Chronic Myelomonocytic Leukemia Evolving From Preexisting Myelodysplasia Shares Many Features With De Novo Disease

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Key Words: Chronic myelomonocytic leukemia; Myelodysplastic syndrome; Evolution; Overall survival

Abstract

The majority of chronic myelomonocytic leukemia (CMML) cases arise de novo; cases evolving from preexisting myelodysplasia (MDS) or myeloproliferative diseases have not been well-studied. We conducted the present study to determine the clinicopathologic features and to study possible underlying molecular and cytogenetic mechanisms involved in this evolution. Between April 1995 and November 2005, we identified 120 CMML cases, of which 20 (16.7%) had a previous diagnosis of MDS. Of the 20 patients with MDS, 6 had relative monocytosis at diagnosis. At the time of MDS to CMML evolution, mutations in JAK2 (V617F), FLT3 (ITD), K-ras-2, or N-ras were not acquired, and only 1 (6%) of 17 evaluable cases showed cytogenetic progression. The median time to evolution from MDS to CMML was 29 months, and the median survival following CMML development was 13 months. Three cases (17%) transformed to acute myeloid leukemia.

Myelodysplastic syndrome (MDS) is a group of disorders characterized by dys hematopoiesis in the bone marrow (BM) and cytopenias in the peripheral blood.¹² Chronic myelomonocytic leukemia (CMML) was incorporated into the French-American-British (FAB) classification as an MDS and distinguished only by the presence of peripheral blood monocytosis (monocyte count, >1,000/µL [1.0 × 10⁹/L]).³ However, some patients with CMML may have leukocytosis and splenomegaly in addition to monocytosis, resembling a myeloproliferative disease (MPD) rather than an MDS. For these reasons, the FAB classification divided CMML into 2 subgroups: myelodysplastic (MD; WBC count, ≤13.0 × 10⁹/L) and myeloproliferative (MP; WBC count, >13.0 × 10⁹/L) types.³

The principal clinical difference between CMML and other MPDs is the presence of ineffective hematopoiesis, manifesting as more frequent anemia and thrombocytopenia. A fundamental biologic characteristic shared by CMML and MPD is progenitor hypersensitivity to growth factors,⁴,⁵ but the pathways mediating this likely differ, as does the lineage specificity. In the recent World Health Organization (WHO) classification, CMML has been separated from MDS into a newly created group—myelodysplastic/chronic myeloproliferative disease (MDS/MPD), defined as a clonal disorder of BM stem cells with persistent peripheral monocytosis. Based on the number of BM blasts, CMML is divided further into 2 broad categories: CMML-1, with fewer than 10% blasts, and CMML-2, with blasts from 10% to 19%.⁶,⁷

The majority of patients with CMML, including patients with the FAB MD type, have absolute monocytosis at the time of diagnosis. However, a few case studies have shown that in some patients with MDS, there is a dynamic evolution...
of increasing monocyte counts, suggesting that some CMML cases can evolve from a preexisting MDS. The preexisting MDS types described in these studies included many FAB subtypes; however, the exact frequency, clinical significance, and molecular and cytogenetic alterations in this evolution are unclear.

The present study is a retrospective evaluation of a large series of CMML cases (as defined by the WHO classification) diagnosed and treated at 2 institutions. Our aim was to study the clinicopathologic features and clinical outcome of patients with CMML whose disease evolved from a preexisting myeloid disorder in comparison with patients with de novo CMML. The possible underlying molecular pathogenesis involved in this evolution, such as chromosomal abnormalities, FLT3, JAK2 V617F, and RAS (K-ras-2 and N-ras) gene mutations, also was studied.

Materials and Methods

Cases

We retrieved slides and clinical data for all consecutive CMML cases diagnosed at Rush University Medical Center (RUSH, Chicago, IL) and University of Massachusetts Medical Center (UMASS, Worcester) during a 10-year period (April 1995-November 2005). The CMML diagnosis was made on a pretherapy BM aspirate and biopsy. All samples were reviewed by hematopathologists at RUSH and UMASS. In most cases, the BM samples had been first diagnosed using FAB criteria and then were reclassified according to the WHO criteria, based on morphologic features, laboratory features, cytogenetic findings, and clinical follow-up. During the MDS phase, cases that later evolved into CMML had been treated with supportive care only, such as transfusion or growth factors, and had not received cytotoxic chemotherapy. We took special caution to exclude possible secondary causes of monocytosis by strictly applying the WHO criterion requiring that absolute monocytosis persist for at least 3 months. Prognosis was calculated with the International Prognostic Scoring System (IPSS), which included categories of low, intermediate 1, intermediate 2, and high.

