Acute myeloid leukemia with t(4;12)(q12;p13): an aggressive disease with frequent involvement of PDGFRA and ETV6

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ABSTRACT

We describe the clinical, morphologic, immunophenotypic and molecular genetic features of 15 cases of acute myeloid leukemia (AML) with t(4;12)(q12;p13). There were 9 men and 6 women, with a median age of 50 years (range, 17–76). Most patients had hypercellular bone marrow with a median blast count of 58% and multilineage dysplasia. Flow cytometry analysis showed myeloid lineage with blasts positive for CD13, CD33, CD34, CD38, CD117 and HLA-DR. Interestingly, aberrant CD7 expression was detected in 12/14 cases, and myeloperoxidase was either negative (3/15) or positive in only a small subset of the blasts (12/15). t(4;12)(q12;p13) was detected at time of initial diagnosis in 4 and at relapse or progression in 9 patients. The initial karyotype was unknown in 2 cases. FISH analysis showed PDGFRA-ETV6 rearrangement in all 7 cases assessed. FLT3 ITD was detected in 2/11 cases and IDH2 and JAK2 mutation were each detected in 1/2 cases assessed. There were no mutations of KRAS (0/8), NRAS (0/8), CEBPA (0/3), KIT (0/3), NPM1 (0/3) or IDH1 (0/2). All patients received aggressive multiagent chemotherapy; 7 patients additionally received stem cell transplantation. With a median follow-up of 10 months (range, 6–51), 13 patients died of AML, 1 patient had persistent disease, and 1 patient was lost to follow-up. In summary, AML with t(4;12)(q12;p13) is usually associated with myelodysplasia, aberrant CD7 expression, weak of absent myeloperoxidase expression, frequent PDGFRA-ETV6 fusion, and an aggressive clinical course. The molecular findings suggest that there may be a role for tyrosine kinase inhibitors in patient management.

INTRODUCTION

Conventional cytogenetic analysis plays a pivotal role in the risk stratification of acute myeloid leukemia (AML). The karyotype predicts response to induction therapy, risk of relapse, and overall survival. Acute myeloid leukemia with t(4;12) (q12;p13) has rarely been reported. Harada et al. described three cases of AML with t(4;12) (q12;p13) as the sole cytogenetic abnormality in 1995 [1]. He stated that the incidence of t(4;12)-positive AML was 0.6%. In 1999, Cools et al. identified the genes involved in this translocation: ETV6 (ETS Translocation
Variant 6, at 12p13) and BTL (Brl-like Translocated in Leukemia, later renamed CHIC2, at 4q12) [2]. Scattered case reports and small case series have been reported since then with a total of 25 cases reported to date [1–17]. Some reports have emphasized the suboptimal responses of patients treated using only standard chemotherapy regimens, with the chromosomal abnormality reappearing shortly after achievement of complete remission [4, 12, 13, 15]. The partner genes involved in this translocation were only explored in a few studies, and the molecular abnormalities were rarely explored. Here we report the clinical, morphologic, immunophenotypic and molecular genetic features of 15 AML cases with t(4;12) (q11:p13), the largest series to date.

RESULTS

Clinical findings

We identified 15 patients with AML associated with t(4;12) (q12;p13) seen at our institution from January 1, 1990 to December 31, 2016. The clinical and laboratory data are summarized in Table 1. There were 9 men and 6 women with a median age of 50 years (range, 17–76) at time of initial diagnosis. Upon presentation at our institution, laboratory evaluation showed anemia in 14 patients (median hemoglobin, 9.7 g/dL; range, 6.7–13.1 g/dL; reference range, 14.0-18.0 g/dL for men and 12.0–16.0 g/dL for women); thrombocytopenia in 11 patients and thrombocytosis in 1 patient (median platelet count, 42 × 10^9/μL; range, 4-748×10^9/μL); and leukopenia in 7 and leukocytosis in 3 patients (median white blood cell count, 4.7 × 10^9/μL; range, 1.2–87.3 × 10^9/μL; reference range, 4.0-11.0 × 10^9/μL). The serum lactate dehydrogenase level was elevated in 10 of 11 patients assessed (median, 340–87.3 × 10^9/μL; reference range, 140–440 × 10^9/μL); and leukopenia in 7 and leukocytosis in 5 patients (median white blood cell count, 4.7 × 10^9/μL; range, 1.2–87.3 × 10^9/μL; reference range, 4.0-11.0 × 10^9/μL). The results of conventional cytogenetic and FISH analyses were performed using break-apart probes for PDGFRA and ETV6 on cultured bone marrow cells, respectively. For PDGFRA, tri-color break-apart probe (FIP1L1 in green, CHIC2 in red and PDGFRA in white) was used.

