Acute Myeloid Leukemia and Other Types of Disease Progression in Myeloproliferative Neoplasms

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Key Words: Progression; Accelerated phase; Blast phase; Acute myeloid leukemia; Acute lymphoblastic leukemia; Myeloproliferative neoplasms; Chronic myelogenous leukemia; BCR-ABL1 positive; Primary myelofibrosis; Essential thrombocythemia; Polycythemia vera; Chronic neutrophilic leukemia; Systemic mastocytosis; Myelodysplastic syndromes

ABSTRACT

Objectives: This session of the Society for Hematopathology/European Association for Haematopathology workshop focused on disease progression in myeloproliferative neoplasms (MPNs).

Methods: The session included typical and unusual presentations of chronic myelogenous leukemia (CML), BCR-ABL1 positive; Philadelphia chromosome-negative (Ph-neg) MPNs; and mastocytosis.

Results: Cases of CML illustrated various manifestations of progression, with emphasis on criteria defining stages of the disease. Issues were discussed related to the patterns of recurrence in patients receiving tyrosine kinase inhibitor therapy, including leukemic transformation occurring in a Ph-neg clone. Ph-neg MPN cases highlighted diagnostic approaches used to establish accelerated and blast phases, including cases with significant myelofibrosis and when an adequate bone marrow aspirate smear is not available. The session also included rare cases of aggressive mastocytosis.

Conclusions: There was agreement that a definitive diagnosis can be challenging in the absence of documented review of prior diagnostic material and clinical history.

Myeloproliferative neoplasms (MPNs) are chronic hematopoietic stem cell malignant neoplasms defined by a proliferation of select myeloid lineages. This group of diseases includes chronic myelogenous leukemia (CML), BCR-ABL1 positive; primary myelofibrosis (PMF); essential thrombocythemia (ET); polycythemia vera (PV); chronic neutrophilic leukemia (CNL); chronic eosinophilic leukemia, not otherwise specified (CEL-NOS); systemic mastocytosis (SM); and poorly defined MPN that are unclassifiable (MPN-U).¹ The clinical course of these neoplasms is highly variable and ranges from a slow protracted course to a progressive course with transformation to an acute leukemic phase. Targeted therapies developed in the past decade have significantly modified the natural history of these disorders and brought about new patterns of disease evolution and recurrence. Disease progression in MPNs was one of the topics of the 2013 Workshop of the Society for Hematopathology/European Association for Haematopathology and is the focus of this report.

Disease Progression in CML

CML is an MPN originating in a hematopoietic stem cell carrying the BCR-ABL1 fusion gene. BCR-ABL1 is a result of the t(9;22)(q34;q11.2) in which ABL1 and BCR genes on chromosomes 9 and 22, respectively, are split and form a fusion gene residing on the minute derivative chromosome 22, also known as the Philadelphia chromosome (Ph). The BCR-ABL1 fusion leads to a constitutively activated tyrosine kinase and cytokine-independent myeloid proliferation. The annual incidence of CML is one to two cases
per 100,000 persons per year. The median age at diagnosis is 50 to 60 years; however, pediatric and adolescent patients have been reported. The natural history of the disease prior to the availability of tyrosine kinase inhibitors (TKIs) was bi- or triphasic with a chronic phase (CP) followed by an accelerated phase (AP) or blast phase (BP). Survival rates and prevention of progression to the advanced phase have dramatically improved in the era of TKIs.2 This therapy is not curative and requires long-term maintenance to suppress the Ph-positive clone since a CML stem cell persists despite targeted treatment.3,4 With the advent of TKIs, additional mechanisms of disease progression have emerged. These mechanisms include tyrosine kinase domain (TKD) mutations and the emergence of BCR-ABL1–negative clones. The discussion of the former is beyond the scope of this report. The latter and other typical and unusual presentations of CML progression have been illustrated by the workshop cases and are discussed in the following paragraphs.

