Clinical Significance of Cytogenetics in Myeloproliferative Disorders

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Abstract
A complicating feature of the myeloproliferative disorders is the frequent overlap of clinical, laboratory, and morphologic findings among the specific disorders. Cytogenetic and/or fluorescence in situ hybridization studies are performed to obtain evidence of abnormal clones, classify disease, and assess prognosis. These studies help to establish which hematopoietic compartments are affected. For chronic myelogenous leukemia, the BCR/ABL fusion gene allows an unequivocal diagnosis when it occurs with characteristic morphologic and clinical findings. Another activated fusion tyrosine kinase (FIP1L1-PDGFRα) has been recently identified in chronic eosinophilic leukemia/hyper eosinophilic syndrome. In contrast, no specific chromosomal or molecular markers have been identified for the remaining MPD, although recurring cytogenetic abnormalities have been reported. In this review, we discuss the cytogenetic anomalies and their associated clinical significance in myeloproliferative disorders.

Myeloproliferative disorders (MPD) are characterized by proliferation of 1 or more lineages of the myeloid/erythroid series. The World Health Organization classifies MPD into: chronic myelogenous leukemia (CML), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia/hyper eosinophilic syndrome (CEL/HES), polycythemia vera (PV), chronic idiopathic thrombocytopenia purpura (CITP), essential thrombocythemia (ET), and unclassifiable chronic MPD (MPD-U).1

Cytogenetic and/or fluorescence in situ hybridization (FISH) studies should be performed in the work-up of MPD to detect abnormal clones, classify disease, and obtain prognostic information. Cytogenetics can detect therapy-related leukemias, monitor remission, and establish success of engraftment post-bone marrow transplant. These studies also help in establishing which hematopoietic compartments are affected. However, most myeloid disorders do not have a specific karyotype and detected abnormalities may not characterize primary pathogenetic events at all times.2 Cytogenetic subclones can arise due to genetic instability and may not correlate with disease progression.3,4 This review presents current information on cytogenetic findings in MPD and defines the association of karyotype, clinical features, and natural history.

Chronic Myelogenous Leukemia (CML)

Chronic myelogenous leukemia is the most common MPD and originates in an abnormal pluripotent myeloid stem cell, characterized by uncontrolled production of maturing granulocytes, predominantly neutrophils, but also eosinophils and basophils. The karyotypic hallmark of CML is the Philadelphia chromosome (Ph). Ph is the first cytogenetic abnormality identified in a human malignancy and is present in more than 90% of CML cases. Approximately 5% to 10% of CML patients do not have cytogenetic evidence of Ph, but possess a BCR/ABL translocation, and these cases are identical in clinical and pathological respects to Ph positive CML. Ph, first described in 1960, is the result of a reciprocal translocation between chromosomes 9 and 22, (t(9:22)).5 This translocation creates a transcriptionally active fusion gene between the 3’ portion of the ABL oncogene from 9q34 and the 5’ portion of BCR gene at 22q11.2.6 The fusion of BCR and ABL can be detected by conventional cytogenetics, polymerase chain reaction (PCR), or FISH.

The BCR-ABL translocation is the only distinct chromosomal abnormality seen in the chronic phase (CP) of CML. The BCR-ABL gene on Ph encodes for a chimeric protein with constitutive protein tyrosine kinase (PTK) activity that is necessary for transforming capacity and is also involved in leukemogenesis through its interference with signal transduction, the regulation of apoptosis, and cell proliferation.7 In Ph-positive CML, this abnormal gene encodes a novel bcr-abl mRNA that is translated to an aberrant bcr-abl protein (p210bc-abl), which has a much higher PTK activity than normal c-abl protein (p145bc-abl).6 This observation is crucial as it has allowed the development of targeted therapy for CML, which has significantly affected the management of this malignancy.