Morphologic findings

The World Health Organization diagnostic category for the study group included: 6 cases of AML with myelodysplasia-related changes that evolved from polycythemia vera (n = 2), myelodysplastic syndrome (n = 2), chronic myelomonocytic leukemia (n = 1), and 1 de novo; 4 cases of AML without maturation (French-American-British [FAB] classification M1); 2 cases of AML with minimal differentiation (FAB M0), and 1 case each of acute myelomonocytic leukemia (FAB M4) and acute erythroid leukemia (FAB M6). The classification of 1 case is unknown and slides were not available for review.

The bone marrow was generally hypercellular (median cellularity, 75%), with a median blast count of 58% (range, 34%–91%). In most cases, the blasts were small to intermediate in size with fine chromatin, occasional small nucleoli, and scant basophilic cytoplasm. Dysplasia was observed in 10 cases; the other 5 cases had too few maturing cells to assess dysplasia (Figure 1). Only 1 case, an AML arising from polycythemia vera, showed eosinophilia (case 4). None showed basophilia.

Immunophenotypic findings

Immunophenotypic analysis by flow cytometry demonstrated the presence of a distinctive myeloid blast population in all cases. The blasts expressed CD13 (14/15, 93%), CD33 (14/15, 93%), CD34 (14/14, 100%), CD38 (14/14, 100%), CD117 (14/14, 100%) and HLA-DR (13/14, 93%). The blasts in small subsets of cases were also positive for CD64 (6/14, 43%), CD15 (3/14, 21%), CD14 (1/14, 7%) and CD56 (1/14, 7%). All cases were negative for CD3 (surface or cytoplasmic), CD5, CD10, CD19, CD20, and terminal deoxynucleotidyl transferase (TdT). Myeloperoxidase was either negative (n = 3) or positive in only a small subset of blasts (n = 12). Aberrant CD7 expression was detected in 12/14 (86%) cases. Interestingly, acquisition of the t(4;12) was associated with up-regulation of CD7 expression (cases 5, 6 and 7) and down-regulation of myeloperoxidase expression (cases 3, 5 and 7) in patients who acquired the t(4;12) during relapse or disease progression.

Cytogenetic findings

The results of conventional cytogenetic and FISH analyses are summarized in Table 2. The t(4;12) (q12;p13) was identified at time of initial diagnosis in 4 patients (cases 4, 8, 11 and 14), at time of progression from MDS to AML in 3 patients (cases 1, 7 and 9), and at time of relapse in 6 patients (cases 3, 5, 6, 10, 12 and 15). The initial karyotypes were unknown in two cases (cases 2 and 13).

The t(4;12)(q12;p13) was present as the sole cytogenetic abnormality in 7 patients (cases 4, 5, 8, 10, 11, 12 and 14) (Figure 2), as one of two abnormalities (simple abnormal karyotype) in 3 patients (cases 1, 2 and 14), and as a part of complex karyotype (≥ 3) in 5 patients (cases 3, 6, 7, 9 and 13). Two patients in whom t(4;12) was a sole abnormality at initial diagnosis subsequently acquired additional cytogenetic aberrations at time of relapse (case 4) or progression (case 10).

FISH analyses were performed using break-apart probes for PDGFA and ETV6 on cultured bone marrow cells, respectively. For PDGFA, tri-color break-apart probe (FIP1L1 in green, CHIC2 in red and PDGFA in white) was used.
Table 1: Clinical features, treatment, and outcome of AML patients with t(4;12)(q12;p13)