Clinical Features

Approximately 20% to 50% of patients with CML are asymptomatic at the time of initial diagnosis, which is often made on routine physical examination with a CBC demonstrating an elevated WBC count.2,5 Approximately 90% of patients present in CP and, when symptomatic, show fatigue, malaise, weight loss, night sweats, and abdominal pain related to splenomegaly.2,6 Less common findings include bleeding or thrombosis related to abnormal platelet counts and/or function, gouty arthritis due to elevated levels of uric acid, gastric ulcers due to increased histamine levels, and leukostasis due to high WBC, leading to dyspnea, confusion, and priapism. In most untreated patients with CML, the CP is typically prolonged, is slowly progressive, and gradually evolves into the AP. Approximately 20% of patients in CP will progress directly into BP without a transition through AP.2 Both AP and BP are characterized by more prominent symptoms, and the latter may include extramedullary blast proliferation (myeloid sarcoma) most commonly in lymph nodes, skin, and soft tissues.

Morphologic, Immunophenotypic, and Genetic Features of CP

Patients with CP-CML typically have leukocytosis (12-500 × 109/L; median, 100 × 109/L) with neutrophilia and a left shift with overrepresentation of myelocytes (“myelocyte bulge”) and segmented neutrophils. Rare blasts can be seen. Dysgranulopoiesis is absent. Absolute basophilia is a constant feature. Absolute eosinophilia and monocytosis also may be present. Monocytes typically constitute less than 3% of the peripheral blood (PB) differential count, and p190 fusion protein (minor breakpoint region of BCR gene, exons 1-2) is associated with significant monocytosis resembling that seen in chronic myelomonocytic leukemia. Mild to moderate normocytic normochromic anemia is typically present, and poikilocytosis, including teardrop forms and/or nucleated RBCs, is associated with bone marrow (BM) fibrosis. The platelet count can be normal or elevated. Thrombocytosis can be observed in patients with CML associated with p230 fusion protein, when the breakpoint occurs in the u-BCR region.

BM is hypercellular due to markedly increased granulopoiesis with a prominent left shift. The left shift is easily appreciated in the BM biopsy samples due to increased immature precursors along bony trabeculae. Eosinophilia may be present. Blasts constitute less than 5% of a BM differential count in CP; however, the exact cutoff signifying progression to AP varies in different classification schemes.1,7,8 Erythropoiesis is decreased. The characteristic “dwarf” megakaryocytes are small with hypolobated nuclei. The number of megakaryocytes ranges from decreased to markedly increased. In a proportion of patients, aggregates and sheets of megakaryocytes are seen that are often accompanied by reticulin fibrosis and may herald disease progression. Pseudo-Gaucher cells and sea-blue histiocytes may be present.

Immunophenotypic abnormalities reported in CP-CML include decreased expression of CD16 and CD32 on mature neutrophils and aberrant expression of CD56 by the myeloid series.9 Myeloid blasts can show decreased expression of CD62L and aberrant expression of both CD56 and CD7. The major diagnostic utility of flow cytometric or immunohistochemistry-based immunophenotyping is limited to AP or BP, when the determination of lineage of the blast population is necessary. In a limited number of cases with prominent reticulin fibrosis and hemodilute aspirate smears, a CD34 immunohistochemical stain can assist in quantifying blasts in sections of BM biopsy and aspirate clot specimens.

The defining t(9;22)(q34;q11.2) can be seen in BM and PB cultures of more than 90% of patients with CP-CML. A small subset of patients shows variant translocations involving additional chromosome(s), such as 1p36.1, 3p21, 11q13, 12p13, and 17q25.10 Finally, in rare cases, patients with CML have cryptic abnormalities that cannot be appreciated by conventional cytogenetics. In these cases, BCR-ABL1 genes can only be identified by fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR). The most common additional chromosomal abnormalities observed at the time of diagnosis of CP-CML are loss of chromosome Y, gain of chromosome 8, a second Ph chromosome, and isochromosome 17q. Genomic microarray studies have provided additional insights into the spectrum of abnormalities in CP-CML. Single-nucleotide polymorphism array analysis showed clonal alterations in approximately 20% of patients. Most genomic losses are observed.
at or around the BCR and ABL1 genes. Interestingly, select gene polymorphisms, such as the T allele of BIM exon 5, were associated with delayed major molecular response to imatinib therapy, potentially leading to more frequent TKD mutations and TKI resistance.

In summary, diagnosis of CP-CML is typically straightforward, with most patients showing characteristic morphologic and laboratory features. The potential caveats include rare atypical presentations resembling other myeloproliferative or myelodysplastic/myeloproliferative neoplasms such as (1) CNL, when leukocytosis includes predominantly segmented neutrophils with no or minimal left shift; (2) ET, in cases with marked thrombocytosis and minimal leukocytosis; and (3) chronic myelomonocytic leukemia, in CML cases with significant monocytosis. Less often, a diagnosis of CML is made when a patient initially presents in the AP or BP.