Recently, a novel agent, imatinib mesylate, specifically designed to inhibit the PTK activity of BCR-ABL, has been assessed in several large clinical trials. A randomized study in newly diagnosed CML patients showed that complete hematologic response (CHR) (97% versus 69%, p<0.001), major cytogenetic response (MCR) (87% versus 34%, p<0.001), and complete cytogenetic response (CCR) (76% versus 14.5%, P<0.001) were all superior with imatinib compared to a combination of interferon-α and low-dose cytarabine.8 Freedom from progression to accelerated phase (AP) or blast crisis (BC) was significantly longer in the imatinib group in this study. Treatment with imatinib mesylate in AP patients results in an overall hematologic response rate of 71%, CHR of 37%, MCR of 28%, and CCR of 19%.9 Imatinib has less impressive effect in patients with BC and higher rates of relapse have been observed in lymphoid BC.10,11 The CCR after imatinib therapy is associated with prolonged disease free survival, which is the goal of therapy for these patients.

Chronic myelogenous leukemia in AP or BC tends to be resistant to most forms of therapy and responses usually are short-lived while responders tend to survive longer than non-responders. Blastic transformation inevitably complicates the course of Ph-positive, BCR-positive CML in an average of about
5 years in untreated patients. Imatinib mesylate appears to be the appropriate treatment for older CML patients or younger patients without a suitable bone marrow donor. However, the only potentially curative treatment for younger patients in CP with a matched sibling donor is allogeneic hematopoietic cell transplantation (HCT). The phase of the disease at the time of HCT directly correlates with the results obtained with HCT; among patients in CP, HCT within the first year results in the best outcomes.12

BCR-ABL is the most commonly and extensively studied tumor-specific translocation in the hematopoietic malignancies. Its presence can be an important indicator of residual disease or relapse after treatment. Seong and colleagues13 compared routine G-band cytogenetic analysis (CA) and FISH in detection of relapse of CML with long-term colcemid exposure. In this study of 51 CML patients who received allogeneic HCT, 12 patients were initially Ph-positive by hypermetaphase FISH and negative by CA. Significant numbers of Ph-positive cells were detected in 7 of these 12 patients more than 3 months after HCT. All 7 of these patients ultimately became Ph-positive by CA at a median interval of 101 days subsequently leading to a clinical relapse.

The PCR method is a more sensitive tool for detection of BCR-ABL transcripts and is useful for the identification of minimal residual disease (MRD). Radich and colleagues14 reported in a study of 346 CML patients that PCR-positivity occurring 6 to 12 months after HCT, especially if they were persistent, was highly predictive of eventual relapse (42% versus 3% for PCR-negative patients, P=<0.0001). However, PCR-positive results obtained within the first 6 months of HCT or after 36 months were not predictive of relapse in this study. Quantitative PCR-positive results at 3 to 6 months after HCT15 and an increasing tumor signal with time16 have both been shown to be predictive of impending relapse. In 2000, Serrano and colleagues17 showed that lineage-specific mixed chimerism preceded cytogenetic relapse by 2 to 12 months and p190BCR-ABL positivity preceded cytogenetic relapse by 1 to 6 months indicating the highly predictive nature of these 2 variables for impending relapse of CML. However, a clear consensus for the implications of PCR positivity on disease dynamics has yet to be established.

Ph-Negative, BCR-ABL Positive CML

Small percentage of patients (5% to 10%) has clinical features of CML, but lack Ph by cytogenetic analysis. However, about one-half of these patients have complex chromosomal rearrangements masking a (t(9,22)) translocation. Other subsets are Ph-negative by karyotype but have evidence of BCR-ABL gene fusion by metaphase or interphase FISH analysis or RT-PCR. The clinical features of both groups of these patients are similar to those with typical Ph positive CML. One-third of the Ph-negative patients also lack molecular evidence of BCR-ABL fusion and these patients have distinct clinical features, including short survival, poor response to therapy, absence of basophilia, and frequent thrombocytopenia. The disease is distinct from CML, and more often resembles that of myelodysplastic syndromes.1