<table>
<thead>
<tr>
<th>#</th>
<th>Sex/Age</th>
<th>Diagnosis (WHO)</th>
<th>Prior history</th>
<th>WBC (10^3/μL)</th>
<th>Hb (g/dL)</th>
<th>Platelet (10^3/μL)</th>
<th>LDH (IU/L)</th>
<th>β2M (mg/L)</th>
<th>Chemotherapy</th>
<th>BMT</th>
<th>Outcome (FU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/65</td>
<td>AML-MRC</td>
<td>MDS</td>
<td>2.9</td>
<td>10.3</td>
<td>99</td>
<td>332</td>
<td>7.3</td>
<td>I, C, Fl</td>
<td>Yes</td>
<td>DOD (8)</td>
</tr>
<tr>
<td>2</td>
<td>F/36</td>
<td>AML with minimal differentiation</td>
<td>–</td>
<td>45.0</td>
<td>8.0</td>
<td>16</td>
<td>7139</td>
<td>NA</td>
<td>I, C</td>
<td>Yes</td>
<td>DOD (7)</td>
</tr>
<tr>
<td>3</td>
<td>M/35</td>
<td>Acute erythroid leukemia</td>
<td>–</td>
<td>1.4</td>
<td>10.4</td>
<td>4</td>
<td>217</td>
<td>NA</td>
<td>I, C</td>
<td>Yes</td>
<td>DOD (16)</td>
</tr>
<tr>
<td>4</td>
<td>F/45</td>
<td>AML-MRC</td>
<td>PV</td>
<td>9.8</td>
<td>13.1</td>
<td>201</td>
<td>580</td>
<td>2.9</td>
<td>I, C, Mi, E, Fl, G-CSF</td>
<td>Yes</td>
<td>DOD (51)</td>
</tr>
<tr>
<td>5</td>
<td>M/48</td>
<td>AML without maturation</td>
<td>–</td>
<td>87.3</td>
<td>10.4</td>
<td>57</td>
<td>1032</td>
<td>4.1</td>
<td>I, C, Fl, Cy, Top, ABT-751</td>
<td>No</td>
<td>DOD (9)</td>
</tr>
<tr>
<td>6</td>
<td>F/58</td>
<td>AML with minimal differentiation</td>
<td>–</td>
<td>3.1</td>
<td>10.7</td>
<td>338</td>
<td>495</td>
<td>1.8</td>
<td>I, C</td>
<td>Yes</td>
<td>DOD (51)</td>
</tr>
<tr>
<td>7</td>
<td>M/57</td>
<td>AML-MRC</td>
<td>MDS</td>
<td>8.6</td>
<td>8.6</td>
<td>39</td>
<td>2679</td>
<td>2.5</td>
<td>Dec, C, Clo</td>
<td>No</td>
<td>DOD (8)</td>
</tr>
<tr>
<td>8</td>
<td>F/76</td>
<td>AML without maturation</td>
<td>–</td>
<td>2.6</td>
<td>6.7</td>
<td>95</td>
<td>692</td>
<td>5.3</td>
<td>I, C, PKC-412</td>
<td>No</td>
<td>DOD (6)</td>
</tr>
<tr>
<td>9</td>
<td>M/67</td>
<td>AML-MRC</td>
<td>CMML</td>
<td>3.9</td>
<td>8.4</td>
<td>29</td>
<td>875</td>
<td>2.5</td>
<td>E, R, I, C, My, RAD-001</td>
<td>No</td>
<td>DOD (7)</td>
</tr>
<tr>
<td>10</td>
<td>F/54</td>
<td>Acute myelomonocytic leukemia</td>
<td>–</td>
<td>1.2</td>
<td>9.2</td>
<td>17</td>
<td>694</td>
<td>NA</td>
<td>I, C, My, Fl, Das, AZD-1152</td>
<td>No</td>
<td>DOD (12)</td>
</tr>
<tr>
<td>11</td>
<td>M/17</td>
<td>AML-MRC</td>
<td>–</td>
<td>3.7</td>
<td>9.2</td>
<td>38</td>
<td>461</td>
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<td>C, Dan, E</td>
<td>No</td>
<td>Lost FU</td>
</tr>
<tr>
<td>12</td>
<td>M/41</td>
<td>AML without maturation</td>
<td>–</td>
<td>19.5</td>
<td>12.6</td>
<td>171</td>
<td>3025</td>
<td>4.0</td>
<td>Dan, I, C, My, Mi, E, Top, OSI-211</td>
<td>No</td>
<td>DOD (19)</td>
</tr>
<tr>
<td>13</td>
<td>F/50</td>
<td>AML-unknown</td>
<td>–</td>
<td>11.5</td>
<td>9.1</td>
<td>17</td>
<td>1779</td>
<td>7.8</td>
<td>I, C, Fl, My, E, Cy, Dec</td>
<td>No</td>
<td>DOD (11)</td>
</tr>
<tr>
<td>14</td>
<td>M/24</td>
<td>AML without maturation</td>
<td>–</td>
<td>23.3</td>
<td>9.7</td>
<td>42</td>
<td>595</td>
<td>2.9</td>
<td>Clo, I, C</td>
<td>Yes</td>
<td>DOD (6)</td>
</tr>
<tr>
<td>15</td>
<td>M/56</td>
<td>AML-MRC</td>
<td>PV</td>
<td>4.7</td>
<td>9.7</td>
<td>748</td>
<td>1093</td>
<td>2.8</td>
<td>I, C, My, Ana, Rux</td>
<td>Yes</td>
<td>PV (40)</td>
</tr>
</tbody>
</table>