Morphologic Analysis

All cases had representative trephine biopsy specimens and BM aspirate smears available for reevaluation. Perls reaction for iron was performed on aspirate and silver impregnation stain for reticulum on biopsy tissue. For the diagnosis of dysplasia in the BM, the features of dyserythropoiesis, dysgranulopoiesis, and dysmegakaryopoiesis had to be present in at least 10% of cells of the respective lineage by estimation. Dysplasia was defined as unilineage if it involved a single lineage, whereas multilineage dysplasia was considered when at least 2 lineages showed those features. The blasts were counted manually. When the aspirates were of poor quality, we used information from flow cytometric evaluation and CD34 immunohistochemical analysis performed on BM biopsy specimens to enumerate the number of blasts.

Cytogenetic Analysis

Fixed preparations obtained from cultured (24 and 48 hours) BM samples were G-banded, and a minimum of 20 metaphase spreads were examined. The criteria defined by the International System for Human Cytogenetic Nomenclature were used for identification of abnormal clones.

Patient Samples and Isolation of Genomic DNA

All patients provided informed consent with institutional review board approval at each institution. BM samples from 68 patients with CMML, including 57 patients with de novo CMML and 12 patients with evolved CMML from preexisting MDS, were available for analysis. Of 20 MDS to CMML cases, only 4 paired samples (MDS phase and CMML phase) were available. Genomic DNA was isolated from the frozen BM samples using the QIAamp DNA Tissue Kit (Qiagen, Valencia, CA), according to the manufacturer’s instructions.

JAK2 V617F Sequence Analysis

Genomic DNA was amplified by polymerase chain reaction (PCR), and confirmed by electrophoresis on an ethidium bromide–impregnated 1% agarose gel. The Qiagen HotStar Taq PCR Kit was used to generate the PCR products per the manufacturer’s recommendations. Each 50-µL PCR reaction contained approximately 25 ng of DNA template, 5 µL of 10× PCR buffer (final concentration magnesium chloride, 1.5 mmol/L), 1.25 U of HotStar Taq DNA polymerase (Qiagen), 200 µmol/L of deoxynucleoside triphosphates (dNTPs), and 0.5 µmol/L of each of sense and antisense primers (5′-TCCTACAGTGGTTCAGGTCA-3′ and 5′-TCCTACAGTGGTTTCAGGTCA-3′, respectively). PCR cycling parameters were as follows: 1 cycle of 94°C for 15 minutes; 35 cycles of 94°C for 30 seconds, 62°C for 45 seconds, and 72°C for 45 seconds; followed by 1 cycle of 72°C for 5 minutes.

PCR products were cleaned with the QIAquick PCR purification Kit (Qiagen). Fluorescent dye chemistry sequencing was performed using the same primers used for amplification on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems, Foster City, CA). DS Gene 1.5 (ACcelerex, San Diego, CA) and GenBank Accession NM_004 972 (JAK2 messenger RNA) and the corresponding region from the NC_000 009 chromosome 9 contig were used for sequence analysis.
PCR Assay for the Detection of FLT3/ITD in Genomic DNA

The PCR reaction was carried out with a 50-µL mixture containing 50 ng of DNA, 200 µmol/L of dNTPs, 1× PCR buffer, 1.5 mmol/L of magnesium chloride, 2.5 U of HotStart Taq DNA polymerase, and 0.5 µmol/L of the forward primer, 5'-CAATTTAGGTATGAAAAGCC-3', and reverse primer, 5'-GTACCTTTTCAGCATTTTGAC-3', which completely covered the JM domain through the TK 1 domain, on a DNA thermal cycler (PTC200, MJ Research, Ramsey, MN), using a program consisting of denaturation at 94°C for 30 seconds, annealing at 59°C for 45 seconds, and extension at 72°C for 1 minute for 35 cycles, with an initial preheating at 95°C for 15 minutes and a final extension at 72°C for 5 minutes.16

RAS Mutational Analysis

PCR-based DNA pyrosequencing of K-ras-2 and N-ras was done in separate reactions for exon 1 (including codons 12 and 13) and exon 2 (including codon 61). For codons 12 and 13, the order of nucleotide dispensation during pyrosequencing was varied to increase the sensitivity of mutation detection to approximately 1 in 15 mutation-bearing cells. PCR was done in 50-µL volumes with 1× PCR buffer, 2.5 mmol/L of magnesium chloride, 0.25 mmol/L of dNTP mix, 0.2 µmol/L of forward primer, 0.2 µmol/L of reverse primer, 1.25 U of Taq polymerase (ABI AmpliTaq Gold, Applied Biosystems), and 0.1 µg of template DNA. Thermal cycling was performed with initial preheating at 95°C for 2 minutes, followed by 40 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 10 minutes.