Derivative Chromosome 9 Deletions

Large deletions at the t(9;22) breakpoint may identify a poor prognosis subgroup of patients with CML and may provide some explanation for considerable heterogeneity in presenting clinical features and in the time to evolution to BC. In 2000, Sinclair and colleagues18 reported large deletions, spanning several megabases, adjacent to the translocation breakpoint on the derivative 9 chromosome in a substantial minority of CML patients by FISH analysis. These deletions were more prevalent in patients with variant Ph chromosomes. This study showed that the deletion status was an important indication of poor prognosis independent of age, sex, percentage of peripheral blood blasts, and platelet count in a group of CML patients treated mainly with interferon, while only a minority had received treatment with imatinib. Hunter and colleagues19 in 2001 reported that the frequency of these deletions was similar at diagnosis and after disease progression.

In 2003, Hunter and colleagues20 evaluated prognostic information in imatinib treated CML patients with derivative chromosome 9 deletions compared to Ph-positive CML patients without deletions. In both CP (P=0.02) and AP (P=0.02), the time to progression on imatinib treatment was significantly shorter for patients with deletions compared to those without deletions. Hematologic and cytogenetic responses were uniformly lower in patients with deletions both in CP and in AP in this study. However, no difference in survival between imatinib treated patients with and without deletions was noted. These findings suggest that imatinib does not completely reverse the poor prognosis associated with derivative chromosome 9 deletions and that survival differences may eventually become apparent with longer follow-up. Although clinical significance is uncertain at this time, early allogeneic HCT should be considered in patients with derivative chromosome 9 deletions.

Secondary Chromosomal Abnormalities and Cytogenetic Clonal Evolution

There is a well-established association between clonal chromosome evolution and disease progression in CML. Secondary chromosomal abnormalities occur approximately 3 to 6 months prior to transformation to blast crisis. The most frequent of these include +8, i(17q), +19, +21, and additional Ph variants.21 Less frequently +der(22), +17, -17, -7, -Y have been reported with disease progression in CML. Approximately 1% of BC CML patients have t(3;21)(q26;q22).

The t(3;21)(q26q22) is usually found in BC of CML or myelodysplastic syndrome-derived leukemia. This translocation produces an AML1/EVI-1 fusion protein that contains an amino-terminal half of AML1 including a runt homology domain fused to the entire zinc finger EVI-1 protein. The AML1/EVI-1 fusion protein possesses functions of differentiation block and stimulation of proliferation. The runt homology domain in AML1 controls the ability of differentiation block and the zinc finger domain in the EVI-1 portion controls stimulation of the proliferation. This translocation may have an important role in leukemic progression of CML by these dual functions as a transcription factor.22

In 2002, O’Dwyer and colleagues23 studied the role of clonal evolution in 71 CML patients in AP treated with 600 mg of imatinib mesylate. This study showed that good responses to imatinib mesylate are still possible in patients with clonal evolution as the sole criterion of disease progression. However, it demonstrated that patients with clinical features of AP CML and cytogenetic clonal evolution are at high risk of treatment failure when treated with imatinib mesylate alone and should be considered candidates for additional treatments such as HCT.
Many CML patients with initial responses to imatinib mesylate ultimately relapse while a small proportion of treatment naïve patients with CML in CP are resistant to treatment with imatinib mesylate. Although, the mechanism of resistance as well as methods to combat them are yet to be established, drug resistance has been correlated with molecular or cytogenetic causes like reactivation of BCR-ABL signal transduction, amino acid substitutions that result in changed conformation of the ATP binding site, and additional chromosomal aberrations.\textsuperscript{22-24} A clonal selection has been suggested by several reports noting an increasing proportion of mutated cells during treatment as compared to presence of a small proportion of mutated leukemic cells prior to treatment.\textsuperscript{25,26}