*Follow-up (months) from time of initial diagnosis

Abbreviations: ABT-751, microtubule inhibitor; AML, acute myeloid leukemia; Ana, anagrelide; AZD-1152, aurora kinase inhibitor; β2M, β2-microglobulin; BMT, bone marrow transplantation; C, cytarabine; Clo, clofarabine; CMML, chronic myelomonocytic leukemia; Cy, cyclophosphamide; Das, daunatinib; Dau, daunorubicin; Dec, decitabine; DOD, died of disease; E, etoposide; F, female; FAB, French-American-British; Fl, fludarabine; FU, follow-up; Hb, hemoglobin; HU, hydroxyurea; I, idarubicin; LDH, lactate dehydrogenase; M, male; MDS, myelodysplastic syndrome; Mi, mitoxantrone; MRC, myelodysplasia-related changes; My, mylortag; NA, not available; OSI-211, liposomal topoisomerase inhibitor; PKC-412, midostaurin; PV, Polycythemia vera; R, rituxan; RAD-001, mTOR inhibitor; Rux, Ruxolitinib; Top, topotecan; WBC, white blood cell; WHO, World Health Organization

aqua) showed 1 triple fusion, 1 green-red fusion, and 1 separate aqua signal, which confirmed PDGFRα gene rearrangement (translocation of aqua signal to derivative chromosome 12). For ETV6, translocation of red signal to derivative chromosome 4 confirmed ETV6 rearrangement. In summary, all 7 cases assessed (cases 1, 4, 5, 6, 7, 10 and 11) showed both PDGFRα and ETV6 rearrangements (Figure 2).

**Molecular findings**

Molecules studies revealed a FLT3 internal tandem duplication (ITD) in 2 of 11 cases assessed (cases 5 and 13). IDH2 and JAK2 mutation was each detected in 1 of 2 cases assessed (case 15). No cases assessed showed mutations of KRAS (n = 8), NRAS (n = 8), CEBPA (n = 3), KIT (n = 3), NPM1 (n = 3), or IDH1 (n = 2) (Table 2).

**Clinical outcome**

All patients received multiagent chemotherapy; 5 patients also were treated with investigational drugs including ABT-751 (microtubule inhibitor), AZD-1152 (aurora kinase inhibitor), OSI-211 (liposomal topoisomerase inhibitor), PKC-412 (midostaurin, tyrosine kinase inhibitor), and RAD-001 (mTOR inhibitor). Seven patients additionally underwent hematopoietic stem cell transplantation. Two patients received tyrosine kinase inhibitor therapy: patient 10 received dasatinib and patient 15 received ruxolitinib. Of the 14 patients with clinical follow-up data, 4 patients were refractory to therapy and 10 patients relapsed shortly after first complete remission with a median time to first relapse of 4 months. With a median follow-up of 10 months from time of initial diagnosis (range, 6–51) or 6 months from the occurrence of t(4;12) (range, 2–51), 13 patients died of AML, 1 patient (case 15) had persistent disease, and 1 patient was lost to follow-up. Among the 14 patients with follow-up data available, 7 (50%) patients died within 1 year, 10 (71%) patients died within 2 years, and 13 (93%) patients died within 5 years.

**DISCUSSION**

The t(4;12)(q12;p13) is a rare recurrent cytogenetic abnormality in AML, reported previously in 25 cases as case reports or small series, with limited molecular genetic analysis [1–17]. We present the clinical, morphologic, immunophenotypic and molecular genetic features of 15 AML cases with t(4;12)(q12;p13) evaluated at a single institution, the largest series to date.
In the initial study of 3 cases of AML with t(4;12), Harada and colleagues described the blasts as having “pseudo-lymphoid” morphology and a background of trilineage dysplasia, basophilia, and eosinophilia were common. The results in this series, in part, confirm these findings. The blasts were small to intermediate in size and myeloperoxidase expression was present in only a subset of blasts. These blasts do resemble, in part, lymphoblasts. We also observed multilineage dysplasia in all cases in which the number of maturing hematopoietic cells was adequate to evaluate. In addition, some patients had a history of a myelodysplastic syndrome or myeloproliferative neoplasm. However, no cases in this study were associated with basophilia and only one patient with a history of polycythemia vera had eosinophilia.