Following generation of single-stranded DNA template by avidin-sepharose bead purification, pyrosequencing was performed with PyroGold reagents (Biotage, Uppsala, Sweden) using 8 to 15 µL of PCR product. PCR primers were as follows: K-ras-2 -12,13, forward, 5'-AGG CCT GCT GAA AAT GAC TG-3'; reverse, 5'-biotin-ATT GTT GGA TCA TAT TCG T-3'; K-ras codon 61, forward, 5'-GAT TCC TAC AGG AAG CAA GT-3'; reverse, biotin-CCA CCT ATA ATG GTG AAT ATC T-3'; N-ras codons 12,13, forward, 5'-GTT CTT GCT GTT GGA TCA TAT TCG T-3'; K-ras codon 61, forward, 5'-GAT TCC TAC AGG AAG CAA GT-3'; reverse, biotin-CCA CCT ATA ATG GTG AAT ATC T-3'; N-ras codons 12,13, forward, 5'-GTT CTT GCT GTT GGA TCA TAT TCG T-3'; reverse, 5'-biotin-CTC TAT GTG GGG ATA ATC TAT TCC TAC AGG AAG CAA GT-3'; reverse, 5'-biotin-CTC TAT GTG GGG ATA ATC TAT TCC TAC AGG AAG CAA GT-3'; reverse, 5'-biotin-CGT TAG AGG TTA ATA TCC GCA-3'; and reverse, 5'-biotin-CGT TAG AGG TTA ATA TCC GCA-3'.

Statistical Analysis

One-way analysis of variance with Bonferroni correction was used in the comparison of continuous variables among the different groups, and the Fisher exact test and χ² test were applied for categorical variables. Patient survival was estimated by the Kaplan-Meier method from the date of diagnosis until death from any cause or until the last patient follow-up. Survival curves were compared statistically by using the log-rank test.

Results

Patients

Between April 1995 and November 2005, a total of 120 CMML cases were diagnosed at RUSH and UMASS based on the initial FAB classification and subsequent WHO revision. The median age of patients with CMML was 68 years (range, 37-92 years); the male/female ratio was 2.3:1. Among all patients with CMML, 22 had a preexisting myeloid disease, considered as evolved CMML. In contrast, the remaining 98 cases were considered de novo CMML, defined as persistent monocytosis (monocyte count, >1,000/µL [1.0 × 10⁹/L], >3 months) at initial diagnosis, in addition to the other inclusion and exclusion criteria for establishing a diagnosis of CMML.

Clinicopathologic Features of CMML Evolved From Preexisting MDS: MDS Phase vs CMML Phase

Of the 120 CMML cases, 22 (18.3%) had preexisting MDS or MPD, including 19 MDS, 1 refractory anemia with excess blasts in transformation (RAEB-t; FAB/acute myeloid leukemia (AML; WHO), 1 MDS/MPD-unclassifiable (U), and 1 MPD-U. The MDS to CMML cases included 6 cases of refractory anemia (RA), 2 of RA with ringed sideroblasts (RARS), 8 of RAEB, 1 of RAEB-t, 2 of MDS-U, and 1 RA with fibrosis according to the FAB classification, and, according to the WHO criteria, 3 RA cases, 5 RA with multilineage dysplasia (RCMD), 1 RARS, 1 RCMD-RS, 1 MDS-U, 6 RAEB-1, 2 RAEB-2, and 1 AML. Case 7 (RAEB-t/AML) was included in this study because the patient had a clinical course more akin to RAEB-2, and his blast count never exceeded 20%. Because the MDS cases accounted for the majority of the preexisting myeloid diseases in the evolved CMML group and the hematologic characteristics differed from those of MDS/MPD-U and MPD-U, the study focused on CMML evolving from MDS (MDS to CMML). The FAB/WHO classification and clinicopathologic features of 19 MDS and 1 RAEB-t/AML cases are shown in Table 1.