How Do We Define Disease Progression in CML?

Staging of CML at the time of initial diagnosis and classification of disease progression have been topics of ongoing discussions. Most published classification schemes rely on a combination of pathologic, laboratory, and clinical parameters. However, the cutoff criteria for advanced stage disease vary, and clinical validation of various staging systems is complex due to the ongoing changes in therapeutic regimens that have become available in the past 15 years. The 2008 World Health Organization (WHO) classification, most commonly used by pathologists, adopted staging criteria based on a literature review and the consensus of the Clinical Advisory Committee.

Table 1. 2008 WHO Classification Criteria for Disease Progression in CML

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Table 2. 2008 WHO Classification Criteria for Disease Progression in CML

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AP, accelerated phase; BM, bone marrow; BP, blast phase; CML, chronic myelogenous leukemia; PB, peripheral blood; WHO, World Health Organization.

What Are the Manifestations of Disease Progression in Patients With CML? How Are They Modified by TKI Therapy?

According to the WHO classification, the objective morphologic criteria of AP include the presence of 10% to 19% blasts in PB or BM and basophilia of more than 20% (Table 1). However, there is a concern that these parameters also may indicate more progressive disease. Other features such as megakaryocytic proliferation and fibrosis are more subjective and may require comparison with an original diagnostic BM specimen since approximately 30% of patients with CML have marked reticulin fibrosis at the time of original diagnosis. The morphologic features of AP frequently coincide with other staging criteria, such as progressive therapy-resistant leukocytosis, cytopenias, and/or splenomegaly. Case 140 illustrated the common coexistence of multiple features of AP and an interesting evolution of disease on imatinib therapy. This 82-year-old man originally presented with AP-CML with a WBC count of 200 × 10^9/L, 11% blasts in BM, and significant BM fibrosis. After 5 months of low-dose imatinib, the patient developed a prominent BM and PB basophilia of more than 20% Image 1. The BM blast count at that time decreased to 7%, which is below the threshold of 10% defining AP-CML. Flow cytometry immunophenotyping confirmed the presence of CD34- and/or CD117-positive precursors and a substantial population of atypical basophils positive for CD117; low-density CD45 and low side scatter; decreased-density CD123, CD13, and CD33; and negative for HLA-DR (Image 1). Of note, normal basophils are positive for dim CD45, CD123, CD9, CD22, bright CD38, dim CD33, dim CD25, and CD36 and negative for HLA-DR, CD34, CD117, CD64, and CD19.
Abnormal basophils frequently show decreased-density CD38 and remain positive for CD34 or CD117, as seen in this case. The recognition of basophils is also important due to an overlap with a blast gate and a potential to overestimate blast percentage. In summary, this case illustrates that the features of AP-CML may change over time and that several characteristics of disease progression may be present simultaneously in a single case.

Similarly, case 201 showed several features of AP-CML in BM and PB. The patient had progressive fatigue, weight loss, low back pain, and “saddle” anesthesia. The CBC showed leukocytosis with 16% blasts and 25% basophils, anemia, and thrombocytopenia. The computed tomography scan showed a prominent soft tissue mass invading the right acetabulum and iliocostalis, diffuse “osseous metastases” with an epidural mass, and splenomegaly. BM was in aspirable. The BM biopsy specimen showed hypocellular BM with osteomyelosclerosis and collagen deposition (MF-3). No increase in blasts was seen on CD34 immunostain. The karyotype and FISH confirmed the presence of t(9;22)(q34;q11.2)/BCR-ABL1. Based on BM and PB parameters alone, this case would be classified as AP-CML. However, a biopsy specimen obtained from the epidural tumor showed sheets of intermediate-size cells with vesicular chromatin, prominent nucleoli, and scant cytoplasm positive for CD34, CD33, lysozyme, CD43, and CD45 diagnostic of myeloid sarcoma. Taken together, this case is best classified as BP-CML. CML presents in BP in less than 5% of cases. Extramedullary BP is even less common in patients with CML and more frequently occurs in acute myeloid leukemia (AML) at the time of initial diagnosis or relapse. Central nervous system (CNS) relapse of CML and AML in a setting of hematopoietic stem cell transplantation (HST) has been reported. Case 327 was a 34-year-old woman diagnosed with CP-CML, treated with nilotinib, who developed lymphoblastic BP; unusual BP with t(15;17) in a patient previously diagnosed with CML in cytogenetic remission; BP; developed shortly after cessation of imatinib; despite CR after ATRA and arsenic therapy, patient died shortly after the diagnosis of BP-CML.
therapy, the patient developed right-side facial numbness and diplopia and was shown to have a left temporal enhancing mass with evidence of relapse in cerebrospinal fluid. Concurrent BM examination showed trilineage hematopoiesis, a normal male (donor) karyotype, and no evidence of BCR-ABL1. Dasatinib has been reported previously to show efficacy in preventing recurrent CNS disease due to its high blood-brain barrier penetrance. Cases of isolated CNS relapse in patients taking dasatinib have been related to the emergence of dasatinib-resistant BCR-ABL1 mutated clones, yet another mechanism of disease progression in CML treated by TKIs.