Diagnosis and Monitoring of CML

It is imperative that a cytogenetic or molecular diagnosis is established for a diagnosis of CML, by either the demonstration of Ph by conventional cytogenetic techniques or the demonstration of the BCR-ABL fusion. Diagnosis of CML-AP may be made when one or more of the following are present\textsuperscript{1}:

- Blasts 10% to 19% of white blood cells in peripheral blood and/or of nucleated bone marrow cells
- Peripheral blood basophils ≥ 20%
- Persistent thrombocytopenia (< 100,000/µL) unrelated to therapy or persistent thrombocytosis (> 1,000,000/µL) unresponsive to therapy
- Increasing spleen size and increasing white blood cell count unresponsive to therapy
- Cytogenetic evidence of clonal evolution
- BP of CML can be diagnosed if one or more of the following is present\textsuperscript{1}:
  - Blasts ≥ 20% of peripheral blood white cells or of nucleated bone marrow cells
  - Extramedullary blast proliferation
  - Large foci or clusters of blasts in the bone marrow biopsy

The monitoring of response to therapy in CML patients is illustrated in Table 1.

Chronic Neutrophilic Leukemia (CNL)

Chronic neutrophilic leukemia is a rare chronic myeloproliferative disorder that is characterized by a sustained, mature neutrophilic leukocytosis with minimal or no circulating immature granulocytes, bone marrow hypercellularity due to neutrophilic granulocyte proliferation, and absence of peripheral blood monocytosis, basophilia, and eosinophilia. No specific chromosomal abnormality has been associated with CNL. The origin of the neoplastic clone in CNL is unknown. Cytogenetic and molecular analyses are negative for the Philadelphia chromosome and the BCR/ABL fusion gene, distinguishing it from CML. Cytogenetic studies are normal in nearly 90% of patients. Sporadic reports of +8, +9, del(20)(q11q13), del(11)(q14), +21, and complex karyotypes have been described in the literature.\textsuperscript{27-31} Deletion of long arm of chromosome 20 appears to be the most frequent cytogenetic change in patients with CNL.\textsuperscript{6}

Elliott and colleagues\textsuperscript{32} performed conventional cytogenetic and FISH analyses on 6 patients diagnosed with CNL. FISH was performed using DNA probes to detect abnormalities of chromosomes 5, 7, 8, 9, 11, 20, and 22. At initial diagnosis, the cytogenetic analysis and FISH were normal for all 6 patients. Clonal chromosome evolution occurred during the course of disease in 2 patients (+21 and del(12)). Both patients were treated with hydroxyurea prior to detection of these abnormalities.

<table>
<thead>
<tr>
<th>Table 1. Monitoring Response to Therapy in CML</th>
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<tbody>
<tr>
<td>First Year</td>
</tr>
<tr>
<td>Every 3 months</td>
</tr>
<tr>
<td>Blood Sample</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Marrow Sample</td>
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</table>

X denotes the specific test to be performed at that time point.
Abbreviations: CBC - complete blood count, FISH - fluorescence in situ hybridization, PCR - polymerase chain reaction.

Chronic Eosinophilic Leukemia/Hypereosinophilic Syndrome (CEL/HES)

Hypereosinophilic syndrome is defined as persistent eosinophilia (≥ 1,500/mm\textsuperscript{3}), for which no underlying cause can be found, and which is associated with signs of organ involvement and dysfunction. CEL is characterized by an autonomous, clonal proliferation of eosinophilic precursors resulting in persistently increased numbers of eosinophils in the blood (≥ 1,500/mm\textsuperscript{3}), bone marrow, and peripheral tissues.\textsuperscript{1} Although no specific cytogenetic abnormality is associated with CEL, the detection of any MPD-associated clonal anomaly helps to distinguish CEL from reactive diseases causing increased eosinophils. The presence of any cytogenetic abnormality has been associated with a poor prognosis in CEL patients.\textsuperscript{33} However, there have been published case reports that have responded well to therapy despite abnormal chromosomes.\textsuperscript{34-37}

