

**Discussion**

To our knowledge, this is the largest study assessing the frequency of SM with AML with t(8;21) to date. In this study of 40 patients, we identified 4 (10%) with SM and 1 patient with MML, diseases that were not identified at the original leukemia diagnosis. One additional patient showed an atypical interstitial mast cell infiltrate but lacked appropriate material to perform KIT mutational analysis and establish a diagnosis of SM. Eight (20%) patients showed mast cell hyperplasia, a benign finding but important to recognize in the differential diagnosis of an interstitial mast cell process.

Regarding the origin of the mast cell burden in SM-AML, several studies suggest that the mast cells may be derived from the leukemic clone. Sperr et al.27 examined a case of SM-AML from a 65-year-old woman diagnosed with AML M4 (prior French-American-British classification). This case was positive for the D816V mutation in KIT, and this mutation could be detected in serial trephine studies when mast cells comprised 5% of the hematopoietic elements but not when mast cells represented less than 1% of marrow elements. A subsequent study by Fritsche-Polanz et al.28 examined 101 cases of AML. Seven cases were positive for the D816V mutation in KIT, all of which demonstrated concurrent systemic mastocytosis by tryptase immunohistochemistry. The D816V mutation was present in microdissected mast cells in 4 of 4 cases examined; in addition, the mutation was present in microdissected CD34-positive blasts in 2 of those same 4 cases tested. Pullarkat et al.29 examined 1 case of SM-AML with t(8;21) using FISH and demonstrated that mast cells also possessed the RUNXI-RUNXIT1 translocation. A similar study had been performed earlier by Sperr et al.30 in a case of MML bearing the t(8;21) translocation from a 17-year-old male; likewise, the translocation could be identified within mast cells. Although these advanced techniques could be performed in cases prospectively suspected to be AML with t(8;21), we did not perform such studies in this retrospective analysis.

The prevalence of SM associated with AML with t(8;21) is currently unknown. A recent study by Kristensen et al.31
Mast cell aggregates in systemic mastocytosis associated with acute myeloid leukemia with t(8;21)(q22;q22). Patient 1 showed leukemic burden with increased mast cells in the background, many of which showed atypical type I spindle morphology (A, arrows). Dense mast cell aggregates were present in a perisinusoidal and paratrabecular location (B) with aberrant expression of CD25 (C). This patient had the D816V KIT mutation. Patient 2 also showed dense perivascular mast cell aggregates (D), CD25 expression (E), and the D816V KIT mutation.
A subsequent flow cytometric study on the ensuing induction chemotherapy bone marrow revealed persistent CD25-positive mast cells (F). Patient 3 revealed increased spindle-shaped mast cells on bone marrow aspirate (G, arrow). Mast cells were clustered on a tryptase stain (H), showed membranous expression of CD117 (I), and expressed CD25 (J). This patient also harbored the D816V KIT mutation. Patient 4 demonstrated interstitial (K) as well as perivascular clustered mast cells on closer examination (K, inset).
suggested that SM in CBF leukemias is uncommon. Their study examined the frequency of SM in 20 cases of CBF AML; 4 of 13 cases of AML with t(8;21) and 4 of 7 cases of AML with inv(16) examined carried the D816V mutation assessed by real-time PCR of peripheral blood mononuclear cells. None of the 8 cases had evidence of SM by immunohistochemistry for CD117 and tryptase. This study examined only the cases positive for the D816V KIT mutation in peripheral blood mononuclear cells for the presence of SM in the bone marrow core biopsy specimen. In the study by Fritsche-Polanz et al28 in which SM was present in 7 of 7 D816V-positive AML cases, no CBF-related cytogenetic abnormalities [ie, t(8;21), inv(16)] were seen among those cases, although 5 patients had an original diagnosis of chronic myelomonocytic leukemia. In contrast to this study, we assessed AML with t(8;21) only, and our data suggest that the overall prevalence of SM in

![Image 1](cont) These mast cells expressed CD25 by immunohistochemistry (L) and showed atypical type II morphology with bilobed nuclei, partially condensed chromatin, and hypogranular cytoplasm (M, arrows). (A, Wright-Giemsa, x900; B, tryptase, x400; C, CD25, x600; D, tryptase, x200; E, CD25, x400; G, Wright-Giemsa, x900; H, tryptase, x400; I, CD117, x600; J, CD25, x600; K, tryptase, x200; inset, x600; L, CD25, x600; M, Wright-Giemsa, x1,200.)

![Image 2](The differential diagnosis of interstitial mast cell infiltrates associated with acute myeloid leukemia with t(8;21). Ten of 42 cases assessed revealed increased interstitial mast cells as the predominant mast cell burden. Patient 5 revealed a 5% mast cell burden on the original leukemic presentation bone marrow with atypical type II mast cell morphology (A, arrows). After induction chemotherapy, a bone marrow biopsy specimen revealed persistent leukemic disease and an increase in interstitial mast cells (B) comprising 20% of the involved bone marrow with persistent atypical morphology (C, arrows).
AML with t(8;21) is approximately 10%. Further studies are required to compare the frequency of SM in AML with t(8;21) with other recurrent cytogenetic abnormality AMLs, as well as AML with normal cytogenetics.

