Myelodysplastic Syndrome With inv(3)(q21q26.2) or t(3;3)(q21;q26.2) Has a High Risk for Progression to Acute Myeloid Leukemia

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Key Words: Myelodysplastic syndrome; MDS; inv(3)(q21q26.2); t(3;3)(q21;q26.2); De novo MDS; Therapy-related MDS; Acute myeloid leukemia

Abstract

Acute myeloid leukemia (AML) with inv(3) (q21q26.2) or t(3;3)(q21;q26.2) is a distinct subtype in the World Health Organization classification. The natural history of myelodysplastic syndrome (MDS) associated with these cytogenetic aberrations is poorly understood. We studied 17 MDS (11 de novo and 6 therapy related) and 3 chronic myelomonocytic leukemia (CMML) cases associated with inv(3) (q21q26.2) or t(3;3)(q21;q26.2). The de novo cases were further classified as refractory cytopenia with multilineage dysplasia (n = 8) and refractory anemia with excess blasts (n = 3). Isolated inv(3)/t(3;3) was identified in 4 cases, whereas –7/7q (n = 13) and –5/5q (n = 6) were common additional aberrations. Nineteen patients died, including 13 in whom the disease progressed to AML after a median of 7 months. Median survival for patients with de novo disease was similar to that for patients with therapy-related MDS (13 vs 17.5 months). MDS or CMML with inv(3)/t(3;3) are aggressive diseases with a high risk of progression to AML.

The inv(3)(q21q26.2) or t(3;3)(q21;q26.2) is present in approximately 1% of patients with myelodysplastic syndrome (MDS) and in a subset of patients with acute myeloid leukemia (AML) and the blast phase of myeloproliferative neoplasms.1-4 Patients with inv(3)/(3;3)-associated myeloid neoplasms often have anemia. The platelet count may be normal or increased. The bone marrow typically shows increased numbers of small hypolobated megakaryocytes and multilineage dysplasia.1,2

In the current World Health Organization (WHO) classification of myeloid neoplasms, AML associated with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) is recognized as a distinct entity, included with the group of AMLs with recurrent genetic abnormalities.3 These AML cases can arise de novo or in patients with MDS. Conventional cytogenetic analysis often shows additional cytogenetic aberrations such as loss of chromosome 7, 5q deletion, or a complex karyotype.2,4 Patients with AML associated with inv(3)(q21q26.2)/(3;3) (q21;q26.2) frequently have disease refractory to conventional chemotherapy regimens and a short overall survival (OS).1,5

In the WHO classification, for some types of AML with recurrent cytogenetic abnormalities, the detection of the cytogenetic or molecular abnormality defines the presence of disease, irrespective of blast count. For example, patients with acute promyelocytic leukemia associated with t(15;17) (q22;q21)/PML-RARA have the disease even when the blast/promyelocyte count is less than the 20% threshold. The WHO classification is much more circumspect for patients with inv(3)(q21q26.2)/(3;3)(q21;q26.2), stating that patients with fewer than 20% blasts “must be monitored
closely for development of more definite evidence of AML. Therefore, such cases are classified as MDS. Clearly, a greater understanding of the natural history of patients with MDS associated with inv(3)(q21q26.2) is needed.

In this study, we analyzed cases of MDS associated with inv(3)(q21q26.2) or t(3;3)(q21q26.2) to better appreciate their clinical and pathologic features and to assess their natural history.

Materials and Methods

Cases of MDS associated with inv(3)(q21q26.2) or t(3;3)(q21q26.2) detected by conventional cytogenetic analysis were obtained from the files of the Department of Hematopathology, M.D. Anderson Cancer Center, Houston, TX, between January 1996 and June 2008. The medical records of each case were reviewed. This study was performed with approval from the institutional review board of our institution.

Air-dried bone marrow aspirate smears and biopsy touch imprints were stained with Wright-Giemsa. Bone marrow biopsy and aspirate clot specimens were fixed in formalin, and cut slides were stained with H&E.