The emergence of additional cytogenetic abnormalities in a BCR-ABL1–positive clone is not uncommon upon CML progression and occurs in approximately 60% of patients. Chromosome gains or losses can occur in advance of morphologic evidence of progression. The most common unbalanced abnormalities are +8, additional Ph chromosome, t(17q), +19, +21, −Y, and −7. Less commonly, recurrent cytogenetic abnormalities typically seen in de novo AML, such as t(8;21), inv(16), t(15;17), and inv(3q)/t(3;3), have been reported. Case 127 is an example of BP with t(15;17)(q24.1;q21.2)/PML-RARA that has been reported elsewhere. The patient was initially diagnosed with CP-CML and achieved complete

Image II Accelerated phase chronic myelogenous leukemia defined by more than 10% blasts in bone marrow (BM) develops marked basophilia during therapy with imatinib. A, Initial BM showed 11% blasts and 4.5% basophils. B, Flow cytometry immunophenotyping demonstrated a prominent population of blasts (in red) and immunophenotypically aberrant basophils (in green). C, Subsequent BM showed mild decrease in the number of blasts and a significant increase in basophils (30%) and eosinophils (21%). D, Similar features were seen by flow cytometry. (Case 140, courtesy of B. Sander, MD, PhD.)
cytogenetic remission on imatinib. Imatinib was discontinued for unknown reasons, and 1 month later, the patient developed coagulopathy and leukocytosis with numerous promyelocytes with morphologic features similar to that of a microgranular variant of acute promyelocytic leukemia (APL) Image 4. Flow cytometry showed an immunophenotype seen frequently in the microgranular variant of APL with blasts/promyelocytes positive for CD2, CD13, CD15, CD33, CD34 (dim, subset), CD38, CD56, CD64, CD117, and myeloperoxidase. A complex karyotype with t(15;17) (q24.1;q21.2) and t(9;22)(q34;q11.2) was seen in 20 metaphases. The patient achieved complete remission on all-trans retinoic acid and arsenic therapy with FISH negative for BCR/ABL1 and PML/RARA. Reverse transcriptase–PCR showed low-level BCR/ABL1 transcripts and was negative for PML/RARA. The patient died 1 month later of multiorgan system failure, an outcome similar to other reported BP-CML cases with t(15;17). Another 43-year-old man (case 8) with BP-CML with a recurrent cytogenetic abnormality, inv(16) (p13.2q22)/CBFB-MYH11, was presented. One year after the diagnosis of CP-CML the patient developed BP with inv(16) (p13.2q22) and t(9;22)(q34;q11.2). Despite a typically favorable prognosis in de novo AML with t(15;17), inv(16), or t(8;21), the prognosis of patients with BP-CML with these recurrent abnormalities is usually poor.22

Although most cases of BP-CML have a myeloid immunophenotype, approximately 30% of patients with CML can develop B-lymphoblastic BP. Rare cases of the T-lymphoblastic immunophenotype also have been reported.22 Case 302 was a 22-year-old woman with CML who developed BP with an unusual B/myeloid mixed phenotype. Flow cytometry immunophenotypic analysis showed that the blasts were positive for B-cell (CD19, CD22) and myeloid antigens (CD13, CD33, CD117), along with CD34, TdT, CD10, and HLA-DR. Myeloperoxidase was detected in a subset of blasts by immunohistochemistry.
Image 3 | Isolated central nervous system relapse of lymphoblastic blast phase chronic myelogenous leukemia in a patient after a hematopoietic stem cell transplant. **A**, Magnetic resonance imaging scan showed a left temporal lobe intra-axial enhancing mass. **B**, Numerous blasts were seen on a cytospin of cerebrospinal fluid. (Case 327, courtesy of X. Zhang, MD, PhD, and colleagues.)