Various cytogenetic abnormalities have been documented in patients with CEL. These include trisomy 8, isochromosome 17, monosomy 7, trisomy 15, and translocations of the long arm of chromosome 5.\textsuperscript{33}

Recently, fusion of the Fip1-like 1 (FIP1L1) gene to the platelet derived growth factor receptor alpha (PDGFRA) gene was described in 9 patients with hypereosinophilic syndrome.\textsuperscript{38} The fusion occurs from the interstitial chromosomal deletion, del(4)(q12q12). This fusion protein is the first description of a gain-of-function fusion protein occurring from a cryptic interstitial deletion between genes rather than a reciprocal chromosomal translocation. Similar to other fusion tyrosine kinases, FIP1L1-PDGFRA is a constitutively active tyrosine kinase that transforms hematopoietic cells in vitro and in vivo.\textsuperscript{38,39} The fusion gene can be detected at the RNA level by reverse transcriptase polymerase chain reaction (RT-PCR), which may be the most reliable method. The chromosomal deletion is not evident by standard cytogenetics, and can only be detected by FISH on interphase or metaphase nuclei, which explains why most HES patients with the fusion have a normal karyotype.

Cools and colleagues\textsuperscript{30} studied 17 patients with a diagnosis of HES (one had transformed acute myelogenous leukemia from a rapidly progressive hypereosinophilic myeloproliferative disorder). Eleven patients with symptomatic HES were treated with
imatinib 100 to 400 mg daily. Nine of these patients had responses lasting more than 3 months with normal eosinophil counts. The FIP1L1-PDGFRA fusion gene was detected in 4 of the 9 responding patients; these 9 patients had normal karyotypes. However, an additional 4 patients with durable response to imatinib lacked FIP1L1-PDGFRA, indicating that yet another unidentified target of imatinib may be accountable for HES in these cases. Four of the 6 patients who did not receive imatinib were also found to have the fusion gene. All patients were BCR-ABL negative on cytogenetic analysis or FISH.

In a combined prospective and retrospective study of 89 consecutive patients presenting with absolute eosinophil counts greater than 1.5 x 10^9/L, FISH-based strategies were performed on bone marrow cells to detect FIP1L1-PDGFRA. Fourteen percent of 81 patients with primary eosinophilia had the specific mutation. None of 57 patients with HES harbored the FIP1L1-PDGFRA, indicating that another imatinib-sensitive kinase is mutated in these cases. That another imatinib-sensitive kinase is mutated in these cases.

Polycythemia Vera (PV)

Polycythemia vera is characterized by uncontrolled red blood cell production independent of the normal mechanisms that regulate erythropoiesis. It is characterized not only by an increase of the total body red cell volume, but also of granulocytes and platelets. Although bone marrow evaluation is not critical for the initial work-up of suspected PV, karyotypic analysis may confirm the diagnosis when a clonal defect is identified and can help distinguish patients with PV from patients with CML who harbor the Philadelphia chromosome. If a chromosomally abnormal clone is detected at initial diagnosis of PV, the prognosis is worse than in typical PV. Karyotype abnormalities at initial diagnosis implies either an atypical, aggressive phenotype and/or later stage disease with shorter survival. Cyto genetic abnormalities are found in only 10% to 20% of patients at initial diagnosis. The most common recurring abnormalities are trisomy 8, trisomy 9, and del (20q-). Sometimes trisomy 8 and trisomy 9 may occur together.

The chromosomal abnormalities in PV are more often seen with disease progression and occur in 80% to 90% of patients with post-polycythemic myelofibrosis and myeloid metaplasia (PPMM). Nearly all PV patients that transform to myelodysplastic syndrome or acute leukemia will develop cytogenetic abnormalities. There is no clear association between a specific abnormality and the risk of progression to either acute myeloid leukemia (AML) or the preterminal stage of marrow fibrosis.