The median age of this group was 67 years (range, 46-83 years), and all were men. The MDS phase of the MDS to CMML cases manifested as cytopenia without absolute monocytosis. Of the 15 patients with available CBC counts, 6 had more than 10% monocytes (0.10) in the peripheral blood (cases 2, 4, 10, 16, 17, and 20, Table 1), although the absolute number of monocytes was less than 1,000/µL (1.0 × 10⁹/L). At the CMML phase (monocyte count, >1,000/µL [1.0 × 10⁹/L] and >3 months), the mean monocyte percentage and absolute...
number were 25% (0.25) and 5,569/µL (5.6 × 10^9/L), significantly increased from 9% (0.09) and 350/µL (0.4 × 10^9/L) at the MDS phase (\(P = .01\) and \(P < .001\), respectively). Similarly, the total WBC count in the CMML phase was statistically higher than that of the MDS phase (mean, 26.0 × 10^9/L vs 4.3 × 10^9/L; \(P < .001\)). The mean hemoglobin level, platelet count, BM cellularity, and blast count were not different between the MDS and CMML phases of the disease. The difference in IPSS risk distribution also was not significant.

**Clinicopathologic Features: De Novo CMML vs Evolved CMML in MDS and CMML Phases**

The median age of patients with de novo CMML was 68 years (range, 37-92 years), which is not different from that of the evolved CMML (MDS to CMML) group at the MDS phase (67 years) or at the time of CMML evolution (69 years). The de novo CMML group included 62 men and 36 women, with a much lower proportion of men than the MDS to CMML group (\(P = .0003\)). The median hemoglobin level (10.1 g/dL [101 g/L]) and platelet count (136 × 10^9/L) were not statistically different from those of MDS to CMML cases at the MDS or CMML phase. The total WBC and absolute monocyte counts were significantly higher than in the MDS to CMML group in the MDS phase (\(P < .001\) for both) but were comparable to evolved CMML in the CMML phase.

Of 20 MDS to CMML cases, 14 evolved into MP-type CMML and 6 into MD-type CMML, as defined by the FAB criterion of a WBC count of more than 13.0 × 10^9/L (13.0 × 10^9/L). Of the de novo CMML cases, 46 were the MP and 52 the MD type. The difference was not statistically significant (\(P = .33\)). BM examination of de novo CMML and MDS to CMML cases showed hypercellular BM. BM blast counts were comparable between de novo CMML and evolved CMML at the MDS and CMML phases, as were other BM morphologic parameters, including degree of dysplasia, reticulin fibrosis, and megakaryocyte morphologic features and distribution. The BM monocyte percentage in MDS to CMML cases in the CMML phase (mean, 13%; range, 3%-28%) was comparable to that in de novo CMML cases (mean, 15%; range, 5%-42%) but higher than that in evolved CMML in the MDS phase (mean, 7%; range, 5%-18%) (\(P = .05\)). No statistically significant difference was found in IPSS risk distribution between de novo CMML and evolved CMML in the MDS or CMML phase.