Bone marrow aspirate specimens were prepared for conventional cytogenetic analysis using methods described previously. Metaphases were banded by the standard GTG method. Chromosome abnormalities were identified according to guidelines for cancer cytogenetics. A subset of cases was assessed for RAS and/or FLT3/ITD or FLT3/D835 mutations using methods described before.

Kaplan-Meier estimates were used to assess OS calculated from the date of diagnosis of MDS. Survival curves were compared by using a double-sided log-rank test (Mantel-Cox). The \( \chi^2 \) and Student \( t \) tests were used to analyze the differences between 2 groups of patients. Results were significant at a \( P \) level of less than .05. GraphPad Prism 5 (GraphPad Software, La Jolla, CA) was used for statistical analysis.

Results

Clinical and Pathologic Features

There were 14 cases of de novo disease and 6 were therapy-related MDS (t-MDS). The clinical and pathologic features patients are summarized in Table 1 and Table 2.

The patients were 10 men and 10 women with a median age of 64 years (range, 15-77 years; median, 65 years in the de novo group and 53 years in the t-MDS group. All patients had anemia (hemoglobin level, range 7.1-11.5 g/dL; median, 9.2 g/dL [92 g/L]; reference range, 14-18 g/dL [140-180 g/L]), 14 patients had thrombocytopenia (platelet count, range, 15-119 × 10^3/μL [15-119 × 10^9/L]; median, 93 g/dL [93 g/L]), and 11 patients had leukopenia (WBC count, range, 1.4-15.0 × 10^9/L [1.4-15.0 × 10^3/μL]).

Table 1

<table>
<thead>
<tr>
<th>Case No./ Sex/Age (y)</th>
<th>Diagnosis*</th>
<th>Therapy for MDS†</th>
<th>Disease Progression*</th>
<th>Time to Progression (mo)</th>
<th>Therapy for AML or High-Grade MDS</th>
<th>Outcome (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/55</td>
<td>RCMD</td>
<td>Supportive</td>
<td>AML</td>
<td>5</td>
<td>Daunorubicin, cytarabine</td>
<td>Died (11)</td>
</tr>
<tr>
<td>2/F/74</td>
<td>RCMD</td>
<td>Epo, G-CSF</td>
<td>AML</td>
<td>7</td>
<td>Idfarubicin, cytarabine, etoposide, troxactitabine</td>
<td>Died (13)</td>
</tr>
<tr>
<td>3/F/55</td>
<td>RCMD</td>
<td>Epo</td>
<td>AML</td>
<td>9</td>
<td>Idfarubicin, cytarabine, IL-11</td>
<td>Died (13)</td>
</tr>
<tr>
<td>4/M/54</td>
<td>RCMD</td>
<td>Supportive</td>
<td>AML</td>
<td>6</td>
<td>Troxactitabine, cytarabine, allo-SCT</td>
<td>Died (13)</td>
</tr>
<tr>
<td>5/M/75</td>
<td>RCMD</td>
<td>Azacitidine, decitabine</td>
<td>AML</td>
<td>10</td>
<td>Clofarabine, cytarabine</td>
<td>Died (15)</td>
</tr>
<tr>
<td>6/M/70</td>
<td>RCMD</td>
<td>Supportive</td>
<td>RAEB-1 (6)</td>
<td>2</td>
<td>Unknown</td>
<td>Died (6)</td>
</tr>
<tr>
<td>7/M/71</td>
<td>RCMD</td>
<td>Supportive</td>
<td>RAEB-2 (13)</td>
<td>8</td>
<td>Idfarubicin, cytarabine, IL-11; clofarabine</td>
<td>Died (28)</td>
</tr>
<tr>
<td>8/M/64</td>
<td>RCMD</td>
<td>Azacitidine, G-CSF</td>
<td>RAEB-1 (8)</td>
<td>6</td>
<td>Unknown</td>
<td>Died (6)</td>
</tr>
<tr>
<td>9/F/60</td>
<td>RAEB-1 (8)</td>
<td>Azacitidine</td>
<td>—</td>
<td>—</td>
<td>Unknown</td>
<td>Died (4)</td>
</tr>
<tr>
<td>10/F/71</td>
<td>RAEB-2 (10)</td>
<td>Supportive</td>
<td>AML</td>
<td>6</td>
<td>Troxactitabine, cytarabine</td>
<td>Died (7)</td>
</tr>
<tr>
<td>11/M/23</td>
<td>RAEB-2 (10)</td>
<td>Supportive</td>
<td>Cord blood SCT</td>
<td>2</td>
<td>Idfarubicin, cytarabine, fludarabine, gemtuzumab ozogamicin, aurora kinase inhibitor, allo-SCT</td>
<td>Died (31)</td>
</tr>
<tr>
<td>12/F/52</td>
<td>CMML-1 (2)</td>
<td>Supportive</td>
<td>CMML-2 (10)</td>
<td>1</td>
<td>Idfarubicin, cytarabine</td>
<td>Died (6)</td>
</tr>
<tr>
<td>13/F/84</td>
<td>CMML-2 (10)</td>
<td>Supportive</td>
<td>AML</td>
<td>3</td>
<td>Unknown</td>
<td>Died (15)</td>
</tr>
<tr>
<td>14/M/75</td>
<td>CMML-2 (10)</td>
<td>Supportive</td>
<td>AML</td>
<td>5</td>
<td>Daunorubicin, cytarabine, thalidomide, Ras inhibitor</td>
<td>Died (21)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses indicate the percentage of blasts in bone marrow aspirate smears.  
† Supportive treatment involved blood component transfusion when needed.