Recurrent chromosomal rearrangements that generate oncogenic fusion genes or deregulate the expression of proto-oncogenes and/or tumor suppressor genes play an essential role in the development and progression of hematologic neoplasms. In our study, the t(4;12) was found as both a primary abnormality at diagnosis and a secondary abnormality associated with relapse or progression. In an earlier study, Cools et al. identified the genes involved in this translocation to be \( ETV6 \) (at 12p13) and \( BTL \) (at 4q12) [2]. The partner genes involved in this translocation were rarely explored since then. In all 7 of our cases assessed by FISH, we identified rearrangements of \( PDGFRA \) and \( ETV6 \), suggesting a role in the pathogenesis of AML with t(4;12). Located as 4q12, \( PDGFRA \) is a member of the class III receptor tyrosine kinase family [18, 19]. It contains an extracellular immunoglobulin-like domain, a transmembrane domain with an inhibitory juxtamembrane WW-like domain, and an intracellular kinase domain [20]. PDGFRA activates intracellular tyrosine kinase signaling pathway by forming homodimer or heterodimer with \( PDGFRB \) [21]. Hematologic malignancies associated with \( PDGFRA \) rearrangement commonly manifest as myeloid or lymphoid neoplasms with eosinophilia [19]. Its most common partner gene is \( FIP1L1 \). An approximately 800 kb interstitial chromosomal deletion juxtaposes \( FIP1L1 \) and \( PDGFRA \) resulting in a gain-of-function fusion protein

Table 2: Cytogenetic and molecular findings of AML with t(4;12)(q12;p13)

<table>
<thead>
<tr>
<th>Case</th>
<th>Karyotype</th>
<th>FISH</th>
<th>Gene Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46,XY,t(4;12)(q12;p13)[7]/46,XY,t(4;12) (q12;p13),del(7)(q21q32)[6]/53,XY,+6,+8,+10,+11,+12,+14,+19[6]</td>
<td>PDGFRA-ETV6</td>
<td>KRAS-, NRAS-</td>
</tr>
<tr>
<td>2</td>
<td>46,XX,t(4;12)(q12;p13),del(9)(q22)[20]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>41-42,XYadd(2)(q44),-3(-q10),t(4;12)(q12;p13),del(5)(p14),del(5)(q13),-7,-9,del(11)(q12),add(14)(p11),-15,-16,-17,-18,-20,-21,-22,+6mar [cp20]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4*</td>
<td>46,XX,t(4;12)(q12;p13)[1]/46,XX [29]</td>
<td>PDGFRA-ETV6</td>
<td>CEBP4-, FLT3-, KIT-, KRAS-, NPM1-, NRAS-</td>
</tr>
<tr>
<td>5</td>
<td>46,XY,t(4;12)(q12;p13)[17]/46,XY [3]</td>
<td>PDGFRA-ETV6</td>
<td>FLT3-ITD+</td>
</tr>
<tr>
<td>6</td>
<td>46,XX,t(4;12)(q12;p13)[1]/46,XX,sl,del(16)(q22)[11]/46,sl,t(1;21)(q11q11.1)[4]/46,XX [3]</td>
<td>PDGFRA-ETV6</td>
<td>FLT3-, KRAS-, NRAS-</td>
</tr>
<tr>
<td>7</td>
<td>45,XY,t(4;12)(q12;p13),del(13;15)(q10;q10),del(20)(q11.2q13.3)[20]</td>
<td>PDGFRA-ETV6</td>
<td>FLT3-, JAK2-, KRAS-, NRAS-</td>
</tr>
<tr>
<td>8</td>
<td>46,XX,t(4;12)(q12;p13)[19]</td>
<td>NA</td>
<td>FLT3-</td>
</tr>
<tr>
<td>9</td>
<td>46,XY,t(4;12)(q12;p13),-7,+mar [13]/46,XY [7]</td>
<td>NA</td>
<td>FLT3-, KRAS-, NRAS-</td>
</tr>
<tr>
<td>10</td>
<td>46,XX,t(4;12)(q12;p13)[11]/46,XX [7]</td>
<td>PDGFRA-ETV6</td>
<td>FLT3-, KIT-, NRAS-</td>
</tr>
<tr>
<td>11</td>
<td>46,XY,t(4;12)(q12;p13)[20]</td>
<td>PDGFRA-ETV6</td>
<td>FLT3-</td>
</tr>
<tr>
<td>13</td>
<td>46,XX,t(4;12)(q12;p13),t(10;13)(q22q12)[12]/46,sl,del(2)(q33q37)[3]/46,XX,t(4;12)(q12;p13),t(10;13)(q22q12)[cp5]</td>
<td>NA</td>
<td>FLT3-ITD+</td>
</tr>
<tr>
<td>14</td>
<td>46,XY,t(4;12)(q12;p13),t(9;14)(p13;q13)[19]/46,XY [1]</td>
<td>NA</td>
<td>CEBP4-, FLT3-, IDH1-, IDH2-, KIT-, KRAS-, NPM1-, NRAS-, IDH2-, JAK2+, CEBP4-, FLT3-, IDH1-, KIT-, KRAS-, NPM1-, NRAS-</td>
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<td>15</td>
<td>46,XY,t(4;12)(q12;p13)[20]</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*These two patients had t(4;12) as a sole abnormality at initial diagnosis and acquired additional cytogenetic aberrations at relapse (case 4) or progression (case 10).