### Table 1

**Clinicopathologic Features of Cases of CMML Evolved From Preexisting MDS (MDS to CMML)**

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Initial Diagnosis: FAB/WHO Classification</th>
<th>WBC Count (× 10^9/L)/ Monocytes (%)</th>
<th>Karyotype</th>
<th>MDS Phase</th>
<th>CMML Phase</th>
<th>MDS to CMML (mo)</th>
<th>CMML to Death (mo)</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/70</td>
<td>RA/RCMD</td>
<td>32.5/64</td>
<td>46,XY</td>
<td>46,XY</td>
<td>8</td>
<td>19</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>2/M/46</td>
<td>MDS-U/RCMD</td>
<td>2.3/14</td>
<td>46,XY</td>
<td>46,XY</td>
<td>6</td>
<td>55</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>3/M/70</td>
<td>RAEB/RAEB-2</td>
<td>22.6/16</td>
<td>46,XY</td>
<td>30</td>
<td>2</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/M/57</td>
<td>RA/RCMD</td>
<td>26.8/08</td>
<td>46,XY</td>
<td>46,XY</td>
<td>2</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/M/83</td>
<td>RAEB/RAEB-1</td>
<td>18/21</td>
<td>NA</td>
<td>46,XY(del17)(p11.2)</td>
<td>33</td>
<td>11</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>6/M/65</td>
<td>RAEB/RAEB-1</td>
<td>8.1/25</td>
<td>46,XY</td>
<td>46,XY</td>
<td>27</td>
<td>14</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>7/M/67</td>
<td>RAEB-t/AML</td>
<td>4.9/36</td>
<td>45,X−Y</td>
<td>46,XY</td>
<td>21</td>
<td>20</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>8/M/75</td>
<td>RAEB/RAEB-1</td>
<td>9.5/26</td>
<td>46,XY</td>
<td>46,XY</td>
<td>16</td>
<td>17</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>9/M/60</td>
<td>RAEB/RAEB-1</td>
<td>6.4/9</td>
<td>46,XY(del7)(q11.2)</td>
<td>20</td>
<td>20</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/M/73</td>
<td>RAEB/RAEB-1</td>
<td>13/17</td>
<td>46,XY</td>
<td>46,XY</td>
<td>10</td>
<td>19</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>11/M/60</td>
<td>RA/RA</td>
<td>11.5/24</td>
<td>46,XY</td>
<td>46,XY</td>
<td>10</td>
<td>19</td>
<td>N</td>
<td></td>
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<tr>
<td>12/M/70</td>
<td>RA/RA</td>
<td>15.1/17</td>
<td>46,XY</td>
<td>46,XY</td>
<td>10</td>
<td>19</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>13/M/70</td>
<td>RA/RCMD</td>
<td>8.6/20</td>
<td>46,XY</td>
<td>46,XY</td>
<td>70</td>
<td>15</td>
<td>Y</td>
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<tr>
<td>14/M/73</td>
<td>RAEB/RAEB-1</td>
<td>22.5/15</td>
<td>46,XY</td>
<td>46,XY</td>
<td>10</td>
<td>19</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>15/M/68</td>
<td>RARS/RCMD-RS</td>
<td>21.8/16</td>
<td>46,XY</td>
<td>46,XY</td>
<td>10</td>
<td>19</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>16/M/58</td>
<td>RAEB/RAEB-2</td>
<td>5.3/11</td>
<td>46,XY</td>
<td>46,XY</td>
<td>7</td>
<td>42</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>17/M/83</td>
<td>RARS/RAEB-1</td>
<td>26.1/20</td>
<td>46,XY</td>
<td>46,XY</td>
<td>51</td>
<td>21</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>18/M/65</td>
<td>RAEB/RAEB-1</td>
<td>4.3/7</td>
<td>46,XY</td>
<td>46,XY</td>
<td>51</td>
<td>21</td>
<td>N</td>
<td></td>
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<tr>
<td>19/M/59</td>
<td>RA/RA</td>
<td>375/20</td>
<td>46,XY(del12)(p11.2;q22)</td>
<td>50</td>
<td>26</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20/M/70</td>
<td>MDS-myelofibrosis/RCMD/U</td>
<td>14/13</td>
<td>46,XY</td>
<td>46,XY</td>
<td>43</td>
<td>8</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; FAB, French-American-British; MDS, myelodysplastic syndrome; N, no; NA, not available; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB-t, RAEB in transformation; RARS, refractory anemia with ringed sideroblasts; RCMD, RA with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; U, unclassifiable; WHO, World Health Organization; Y, yes.

* From the first diagnostic CBC count (or the closest CBC count available).

† Persistent monocytosis of >1,000/µL (1.0 × 10^9/L); the CBC count was recorded when absolute monocytosis appeared for ≥3 mo.

‡ In cases 2, 16, 17, and 18, the patients were alive at the last follow-up. The remaining patients had died.
Cytogenetics: Evolved CMML (MDS to CMML) vs De Novo CMML

Of 20 cases of MDS to CMML, G-banded karyotype was available for 17 patients in the MDS phase and 20 in the CMML phase. Of 98 patients with de novo CMML, the karyotype was available for 89. Of the MDS to CMML cases, the karyotype was normal in 13 (76%) of 17 in the MDS phase and 13 (65%) of 20 in the CMML phase. Of the 17 cases with pre-CMML and post-CMML evolution karyotypes available, only 1 (6%; case 14, Table 1) showed cytogenetic progression. Of the remaining 3 cases, 2 (cases 5 and 20, Table 1) showed alteration of chromosome 17p, a chromosomal abnormality often associated with advanced disease. Case 19 showed t(8;21)(p11.2;q22); however, it involved the short arm of chromosome 8, differing from t(8;21)(q22;q22) in AML-M2. This case had never developed into AML hematologically or clinically.