Diez-Martin and colleagues analyzed chromosomal studies in 104 patients with various stages of PV. Of 63 patients with successful chromosomal analysis during the first 10 years of disease, 27% had an abnormal clone. Of 23 patients who had the disease for more than 10 years, 87% had an abnormal clone. Trisomy 8, +9, and 20q- were detected in some patients during the early part of their disease and were also found among untreated patients. In their analysis, cytogenetics did not predict evolution of disease, but did provide clues to hematologic phenotype, duration of the disease, and consequences of myelosuppressive treatment.

Another study of 104 PV patients revealed chromosomal abnormalities in 13% of untreated patients, 56% of cases treated with radioactive phosphorus (32P) or cytotoxic agents, and in 85% of patients with transformation of disease. The most common karyotype abnormalities detected prior to disease therapy were +8, +9, 13q-, and 20q-.

### Table 2 Common Cytogenetic Abnormalities in Myeloproliferative Disorders

<table>
<thead>
<tr>
<th>MPD</th>
<th>Specific Abnormality</th>
<th>Molecular Biology</th>
<th>% Abnormality</th>
<th>Recurring, Nonspecific Cytogenetic/ Genetic Abnormalities</th>
<th>% Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML, chronic phase</td>
<td>t(9;22)(q34;q11)</td>
<td>BCR/ABL Tyrosine kinase</td>
<td>100</td>
<td>+8, +Ph, +19,(i(17q),t(3;21)(q26;q22) (Evl1/AML1)</td>
<td>80</td>
</tr>
<tr>
<td>CML, accelerated or blast phase</td>
<td>t(9;22)(q34;q11)</td>
<td>BCR/ABL Tyrosine kinase</td>
<td>100</td>
<td>del(20q), +8,+9,del(11q14) +8,del(12p)(q33)p13(TEL/PDGFbr), (17q), 8p11 (FGFR1) 8+, 9+, del(20q), del(13q) 10-20% at initial diagnosis; 80-90% with progression</td>
<td>?</td>
</tr>
<tr>
<td>CML</td>
<td>None</td>
<td>Deletion on 4q12 FIP1L1-PDGFRA</td>
<td>-50</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>ET</td>
<td>None</td>
<td>None</td>
<td>del(13q), +8,del(20q), -7/del(7q), del(11q) 25</td>
<td>-5</td>
<td></td>
</tr>
<tr>
<td>CIMF</td>
<td>None</td>
<td>None</td>
<td>+8, del(13q)</td>
<td>-5</td>
<td>-5</td>
</tr>
<tr>
<td>PV</td>
<td>None</td>
<td>None</td>
<td>?</td>
<td>?</td>
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</table>

Swolin and colleagues\(^{44}\) completed a prospective long-term cytogenetic analyses in PV patients in relation to treatment and clinical course. Of 64 patients, 57 were followed prospectively. Karyotype abnormalities were found in 11 patients at initial diagnosis, and subsequently in an additional 20 patients during the course of the disease. Cytogenetic evaluation after patients developed myeloid metaplasia, myelofibrosis, or leukemia, revealed abnormalities in 71% to 80% of cases. Patients treated with myelosuppressive agents had a significantly greater risk of karyotype abnormalities when compared to those who underwent phlebotomies. A chromosomal abnormality at initial diagnosis did not predict for a greater risk of transformation to leukemia, myeloid metaplasia, and/or myelofibrosis. The karyotype abnormalities had a nonrandom pattern and the most common were +8, +9, and 20q-.\(^{44}\)

In conclusion, karyotype analysis is not required for diagnosis of PV. In the 10% to 20% of patients that cytogenetic abnormalities are detected, the most common are trisomy 8, trisomy 9, and del (20q-). Presence of karyotype abnormalities in PV patients implies an aggressive course or disease progression.