Image 4 | Blast phase chronic myelogenous leukemia with t(15;17)(q24.1;q21.2); PML-RARA. **A**, Hypercellular bone marrow (BM) with sheets of blasts/promyelocytes. **B**, BM aspirate shows features of the microgranular variant of acute promyelocytic leukemia. **C**, Conventional cytogenetic analysis showing concurrent t(15;17)(q24.1;q21.2) and t(9;22)(q34;q11.2), among other abnormalities {46,XX,der(3)t(3;15)(q21;q15),t(15;17)(q24.1;q21.2),t(9;22)(q34;q11.2),der(15)t(3;15),del(17)(q21)[20]. (Case 127, courtesy of C. C. Yin, MD, PhD, and colleagues.)
**BCR-ABL1–Negative Myelodysplastic Syndrome and Acute Leukemia in Patients With CML**

Progression of CML typically occurs in a clone carrying *BCR-ABL1*. However, additional chromosomal abnormalities, including recurrent translocations, can also occur in Ph-negative (Ph-neg) clones. The most common additional chromosomal abnormalities are trisomy 8 and loss of chromosomes 7 and Y. These abnormalities can be transient or related to a development of Ph-neg myelodysplastic syndrome (MDS) or AML and have been reported in the setting of interferon and TKI therapy.

It has been hypothesized that targeting *ABL1*, which is involved in DNA repair, leads to genetic instability and additional chromosomal abnormalities in Ph-neg stem cells. Several patients with CML illustrating this scenario were presented in the workshop.

A Ph-neg clone developed in a 55-year-old man with CP-CML (case 368). The patient received intermittent imatinib therapy and was noted to have anemia and thrombocytopenia along with 4% blasts in the PB smear. The BM was hypercellular with dyserythropoiesis, dysmegakaryopoiesis, and 11% blasts. Conventional karyotyping showed a Ph-neg clone with inv(Y) and monosomy 7. The patient received azacitidine with no response and subsequent induction chemotherapy, upon which he returned to CP-CML.

The Ph-positive clone was accompanied by a minor clone with chromosomal abnormalities seen in the myelodysplastic phase. Case 14 was a 56-year-old man with CP-CML who achieved complete molecular remission on second-line treatment with dasatinib. One year later, the patient developed pancytopenia; at this time, BM was hypercellular with dysplastic myeloid elements and 23% blasts. Conventional cytogenetic analysis demonstrated Ph-neg clones with trisomy 8 and 10. A similar progression was observed in the patient presented in case 147. This 82-year-old woman with CP-CML was treated with modified doses of imatinib and subsequently dasatinib over a period of 5 years. The patient showed progressive cytopenias, and eventually blasts were seen in the PB smear. The BM showed variable cellularity with 32% blasts and no significant dyserythropoiesis or dysgranulopoiesis was seen. Cytogenetic analysis showed two leukemic clones: monosomy 7 and del(6) in 19 metaphases and one metaphase with the Ph chromosome and no other abnormalities.

The above cases underscore the importance of BM examination and continued cytogenetic monitoring of patients with CML on TKI therapy.

**Disease Progression in Ph-Neg MPN**

Ph-neg MPNs include PMF, ET, PV, CNL, CEL-NOS, SM, and MPN-U. Most cases occur in adults, with peak incidence between 50 and 70 years of age.

Select entities such as SM can also be seen in the pediatric population. Others such as ET and PMF are exceedingly rare in children and may follow a different clinical course than those seen in adults. For example, blastic transformation of PMF, which can occur in up to 10% of adult patients with PMF, has not been reported in children.

In addition, spontaneous remission or disease regression...
after a cytoreductive therapy is not uncommon in a pediatric population. This wide variability in the clinical course suggests that pediatric PMF has a variety of etiologies. It has been suggested that a significant proportion of PMF cases diagnosed in children do not represent a clonal hematopoietic disorder or require aggressive therapy. However, the diagnostic and prognostic criteria allowing for treatment stratification are not available for children. This diagnostic dilemma is illustrated in case 340, a 3-year-old girl who had persistent fevers, abdominal pain, petechiae, pancytopenia, and splenomegaly. BM examination showed significant fibrosis with 40% cellularity. Similar to most pediatric patients with PMF, the JAK2V617F mutation was not detected in this tumor. The patient underwent HST and was well at the time of last follow-up.