allo, allogeneic; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; Ep, erythropoietin; G-CSF, granuloocyte colony stimulating factor; IL-11, interleukin-11; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia; SCT, stem cell transplantation.

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500-3,500/μL [0.5-3.5 × 10^9/L]; median, 2,500/μL [2.5 × 10^9/L]; reference range, 4,000-11,000/μL [4.0-11.0 × 10^9/L]).

The bone marrow biopsy specimens in the de novo cases showed normal or hypercellular bone marrow associated with megakaryocytic hyperplasia Image 1A. These megakaryocytes were dysplastic and often had small monolobated or hypolobated nuclei (Image 1A) Image 1B. The bone marrow was hypocellular in 5 cases of t-MDS, and in 1 such case, the initial differential diagnoses included aplastic anemia. Bone marrow aspirate smears also showed dysplasia involving the erythroid and myeloid lineages. The bone marrow blast count ranged from 0% to 10% Image 1C and Image 1D. An iron stain was performed in 10 cases, and 6 cases showed ring sideroblasts in a range of 2% to 10%.

The morphologic classification of de novo cases using the WHO classification was as follows: 8 refractory cytopenia with multilineage dysplasia, 3 refractory anemia with excess blasts (RAEB), and 3 chronic myelomonocytic leukemia (CMML). The bone marrow blast count in 3 cases of RAEB were 8%, 10%, and 10%, respectively. The CMML cases were further classified as 1 CMML-1 with 2% blasts and 2 CMML-2 with 10% blasts each.

Eight de novo cases were managed by supportive care such as transfusion and/or growth factors. Of the 8 patients, 2 received azacitidine, 1 received decitabine, and 1 received azacitidine and decitabine. One patient with RAEB received gemtuzumab ozogamicin, and one patient received cord blood stem cell transplantation after conditioning with total body irradiation and cyclophosphamide.