### Chronic Idiopathic Myelofibrosis (CIMF)

Chronic idiopathic myelofibrosis is a clonal myeloid disorder characterized by a myelophthisic anemia, hepatosplenomegaly from extramedullary hematopoiesis, and dysplastic megakaryocytic hyperplasia with associated bone marrow fibrosis. Due to the difficulty of performing chromosomal analysis on bone marrow samples with myelofibrosis or osteosclerosis, there have been only a few published cytogenetic studies involving large series of CIMF patients. In a study by Sandberg and colleagues,\(^{45}\) 28% of 104 patients had no appropriate metaphase for cytogenetic evaluation. However, when chromosomal studies are successful, an abnormal clone can be detected in 30% to 60% of CIMF patients.\(^{46}\)

Reilly and colleagues\(^{46,47}\) published on a series of 106 cases that were studied to identify the frequency and nature of karyotypic abnormalities as well as assess prognostic significance. Cytogenetic abnormalities were detected in 37/106 (34.9%) of patients at initial diagnosis, while 69/106 (65%) had a normal karyotype. Three recurring patterns were seen in 65% of the abnormal cases: deletion 13q-, deletion 20q-, and partial trisomy 1q. Median survival for patients with normal karyotype was 71 months and for those with normal karyotype 30 months.\(^{46}\) In another series, 15 (32%) of 47 patients were found to have chromosomal abnormalities,\(^{48}\) including 20q-deletion, 13q- deletion, and acquired +21 or 21q+. Again, survival was significantly shorter in the patients with abnormal karyotypes (median survival 30 months) when compared to the 32 patients with normal cytogenetics (median, not reached at 6 years). However, no association was discovered between the type of cytogenetic abnormality and survival.

Besa and colleagues reported that chromosome analysis is the best predictor of androgen response in patients with CIMF. In their study of 23 patients, good response to androgen therapy was correlated with normal karyotype and with longer survival.\(^{49}\) According to Whang-Peng and colleagues,\(^{50}\) acquisition of an abnormal karyotype during the course of the disease may indicate the terminal phase with subsequent development of acute leukemia. Additional aberrations likely express further evolution of the pathological clones or the emergence of new ones.

In the largest series, Tefferi and colleagues\(^{51}\) retrospectively studied 165 patients with CIMF and discovered 48% of patients with an abnormal clone at initial diagnosis. Clonal evolution was found to be more frequent in subsequent analysis. A 20q- deletion, 13q- deletion, and +8 were the most common, each accounting for 15% to 25% of all abnormalities. In a multivariate analysis including other clinical and laboratory variables, an abnormal karyotype did not correlate with a worse prognosis.\(^{51}\)

Due to inadequate analyzable metaphases from bone marrow aspirates of CIMF patients, peripheral blood interphase cytogenetics with FISH probes has been attempted to offer an alternative method for obtaining cytogenetic information. Tefferi and colleagues\(^{52}\) performed a prospective study in 42 patients with CIMF, analyzing both peripheral blood and bone marrow interphase cytogenetics. FISH using probes to detect common anomalies of chromosomes 5, 7, 8, 11, 13, 20, and 21 were completed. The results of FISH were comparable in blood and bone marrow, regardless of the peripheral blood CD34 count. Bone marrow karyotype analysis was unsuccessful in 2 patients due to inadequate number of analyzable metaphases; however, peripheral blood FISH analysis detected a prognostically relevant cytogenetic abnormality. Although 17 of the 40 karyotypically evaluable patients (43%) had clonal cytogenetic abnormalities by karyotype, only 13 (33%) had chromosomal changes detectable by the panel of FISH probes used in this study. Nevertheless, peripheral blood FISH analysis with informative probes can be a practical alternative in patients with inadequate bone marrow samples and in those who may benefit from periodic testing to monitor disease status.\(^{53}\)

In conclusion, no specific genetic defect has been identified in CIMF patients. The most common recurring abnormalities include deletion 13q, deletion 20q, and trisomy +8 and/or +9.