In contrast, the etiology of adult Ph-neg MPNs is better understood and diagnostic criteria more clearly defined. In addition to relatively well-defined morphologic features described below, most cases show clonal genetic abnormalities such as JAK2V617F mutation, JAK2 exon 12 mutations, mutations of the MPL gene, and recently described abnormalities of the calreticulin (CALR) gene. These genetic abnormalities are mutually exclusive and constitute objective diagnostic criteria that are currently incorporated or have been proposed to be included in the WHO classification. Moreover, there is accumulating evidence that

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**Image 6** BCR-ABL1–negative acute leukemias arising in patients with chronic myelogenous leukemia. A, Frequent blasts in bone marrow (BM) aspirate smear. B, Cytogenetic analysis showed trisomy 8 and 10 and no Philadelphia (Ph) chromosome. (A and B, Case 14, courtesy of J. Zhou, MD, PhD, and colleagues.) C, Numerous blasts seen in BM aspirate smear. D, Cytogenetic analysis showed two clones, one of which included the Ph chromosome. (C and D, Case 147, courtesy of T. Polsky, MD, PhD, and colleagues.)
Myelofibrosis and AP in Ph-Neg MPN

All Ph-neg MPNs share a propensity to progress toward a late myelofibrotic stage characterized by splenomegaly and decreased PB counts due to BM failure. The frequency of myelofibrosis (MF) differs among Ph-neg MPN entities. In adults, typical PMF evolves from a prefibrotic to fibrotic phase. In fact, most patients with PMF present with a significant BM reticulin or collagen fibrosis (grade 2 or 3) accompanied by increased vascularity.1 The hematopoiesis can be focally preserved, and atypical megakaryocytes can be seen in tight clusters, often within sinuses and in the vicinity of bony trabeculae. In the most advanced stages, trilineage hematopoiesis is nearly absent, and prominent newly formed bone occupies a significant portion of the BM medullary space. In the absence of a history of disease, the late stages of PMF are challenging to distinguish from the “spent phase” and post-PV MF and rare myelofibrotic stage of ET.35–37

Similar to adult PMF, MF and myeloid metaplasia are common signs of progression in PV (post-polycythemic MF, post-PV MF) and occur in approximately 20% of the polycytemic patients 10 or more years after the original diagnosis. At this stage, BM cellularity, including erythropoiesis and granulopoiesis, decreases, which leads to normalizing of RBC mass. Atypical megakaryocytes can be prominent, and osteosclerosis can occur. A leukoerythroblastic PB smear and splenomegaly due to extramedullary hematopoiesis are common. In contrast, only in rare cases do patients with ET develop significant MF, typically late in the disease course (<1% of cases at 10 years and <10% at 15 years follow-up).38 To distinguish between the late fibrotic stages of PMF, PV, and ET, the WHO classification requires a documentation of a history of these neoplasms. However, a recent publication suggested that select morphologic features such as megakaryocyte morphology are helpful in the differential diagnosis of advanced fibrotic stages of PV and PMF.39 In cases of post-PV MF, a significant proportion of megakaryocytes retain their typical morphologic features seen in the polycythemic phase, including pleomorphic nuclei, with minimal if any maturational defects. There is no significant dysmegakaryopoiesis and no severe alterations in the nucleus-to-cytoplasm ratio. On the contrary, obvious atypical megakaryocytes and tight clustering are hallmarks of PMF in a fibrotic stage (MF grades 2 and 3). Of note, terminal fibrotic stages with diffuse osteomyelosclerosis of post-PV MF and PMF are no longer distinguishable.