In the group of 6 t-MDS cases, 5 patients were originally diagnosed with lymphoma: 3 nodular sclerosis Hodgkin lymphoma, 1 follicular lymphoma, and 1 diffuse large B-cell lymphoma. One patient had ductal carcinoma of the breast. Five patients had relapses or metastases and were treated with multiple cycles of chemotherapy that spanned several years. The intervals from the diagnosis of primary tumor and the diagnosis of t-MDS ranged from 22 to 156 months. The t-MDS cases were treated more actively with various therapeutic agents from the time of initial diagnosis instead

### Table 2
Clinical Features of Six Cases of Therapy-Related MDS Associated With inv(3) or t(3;3)

<table>
<thead>
<tr>
<th>Sex/ Age (y)</th>
<th>Primary Disease</th>
<th>Therapy for Primary Tumor</th>
<th>Diagnosis of Primary Disease to t-MDS</th>
<th>t-MDS to t-AML</th>
<th>Therapy for t-MDS/AML</th>
<th>Outcome (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/75</td>
<td>NSHL; multiple relapses, 36, 61, 68, and 74 mo</td>
<td>VPP/ABDIC, ASHEP, ifosfamide, etoposide, BEAM, aSCT; for relapses, MOPP, gemcitabine, rituximab, bortezomib, RXT</td>
<td>75</td>
<td>8</td>
<td>Mocetinostat, laromustine, hydroxyurea, fludarabine, cytarabine</td>
<td>Died (11)</td>
</tr>
<tr>
<td>F/77</td>
<td>DLBCL and islet cell carcinoma</td>
<td>CHOP</td>
<td>45</td>
<td>—</td>
<td>Unknown</td>
<td>Died (14)</td>
</tr>
<tr>
<td>M/15</td>
<td>NSHL; relapse, 39 mo</td>
<td>DBVE with dexrazoxane, RXT; for relapse, MOPP/ABVD</td>
<td>48</td>
<td>9</td>
<td>Allo-SCT</td>
<td>Died (15)</td>
</tr>
<tr>
<td>F/28</td>
<td>NSHL; relapse, 11 mo</td>
<td>MOPP/ABVD, etoposide and cyclophosphamide, aSCT, RXT</td>
<td>22</td>
<td>9</td>
<td>Topotecan, daunorubicin, cytarabine, fludarabine, truxacitabine</td>
<td>Died (20)</td>
</tr>
<tr>
<td>F/56</td>
<td>FL; relapse, 19 mo</td>
<td>FCR, rituximab-CHOP; for relapse, hyper-CVAD, aSCT</td>
<td>22</td>
<td>11</td>
<td>Decitabine, cytarabine, clofarabine, fludarabine, gemtuzumab ozogamicin, allo-SCT Thalidomide, aSCT</td>
<td>Died (32)</td>
</tr>
<tr>
<td>F/50</td>
<td>Breast carcinoma; relapse, 108 mo; metastases, 135 and 141 mo</td>
<td>Cyclophosphamide, doxorubicin, 5-FU, aSCT; for relapse, tamoxifen, paclitaxel, vinorelbine, cyclophosphamide, cisplatin, BCNU, aSCT, docetaxel, capecitabine, trastuzumab</td>
<td>156</td>
<td></td>
<td></td>
<td>Died (33)</td>
</tr>
</tbody>
</table>

allo-SCT allogeneic stem cell transplantation; aSCT, autologous stem cell transplantation; ASHEP, doxorubicin [Adriamycin], methylprednisolone sodium succinate [Solu-Medrol], high-dose cytarabine [ara-C], cisplatin [Platinol]; BCNU, carmustine; BEAM, BCNU, etoposide, cytarabine, melphalan; CHOP, cyclophosphamide, doxorubicin, vincristine [Oncovin], prednisone; DBVE, doxorubicin, bleomycin, vincristine, etoposide; DLBCL, diffuse large B-cell lymphoma; ESAP, etoposide, methylprednisolone sodium succinate [Solu-Medrol], cytarabine [ara-C], cisplatin [Platinol]; FCR, fludarabine, cyclophosphamide, FL, follicular lymphoma; 5-FU, fluorouracil; hyper-CVAD, hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone alternating with methotrexate and cytarabine; MOPP/ABVD, mechlorethamine, vincristine, procarbazine, prednisone/doxorubicin [Adriamycin], bleomycin, vincristine, dacarbazine; NSHL, nodular sclerosis Hodgkin lymphoma; RXT, radiation therapy; t-AML, therapy-related acute myeloid leukemia; t-MDS, therapy-related myelodysplastic syndrome; VPP/ABDIC hybrid, etoposide [VePesid], cisplatin [Platinol] doxorubicin [Adriamycin], dacarbazine, lomustine [CCNU], and prednisone.

of supportive care. Two patients had allogeneic stem cell transplantation and 1, autologous transplantation.