The cytogenetic abnormalities in relation to FAB/WHO distribution of cases of evolved CMML are shown in Table 1. In the de novo CMML group, 52 (56%) of 93 had a normal karyotype, and 41 (44%) had an abnormal karyotype. The distribution of cytogenetic risk groups was as follows: good, 53; intermediate, 26; and poor, 14. No statistical difference was found in karyotype risk group distribution for the de novo CMML group vs evolved CMML in the MDS phase ($\chi^2$, 1.5) or in the CMML phase ($\chi^2$, 0.67).

### Table 2
Clinicopathologic Characteristics: De Novo CMML vs MDS to CMML Cases*

<table>
<thead>
<tr>
<th></th>
<th>MDS to CMML (n = 20)</th>
<th>CMML Phase</th>
<th>De Novo CMML (n = 98)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>67 (46-83)</td>
<td>69 (48-86)</td>
<td>68 (37-92)</td>
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<tr>
<td><strong>M/F ratio</strong></td>
<td>20:0</td>
<td>—</td>
<td>62:36</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/L)</strong></td>
<td>108 (54-135)</td>
<td>100 (76-137)</td>
<td>101 (72-129)</td>
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<tr>
<td><strong>WBC count (x 10^9/L)</strong></td>
<td>4.3 (1.6-7.0)</td>
<td>26.0 (3.9-226.0)</td>
<td>21.3 (13-71.9)</td>
</tr>
<tr>
<td><strong>Platelet count (x 10^9/L)</strong></td>
<td>93 (26-179)</td>
<td>86 (9-299)</td>
<td>136 (5-556)</td>
</tr>
<tr>
<td><strong>Monocytes (%/x 10^9/L)</strong></td>
<td>9/0.35</td>
<td>25/5.69</td>
<td>28/6.369</td>
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<tr>
<td><strong>BM cellularity (%)</strong></td>
<td>78 (20-100)</td>
<td>80 (35-100)</td>
<td>78 (20-100)</td>
</tr>
<tr>
<td><strong>BM blasts (%)</strong></td>
<td>5.7 (0-20)</td>
<td>6.6 (0-17)</td>
<td>6.3 (0-19)</td>
</tr>
<tr>
<td><strong>Karyotype</strong></td>
<td>Normal</td>
<td>13</td>
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<tr>
<td></td>
<td>Abnormal</td>
<td>4</td>
<td>41</td>
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<tr>
<td><strong>International Prognostic Scoring System score</strong></td>
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<td>Low</td>
<td>6</td>
<td>2</td>
<td>20</td>
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<tr>
<td>Intermediate 1</td>
<td>7</td>
<td>9</td>
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<td>High</td>
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<td>2</td>
<td>9</td>
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<tr>
<td><strong>JAK2 mutation</strong></td>
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<td>0/11</td>
<td>2/57</td>
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<tr>
<td><strong>FLT3 mutation</strong></td>
<td>ND</td>
<td>0/11</td>
<td>1/57</td>
</tr>
<tr>
<td><strong>K-ras-2/N-ras</strong></td>
<td>1/4 K-ras-2</td>
<td>1/12 K-ras-2</td>
<td>2/8 N-ras</td>
</tr>
<tr>
<td>AML transformation</td>
<td>—</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Median survival (mo)</td>
<td>42</td>
<td>13</td>
<td>19</td>
</tr>
</tbody>
</table>

* Data are given as mean (range), number of cases, or number positive/number tested unless otherwise indicated.

# JAK2 V617F and FLT3/ITD, K-ras, and N-ras Mutation Studies

BM samples from 68 patients with CMML, including 57 patients with de novo CMML and 11 patients with MDS to CMML in the CMML phase were studied for JAK2 V617F and FLT3/ITD mutations. Of the 57 de novo CMML cases, FLT3/ITD was detected in only 1 (2%), whereas JAK2 V617F was found in 2 cases (4%). Of the BM samples obtained from 11 MDS to CMML cases in the CMML phase, neither JAK2 V617F nor FLT3/ITD mutation was detected ($P = .3$). Because of the negative results, the BM samples obtained from the MDS phase were not tested. RAS mutations, including K-ras-2 and N-ras, were tested in 12 MDS to CMML cases with BM samples obtained during the CMML phase; of these cases, 4 had BM DNA available from the MDS phase that also was examined. Only 1 of 12 MDS to CMML cases showed a K-ras-2 mutation in the CMML phase, and in this case, the same K-ras-2 mutation was present at the MDS phase. No MDS to CMML case showed an N-ras mutation. In contrast, 2 (25%) of 8 de novo CMML cases showed N-ras mutation, whereas none of the 8 showed a K-ras-2 mutation.