Abbreviations: AML, acute myeloid leukemia; NA, not available
with signal-independent kinase activity and increases cell proliferation and survival [9].

The *ETV6* gene (previously called *TEL*), located at 12p13, is a member of the ETS family of transcription factors. It contains two important domains: the HLH (helix-loop-helix) domain, which mediates protein-protein interactions, and the ETS DNA binding domain [22]. *ETV6* is the major target of translocations involving 12p13 in hematopoietic malignancies, and is frequently rearranged in both myeloid and lymphoid neoplasms [22]. Translocations involving *ETV6* generate oncogenic fusion proteins or ectopic promoters [1, 2, 6]. One study has suggested that the transcriptional activation of *PDGFRA* by the positive effect of *ETV6* translocation may be involved in leukemogenesis of these cases [15].

In keeping with earlier studies, acquisition of the t(4;12) appears to be associated with up-regulation of CD7 and down-regulation of myeloperoxidase expression. Interestingly, in patients with sequential bone marrow specimens who acquired the t(4;12) upon relapse or disease progression, increased CD7 and decreased myeloperoxidase expression coincided with acquisition of the translocation. We speculate that the presence of the fusion protein, via downstream pathways, down-regulate myeloperoxidase and up-regulate CD7 although we have no data to explain the possible mechanisms. Moreover, the minimal expression or complete absence of myeloperoxidase and aberrant CD7 expression, along with the morphology of the blasts, can present a challenging differential diagnosis with mixed phenotype acute leukemia (myeloid/T). However, none of the cases showed conclusive evidence of T-cell lineage manifested by lack of surface or cytoplasmic CD3 expression. Therefore, none of these cases met the diagnostic criteria for precursor T immunophenotype.

Some of the earlier studies of AML with t(4;12) have reported the difficulties of using only standard chemotherapy regimens in treating these patients [4, 12, 13, 15]. Others have suggested that these patients may respond to intensive chemotherapy regimens or hematopoietic stem cell transplantation [2, 13, 16]. Our data also suggest that the t(4;12), either as a sole abnormality or as part of a complex karyotype, portends a poor outcome. In the patient cohort presented, despite treatment with multiagent chemotherapy, new investigational agents, and/or hematopoietic stem cell transplantation, most patients failed to achieve a complete remission or only had a brief remission of short duration with multiple relapses and a poor outcome.

In summary, we have described 15 AML cases associated with t(4;12)(q12;p13), the largest series to date. Our results show that AML with t(4;12) (q12;p13) is a distinct entity with an aggressive clinical course and characteristic, although not specific, morphologic

![Figure 1: Morphologic features of AML with t(4;12)(q12;p13) (case 5).](image-url)

(A). The bone marrow core biopsy shows a hypercellular bone marrow with dysplastic megakaryocytes and increased immature cells (H&E, x400). (B). The aspirate smear shows dysgranulopoiesis and increased blasts (Wright-Giemsa, x1000). (C). The aspirate smear shows dyserythropoiesis and increased blasts (Wright-Giemsa, x1000). (D). The aspirate smear shows numerous blasts that are small to intermediate in size with fine chromatin, occasional prominent nucleoli, and scant basophilic cytoplasm (Wright-Giemsa, x500).
and immunophenotypic findings. The t(4;12)(q12;p13) can occur at initial diagnosis or at relapse or disease progression, and frequently involves PDGFRA and ETV6. There may be a role for tyrosine kinase inhibitor therapy in patients with this disease.