Conventional Cytogenetic and Molecular Findings

The inv(3)(q21q26.2) was identified in 16 cases and t(3;3)(q21q26.2) in 4. In 4 cases, there was an isolated inv(3) or t(3;3), whereas 16 cases had additional cytogenetic aberrations. The –7/7q was the most common abnormality, in 7 de novo cases and all 6 t-MDS cases. In 6 cases, there was the –5/5q, detected concurrently with –7/7q in 5 cases. Five cases had a complex karyotype. The frequency of –7/7q was more common in t-MDS than in de novo cases (7/14 vs 6/6; \( P = .05 \)). The frequency of –5/5q (5/14 vs 1/6; \( P = .61 \)) or a complex karyotype (4/14 vs 1/6 t-MDS; \( P = 1.0 \)) was similar between the 2 subgroups.

The –7/7q and inv(3)/t(3;3) were detected simultaneously in 10 cases and sequentially in 3 cases. In 1 de novo case, the initial cytogenetic analysis revealed only t(3;3). A subsequent analysis performed 3 months later detected 2 abnormal clones: one with isolated t(3;3) and the other with t(3;3) and –7, indicating that –7 was a secondary change. By contrast, in 2 other cases, 1 t-MDS and 1 de novo, detection of –7...
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preceded that of inv(3) by 1 and 6 months, respectively. In the t-MDS case, the inv(3) was identified within 1 of 2 preexisting abnormal clones that harbored −7, indicating that inv(3) evolved as a secondary event. It is interesting that emergence of inv(3) also coincided with morphologic changes: a rising platelet count from 198 to 599 × 10^3/μL (198 to 599 × 10^9/L) in the de novo case and increased bone marrow blasts, from less than 5% to 8%, in both cases.

A RAS mutation was detected in 1 of 15 cases tested (CMML-2), and an FLT3 mutation was not detected in any of 12 cases tested.

Survival Comparison Between De Novo MDS and t-MDS With inv(3)/t(3;3)

Of the 14 patients with de novo diseases, AML developed in 9 patients within 2 to 10 months (median, 6 months). In 3 patients, disease progressed from refractory cytopenia with multilineage dysplasia to RAEB within 2 to 8 months. In 1 patient with CMML-1, CMML-2 developed within 1 month. Patients with AML were treated with combination chemotherapy with or without novel or investigative agents. Two patients also received allogeneic bone marrow transplantation. Despite aggressive treatment, 13 patients died of disease with a median survival of 13 months (range, 4-31 months).

Follow-up of 6 patients with t-MDS revealed that 4 patients progressed to t-AML during a period of 8 to 11 months. All 6 patients died, with a median survival of 17.5 months (range, 11-33 months).

As shown in Figure 1A, the OS of patients with de novo disease was comparable to that of patients with t-MDS (P = .09). There was also no significant difference in the frequency of AML between the 2 subgroups (P = 1). The presence of −7/7q did not confer a worse prognosis in the de novo group (P = .49) Figure 1B, nor did a complex karyotype (P = .71) Figure 1C. We could not perform a statistical analysis in the t-MDS cases owing to a limited number of cases.