Although our study is relatively small compared with multicenter studies assessing for KIT mutations in AML with t(8;21), the frequency of KIT mutations in our study mirrors that noted in other studies.11-15 In addition, we assessed for KIT mutations in exon 8 and exon 17 by reverse transcription PCR followed by direct Sanger sequencing to identify point mutations. Although an advantage of performing such testing is to detect point mutations other than D816V, this method is relatively insensitive for detecting D816V and other point mutations. Although we examined RNA transcripts, in general, Sanger-based methods analyzing DNA generally require 15% to 20% mutation-positive DNA relative to wild-type DNA to

Image 2 (cont) An N822K KIT mutation was identified; the findings are most compatible with myelomastocytic leukemia. Eight cases showed interstitial mast cells ranging from 5% to 15% of the bone marrow elements noted on tryptase immunohistochemistry (D); mast cells were round in morphology (D, inset) and lacked CD25 expression (not shown) consistent with mast cell hyperplasia. Patient 6 revealed an increase in mast cells, predominantly interstitial with loose aggregation around blood vessels (E); higher power assessment revealed atypical spindle-shaped mast cell morphology (E, inset). These mast cells aberrantly expressed CD25 (F). No appropriate tissue was available for KIT mutation analysis, and the findings are atypical but of insufficient criteria for systemic mastocytosis. (A, Wright-Giemsa, ×1,500; B, tryptase, ×200; C, Wright-Giemsa, ×900; D, tryptase, ×400; inset, Wright-Giemsa, ×900; E, tryptase ×200; inset, ×900; F, CD25, ×600.)
yield a positive result. Newer methods, including real-time PCR, peptide–nucleic acid–mediated PCR, and allele-specific PCR, show a much lower limit of detection (lower than 0.01% D816V-positive DNA relative to wild-type DNA) and are thus recommended methods for the detection of SM.\textsuperscript{32-35}

Although the D816V KIT mutation (as well as D816H and D816Y) is known to result in the proliferation of mast cells, much less is known about how other non-D816 mutations contribute to mast cell proliferations. Three cases in our study possessed the N822K KIT mutation, which has been reported infrequently in the literature associated with AML with t(8;21).\textsuperscript{11,12,36} One case was associated with a diagnosis of MML that resulted in a poor patient outcome.\textsuperscript{23,24} Of the other 2 cases, 1 revealed mast cell hyperplasia and 1 showed no mast cell burden on review; interestingly, both of these patients have had clinical remission to date. Thus, it is uncertain what role the N822K mutation plays in general and whether this mutation was directly responsible for the significant mast cell disease burden present in the case of MML.

In addition, it is unclear whether the presence of SM is merely a marker of proliferation in cases of KIT+ AML with t(8;21) or if the mast cell burden contributes to the prognosis in this subgroup of leukemia. Poor outcomes were noted in 2 of our patients with SM-AML, and both of these cases were KIT+. Prospective studies of sufficient size comparing outcome data in cases of KIT+ AML lacking SM, KIT+ AML with concurrent SM, and KIT- AML with concurrent SM would be necessary to determine the contribution of SM in the prognosis of KIT+ AML.

Regarding karyotypic analysis, all 5 cases of SM and MML showed t(8;21) as the only cytogenetic abnormality. However, 1 case with an atypical mast cell infiltrate that could not be characterized further showed a complex karyotype in addition to t(8;21). The complex karyotype is likely related to a clonal evolution of the acute leukemic process as prior cytogenetics showed t(8;21) as the sole abnormality. Since previous core biopsy specimens were not available for review, it is uncertain if the mast cell infiltrate was present at initial presentation or if the mast cell proliferation is related to the complex karyotype. Further studies of AMLs with complex karyotypes would be necessary to clarify each of these findings.

While several studies suggest possible differences in outcomes in D816V KIT+ AML compared with KIT- AML in adult patients, these studies have not demonstrated whether SM was concurrently present.\textsuperscript{11-15} It is also uncertain at this time whether therapy for KIT+ AML with SM should include a tyrosine kinase inhibitor. In prior reviews, cases of SM-AML with t(8;21) tended to be associated with a worse outcome.\textsuperscript{19,20} Thus, it may be prudent to establish a diagnosis of SM earlier with immunohistochemistry rather than awaiting induction chemotherapy, in which the mast cell infiltrate may be more obvious.\textsuperscript{20} Since KIT mutation status is thought to influence overall prognosis of CBF leukemias, including AML with t(8;21), many centers are routinely performing KIT mutation testing in this subgroup of acute leukemias. Because D816V mutations are invariably seen when SM is present in AML with t(8;21), KIT mutation status may serve as an indicator for possible SM when no obvious mast cell burden is noted on initial review. Such cases may benefit from rereview to include immunohistochemistry for occult mast cell infiltrates. It should be noted, however, that cases of SM are occasionally negative for the D816V mutation, necessitating vigilant morphologic assessment for SM at diagnosis.

This study indicates that mast cell infiltrates are sometimes underappreciated at the original diagnosis of t(8;21) leukemia and that the concurrent diagnosis of SM with AML requires a high index of suspicion with morphologic and immunohistochemical evaluation for a neoplastic mast cell population. A subset of cases with mast cell disease resulted in poor outcomes, including death. We recommend routinely assessing for the presence of mast cell disease in all initial diagnoses and subsequent follow-up marrow studies of AML with t(8;21). Longitudinal monitoring of a large cohort of t(8;21) and/or other AML patients with SM will help further establish whether the presence of concurrent mast cell disease truly affects patient prognosis and whether tailored therapy may benefit this subset of patients.

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References