**MATERIALS AND METHODS**

**Case selection**

We searched the database of the Department of Hematopathology at The University of Texas MD Anderson Cancer Center from January 1, 1990 to December 31, 2016 and identified 15 patients with AML associated with t(4;12)(q11;p13). The diagnosis of AML was based on morphologic and immunophenotypic criteria as specified in the revised World Health Organization classification [23]. Clinical and laboratory data were obtained by review of the medical records. The study was conducted under an Internal Review Board-approved protocol.

**Morphologic examination**

We reviewed Wright Giemsa-stained peripheral blood smears, bone marrow aspirate smears and touch imprints, as well as H&E-stained core biopsy and clot sections in all cases. A manual 100-cell or 500-cell differential count was performed on peripheral blood smear and bone marrow aspirate smear, respectively. Cytochemical stains for myeloperoxidase were performed on aspirate smears in most cases using conventional methods.

**Immunophenotypic analysis**

Immunophenotypic analysis using multicolor flow cytometry was performed on bone marrow aspirates as previously described [24]. The panel of monoclonal antibodies used included reagents specific for CD3 (surface and cytoplasmic), CD5, CD7, CD10, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD45, CD56, CD64, CD117, myeloperoxidase, HLA-DR, and terminal deoxynucleotidyl transferase. All antibodies were purchased from Becton Dickinson Biosciences (San Jose, CA). Analysis was performed using a FACScan or FACSCalibur cytometer (Becton Dickinson Biosciences). An isotype-matched control was used for each antibody.

**Conventional cytogenetic and FISH analyses**

In all cases, conventional cytogenetic analysis was performed on metaphase cells prepared from bone marrow aspirates cultured for 24 or 48 hours without mitogens, using standard techniques. Giemsa-banded metaphases were analyzed, and the results were reported.

![Figure 2: Cytogenetic findings (case 11).](image-url)

(A). Chromosomal analysis shows a karyotype of 46,XY,t(4;12)(q12;p13)[20]. (B). FISH analysis using PDGFRA tri-color break-apart probe (FIP1L1 in green, CHIC2 in red and PDGFRA in aqua) shows 1 triple fusion, 1 green-red fusion, and 1 separate aqua signal, which confirms PDGFRA gene rearrangement (translocation of aqua signal to derivative chromosome 12). (C). FISH analysis using ETV6 dual-color break-apart probe shows ETV6 gene rearrangement (translocation of red signal to derivative chromosome 4).
using the International System for Human Cytogenetic Nomenclature, 2016 (ISCN, 2016)

Fluorescence *in situ* hybridization (FISH) analysis was performed on interphase nuclei obtained from cultures of bone marrow aspirates using probes for *PDGFRA* (tricolor break-apart probe, Abbott Molecular/Vysis, Des Plaines, IL) and *ETV6* (dual-color break-apart probe, Abbott Molecular/Vysis), respectively, using standard techniques. The positive cut-off values established in our laboratory are 4.7% for *PDGFRA* rearrangement and 3.2% for *ETV6* rearrangement.

**Molecular studies**

Genomic DNA extracted from bone marrow aspirates was PCR amplified and subject to mutational analysis for *CEBP A, IDH1* (exon 4), *IDH2* (exon 4), *KIT* (exon 17), *NPM1* (exon 12), and *TP53* (exons 2–11) by direct Sanger sequencing on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA), or *JAK2* V617F and codons 12, 13 and 61 of *KRAS* and *NRAS* by pyrosequencing using a PSQ HS 96 Pysosequencer (Biotage, Uppsala, Sweden), as described previously [25].

A fluorescence-multiplex PCR was used to detect ITD and D835 point mutation of the *FLT3* gene using genomic DNA. For D835, the PCR products were digested with EcoRV restriction enzyme that cuts only the wild type sequence. The PCR products were then subjected to capillary electrophoresis on an ABI Prism 3100 Genetic analyzer to distinguish wild and mutant genotypes [25].

**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.

**REFERENCES**