Discussion

The inv(3)/t(3;3) is a rare recurrent cytogenetic abnormality in a subset of cases of AML, MDS, and the blast phase of chronic myeloid leukemia. The 3q21q26.2
chromosomal aberration involves proto-oncogene **EVI1** at 3q26.2.2 or its longer form **MDS1-EVI1** (also known as **MECOM**) and **RPN1** at 3q21, which results in overexpression of **EVI1**, or **MECOM**, and/or an **RPN1/EVI1** fusion transcript.⁴,⁹,¹⁰ The housekeeping gene **RPN1** is suggested to act as an enhancer of **EVI1** expression.¹¹ Up-regulation of **GATA-2** is also identified in some patients, indicating that it is one of the targets of myeloid neoplasms associated with 3q21q26.2.⁹ **EVI1** is implicated in the regulation of various signaling pathways, including transforming growth factor β, c-Jun N-terminal kinase, and activator protein 1 in different models; therefore, it controls cell growth, survival, and transformation.¹²,¹³ Aberrantly expressed **EVI1** was shown to block granulocytic differentiation in 32D cells, and a lethal condition resembling MDS developed in **EVI1**−deficient mice.¹²,¹⁴ Huang et al.¹⁵ also showed in **GATA-2**−deficient mice that **GATA-2** overexpression facilitated aberrant megakaryopoiesis. Recently, Lin et al.¹⁶ demonstrated that **JAK2** was uncommon in myeloid neoplasms with aberrations of 3q21q26.2; therefore, **JAK2** mutation is not the cause of thrombocytosis and megakaryocytic hyperplasia in these cases.

**AML** associated with inv(3)/t(3;3) is characterized by resistance to conventional chemotherapy and a dismal clinical outcome.¹⁷,¹⁸ It can occur de novo or arise from a preceding MDS. In the present study, we demonstrated that MDS cases with inv(3)/t(3;3) are associated with a dismal clinical outcome, characterized by chemoresistance, a high risk of leukemic transformation, and a short survival. Furthermore, de novo MDS and t-MDS cases share common features in their clinical and pathologic manifestations. A recent study demonstrated that AML with inv(3)/t(3;3) is associated with an adverse outcome with a median survival of 9.6 months,¹⁹ which is comparable to median survival rates seen in this study. In addition, in nearly 65% of cases in the present study, the disease progressed to acute leukemia. These findings suggest that MDS and AML associated with inv(3)/t(3;3) may represent a continuum of the same entity. The slightly longer survival in the t-MDS group may have been a result of more active intervention once the diagnosis of t-MDS was established compared with supportive care received by patients with low-grade de novo MDS.

In the current WHO classification, AML in patients with t(8;21), inv(16), or t(15;17) is classified as such even when a blast or promyelocyte count is less than 20%. In our opinion, the results from this study suggest that this rule could be applied to patients with myeloid neoplasms associated with inv(3)/t(3;3). This approach allows for more intensive intervention such as stem cell transplantation, which may potentially yield a better outcome in appropriate candidates.

Similar to what was found in AML associated with the inv(3)/t(3;3) abnormality,⁴,²⁰ patients with MDS also frequently have additional cytogenetic aberrations. The −7/7q and −5/5q were detected in about 65% and 30% of cases, respectively, in this study. The relationship between 3q21q26.2 and −7/7q remains to be elucidated. In our series, chromosome 7 aberrations occurred before, simultaneously, or after the onset of 3q21q26.2 rearrangement. A recent study by Stein et al.¹⁵ sheds light on the link between **EVI1** activation and the development of monosomy. In this study, insertional activation of **MDS1-EVI1** and, subsequently, the development of monosomy 7 and MDS were identified in 2 patients with X-linked chronic granulomatous disease treated with gene therapy. Furthermore, increased genomic instability is identified in MDS1-EVI1−expressing cells, and overexpression of **EVI1** in human diploid fibroblasts leads to increased frequency of centrosomal aberration.

In summary, to our knowledge this is the first study to characterize and compare de novo MDS and t-MDS with inv(3)/t(3;3). The patients have a dismal clinical outcome characterized by short overall and median survival rates and high risk of disease progression to AML. Additional cytogenetic abnormalities, including −7/7q, −5/5q, or complex cytogenetic aberrations, are common findings associated with MDS with inv(3)/t(3;3).

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