### Survival Analysis

The median follow-up time for all CMML cases was 31 months. The median overall survival (OS) of all patients from time of a diagnosis of CMML was 17 months. The median OS...
observed in the MDS to CMML group was 42 months, in contrast with 19 months in the de novo CMML group. Although the OS between the MDS to CMML and de novo CMML groups was not statistically different by the log-rank test, the 18-month predicted survival and median survival for patients with MDS to CMML were superior to those for patients with de novo CMML. In the MDS to CMML group, the median time of evolution from the MDS phase to the CMML phase was 29 months, and the median survival from the CMML phase to death was 13 months. This median survival time was borderline inferior to that of de novo CMML (19 months) by the log-rank test ($P = .0627$).

Figure 1. It is noteworthy that the median survival after CMML evolution in MDS to CMML cases was similar to the OS of MP-type CMML (defined by WBC count $\leq 13.0 \times 10^3/\mu$L [13.0 × 10^9/L]) (15 months) but inferior to MD-type CMML (23 months) ($P = .04$) (survival curves not shown).

Discussion

The FAB classification placed CMML into the MDS subgroup owing to the presence of dysplasia and a variable percentage of myeloblasts. The key distinguishing difference was the presence of persistent monocytosis of at least 1,000/µL (1.0 × 10^9/L). In the FAB classification, it was also proposed to categorize CMML into an MD type (WBC count, $\leq 13.0 \times 10^3/\mu$L [13.0 × 10^9/L]) and an MP type (WBC count, $>13.0 \times 10^3/\mu$L [13.0 × 10^9/L]).3 Although a number of studies have shown inferior median survival in MP-type CMML compared with MD-type CMML, it has been generally accepted that evolution from MD-type CMML to MP-type CMML is a frequent event and that MD-type CMML may represent an early stage of CMML in most cases. It also has been shown that CMML cases with only slight absolute monocytosis and fewer than 5% blasts behave as low-grade MDS, and survival is excellent.

In the present study, we demonstrated that CMML can evolve from MDS without absolute monocytosis, and the proportion of such cases is approximately 17% of all CMML in this series. The median age of this subgroup of patients was similar to that of patients with de novo CMML, but there was a strong male predominance. It is interesting that strong male predominance also was reported in MD-type CMML cases, although this was not statistically significant. It is noteworthy that no statistical differences were observed in BM cellularity, blast percentage, hemoglobin levels, or platelet counts between cases of MDS that eventually evolved into CMML and cases of de novo CMML. Clonal cytogenetic abnormalities were seen in 23% of MDS to CMML cases vs 44% in de novo CMML cases; however, the difference was not statistically significant nor was the distribution of cytogenetic risk groups.

No significant difference in IPSS score at diagnosis was observed between MDS to CMML and de novo CMML cases. Previous studies have shown that the IPSS score used in assessment of MDS prognosis is not useful in CMML. The reason seems to be attributed mainly to the low frequency of karyotypic abnormalities, the rarity of patients with an absolute neutrophil count of less than 1,500/µL (1.5 × 10^9/L).
10^9/L), and the relatively mild anemia in CMML. Our study also showed that despite a similar distribution of IPSS risk groups at diagnosis, patients with MDS to CMML had a longer median survival than patients with de novo CMML (42 vs 19 months).

The fact that CMML can evolve from preexisting MDS suggests a similarity in the pathogenesis of MDS and CMML. Among our 20 MDS to CMML cases in the MDS phase, although all had an absolute monocyte count of less than 1,000/µL (1.0 × 10^9/L), 6 had more than 10% peripheral monocytes (0.10). In a study conducted by Rigolin et al,21 patients with MDS with relative monocytosis (monocyte count, >10% [0.10]) were found to represent 20.8% of 139 consecutive patients with MDS and showed a high incidence of CMML evolution (34.5%) and AML transformation (17.2%). Our results showed that CMML can evolve from MDS with relative monocytosis (>10% [0.10]) and from MDS without relative monocytosis at initial diagnosis. During this evolution, the total WBC count and absolute monocyte count dramatically increased, but other important parameters useful in predicting prognosis in MDS, such as blast percentage, hemoglobin levels, and platelet counts, failed to show significant changes.

Among the CMML cases evolved from MDS, 12 (60%) of 20 could be classified as MP-type CMML (WBC count >13.0 × 10^9/L [13.0 × 10^9/L]) and 8 as MD-type CMML. Three cases eventually transformed to AML (M4 and M5). After CMML evolution, the median survival of this group of patients was 13 months, which was inferior to that for MD-type CMML (23 months) but comparable to that of MP-type CMML (15 months) cases (data not shown), indicating a similarity in progression from MDS to CMML and MD-type CMML to MP-type CMML.