### Essential Thrombocythemia (ET)

Essential thrombocythemia is a chronic hematopoietic stem cell disorder characterized primarily by thrombocytosis. It is characterized by sustained thrombocytosis in the blood and increased numbers of large, mature megakaryocytes in the bone marrow. The major clinical consequences include thrombohemorrhagic and vasomotor symptoms. Essential thrombocythemia is characterized by a long median survival (20 years),\(^{54}\) and a low risk (<10%) of leukemia transformation.\(^{54}\) No consistent chromosomal anomaly is associated with ET. Abnormal karyotype is detected in only 5% to 10% of cases. Karyotypic abnormalities often arise only upon transformation of ET to acute leukemia.\(^{55}\)

The infrequent karyotypic abnormalities described in ET do not have prognostic significance. Marrow cytogenetic analysis is essential to rule out CML (t(9;22)) or myelodyplasia (5q- syndrome). Some patients may have a masked Philadelphia chromosome, and therefore, FISH using probes for BCR and ABL can be performed. Patients with ET who have the t(9;22) translocation follow a disease course similar to that of CML and these patients should be treated as CML.

The Third International Workshop on Chromosomes in Leukemia reported on 170 patients with ET and found only 9 (5.3%) with a chromosomally abnormal clone.\(^{56}\) One patient had a deletion (13)(q22) and subsequently progressed to acute leukemia. The other 8 patients had various anomalies.

Sessarego and colleagues\(^{57}\) reported on 86 patients with ET. Four were classified as ET but were positive for Philadelphia chromosome. Another patient had partial deletion of 13q and...
evolved into leukemia a few months later. Five patients had normal karyotype at diagnosis, but developed acute leukemia transformation after several years. Four of these patients with transformed leukemia developed clonal karyotype abnormalities involving different chromosomal regions.

**JAK2 in PV, CIMF, ET**

The discovery of a single mutation in 2005 in the Janus Kinase (JAK)-2 gene in a high percentage of cases of PV, ET, and CIMF suggests that it may be the underlying molecular mechanism for these disorders. A point mutation in the JAK2 gene (V617F), a member of the tyrosine kinase family, provides novel molecular targets for drug therapy. Ongoing developments in this mutation are eagerly awaited.

**Unclassifiable Myeloproliferative Disorders (MPD-U)**

MPD-U includes hematologic disorders that are similar to one or more of the typical MPD but have some atypical features. Most cases of MPD-U fall into the category of either initial stage of PV, CIMF, or ET in which the characteristic features are not yet fully developed, or late stage, advanced chronic MPD, with marked myelofibrosis and osteosclerosis obscuring the underlying disorder. No specific karyotypic abnormality has been associated with these disorders. Chromosomal anomalies commonly found in any of the MPD can also be detected in MPD-U.

**Summary**

The only MPD associated with any specific chromosomal anomaly is CML, which is linked with t(9;22) (q34;q11.2) or a variant of this abnormality. Recently, identification of a constitutively activated fusion tyrosine kinase on chromosome 4q12, derived from an interstitial deletion, has been associated with HES/CEL, and has become yet another target for imatinib therapy. Although an association exists for other anomalies [e, del(13)(q12q14) with CIMF; del9(20q11), +8, and +9 with PV], these non-specific abnormalities can be detected in various hematologic malignancies. All of the MPDs have the potential to undergo clonal evolution, and cytogenetic or molecular changes usually indicate the onset of an accelerated stage of disease or transformation to an acute process. In addition to being a prognostic tool, cytogenetic analysis should provide a clearer understanding of the molecular biology of MPD. Important future prospects include detection of other specific molecular defects, such as those found in CML and HES/CEL and the JAK2 mutation, and subsequently establishing targeted therapeutic agents.