The prognostic relevance of monocytic proliferation in MDS and MPD was highlighted by studies conducted in a large series of patients.24,25 Recently, Chen et al26,27 reported that the presence of monocytic nodules in MDS BM was associated with adverse outcomes. In the present study, our observation suggests that an absolute increase in monocyte numbers or acquired monocytic proliferation might represent a more advanced stage in the multistep pathogenesis of MDS.

A number of possible underlying biologic, molecular, and cytogenetic mechanisms that might be associated with MDS to CMML evolution were studied. Of 17 paired cases, only 1 case showed cytogenetic progression to CMML evolution. Although another 2 cases showed alteration of 17p, a chromosomal alteration often associated with advanced disease, such changes can be seen in high-risk MDS as well.

Activating mutations in the receptor tyrosine kinase FLT3 are frequent in AML and associated with proliferative features.28,29 In our study, FLT3/ITD was found only in 1 de novo CMML case of 57 patient samples tested and in none of the MDS to CMML cases. These results not only suggest that the FLT3/ITD mutation is not responsible for this evolution, but also indicate that acquired monocytic proliferation is indeed different from AML transformation.

JAK2 is a constitutively active tyrosine kinase, and activation of the JAK2 tyrosine kinase through V617F mutation is a frequent pathogenetic event in MPD, such as polycythemia vera, essential thrombocytopenia, and idiopathic myelofibrosis.30,31 The JAK2 V617F mutation is likely to contribute to the myeloproliferative state because its cellular expression leads to growth factor independence.32 Our results show that only 2 (4%) of 57 de novo CMML cases and none of 11 MDS to CMML cases had the JAK2 V617F mutation. These data confirm the findings of previous studies demonstrating that the JAK2 V617F mutation is infrequent in MDS and CMML.33,34 Furthermore, this suggests that the proliferative signal for monocytic proliferation leading to CMML evolution may be independent of the JAK2/STAT pathway.

Although functional RAS activation has not been studied systematically in CMML cases, mutations of N-ras and K-ras-2 genes are more frequent in CMML than in MDS.18,35 and in MT-type CMML than in MD-type CMML.18 In vitro transfection of mutant RAS into human cord blood CD34 cells promotes a myeloid maturation defect, with relative sparing of monocyte-macrophage lineage.36 Of 12 BM samples from MDS to CMML cases, only 1 showed a K-ras-2 mutation, which was present in that case before CMML evolution. The results indicate that RAS, a molecule that has been postulated to be involved in the monocytic proliferation in CMML and monocytic AML, is not involved in this evolution process.

In cell cultures, BM cells from MDS cases show decreased colony-forming units–granulocyte-macrophage and burst-forming units–erythroid in vitro colony growth,37 whereas CMML cases show normal or high and spontaneous granulocyte-macrophage colony growth.5.38 In 1998, Del Canizo et al11 studied progenitor cell cultures in 80 MDS cases, and a normal or an increased cell growth pattern was present in 6 MDS cases, of which 5 evolved to CMML and 1 to atypical CML. They found that the evolution took place concomitantly with an infectious episode. Their results suggest that in some patients, progenitor cells might harbor high clonogenic capacity, and the proliferation could be triggered by cytokine secretion. Because we did not identify significant cytogenetic progression of molecular alterations that often are associated with proliferation states in the evolution from MDS to CMML, we hypothesize that stem cells in MDS to CMML might have monocytic differentiation or proliferation capacity at disease inception. MDS could represent the early manifestation of disease in these cases, similar to the hypothesis proposed for the evolution from MD-type CMML to MP-type CMML. The factors that might have triggered the monocytic proliferation remain to be determined.
Our data suggest that CMML evolving from a preexisting MDS is not an uncommon event. Patients with MDS to CMML had a relatively indolent course during the MDS phase but showed rapid disease progression once a monocytic proliferation developed, a clinical course similar to the evolution from MD-type CMML to MP-type CMML. No significant cytogenetic or molecular alterations could be associated with this evolution. We hypothesize that stem cells in some patients with MDS have a monocytic proliferation capacity at disease inception that eventually manifests as CMML. It may be clinically relevant to monitor patients with MDS for the development of monocytosis because this seems to be indicative of disease progression and an unfavorable outcome.

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References