Despite the fact that post-PV MF and PMF share severe BM fibrosis as the dominant morphologic characteristic, the underlying pathogenetic mechanisms are most likely different. Post-PV MF represents a late stage of a slowly evolving clonal disease in which many genetic alterations over time lead to a transformation from a “proliferative” neoplasm to a BM failure syndrome. In contrast, PMF shows a propensity to fiber deposition at a much earlier stage of disease and has a shorter clinical course. Post-PV MF has a higher incidence of karyotypic abnormalities and a higher number of somatic mutations than does PMF; the former also shows a higher frequency of complex karyotypes.39,40

Typical cases of AP PV or PMF show an increase in blasts in BM and/or PB, and this diagnosis requires the presence of 10% to 19% blasts. In cases with significant fibrosis, an immunohistochemical stain for CD34 can be helpful to demonstrate blast clustering in a biopsy specimen, especially if BM aspirate smears are hemodilute. Cases 70 and 209 illustrate the progression of MPN, unclassifiable into AP with a megakaryoblastic immunophenotype Image 8A and Image 8B. Both patients had 10% to 19% blasts expressing megakaryocytic markers either by flow cytometry or immunohistochemistry. Such cases typically show advanced BM fibrosis, and the differential count of a hemodilute BM aspirate smear is not reliable. Since megakaryoblasts can be negative for CD34, one has to rely on an approximate blast count based on the morphologic identification of
megakaryoblasts in the BM biopsy specimen. These megakaryoblasts need to be distinguished from dysplastic small hypolobated megakaryocytes. A helpful feature preventing overdiagnosis of leukemic transformation is a lack of sheets of megakaryoblasts in AP. By definition, sheets of megakaryoblasts are required for the diagnosis of megakaryoblastic BP. Disease progression advancing through AP to BP was seen in case 185 Image 8C and Image 8D.
Per clinical notes, this patient had a 9-year history of ET treated with anagrelide, hydroxyurea, and decitabine and had transfusion-dependent anemia. BM was hypocellular and showed MPN with a striking megakaryocytic proliferation and prominent dysmegakaryopoiesis, 3+ reticulin fibrosis, and 16% medium-sized blasts with scant cytoplasm and cytoplasmic blebbing. The blasts were positive for CD42b and CD61 on immunohistochemical stains and expressed CD34 (dim), CD117, CD13, CD33, HLA-DR, CD36, CD56, and CD41 by flow cytometry. Conventional cytogenetic analysis of a subsequent BM specimen showed 46,XY,der(6)t(1;6)(q21;p21). This cytogenetic abnormality has been reported in rare cases of PMF, post-PV, and post-ET MF with myeloid metaplasia. Transformation to AML and prominent BM fibrosis are uncommon in ET and are more frequently seen in PMF. In this case, original diagnostic slides were not available for review, and thus it cannot be excluded that the initial diagnosis was early PMF.

Another well-reported feature of disease progression in PV is the myelodysplastic type of AP. In these patients, the BM resembles MDS with fibrosis. In a proportion of patients, the development of MDS-type AP has been related to exposure to high-dose P32 and alkylators. However, cases without previous therapy also have been reported.
Unusual Morphologic Features in Advanced Ph-Neg MPN

Several unusual morphologic features have been reported in advanced stages of Ph-neg MPN. Preliminary data suggest that these cases represent a bona fide progression and not merely an evolution of morphologic features without prognostic significance. Rare cases of advanced PMF develop a prominent monocytosis with PB and often BM findings simulating those of chronic myelomonocytic leukemia. In a published series, monocytosis was associated with an increased WBC count, decreased hemoglobin level, decreased platelet count, and the presence of circulating blasts. Patients with PMF who developed monocytosis showed a rapid disease progression and decreased survival.

Workshop case 324 illustrated different unusual morphologic findings developing in a patient with a greater than 20-year history of PV treated with phlebotomies, anagrelide, and hydroxyurea. The patient developed post-PV MF and showed no response to treatment with interferon, a JAK2 inhibitor, or panobinostat. The BM biopsy specimen was markedly hypercellular with grade 4 (MF-3) MF and a predominance of segmented neutrophils. Megakaryocytes were increased with pleomorphic cytologic features and variably tight clustering. The patient experienced symptomatic splenomegaly (spleen weight 3,000 g). Histologic sections of the spleen showed extensive extramedullary hematopoiesis with predominant neutrophilic series, focal megakaryopoiesis, and erythropoiesis. Blasts were not increased as seen on the BM biopsy and spleen sections. A PB smear showed rare blasts and more than 10% of immature myeloid precursors. The authors reviewed additional cases of PV with neutrophilia and more than 10% of immature myeloid precursors. All cases showed sustained (>3 months) neutrophilia that developed in the post-PV MF stage, often many years after the disease onset. Reactive etiologies were excluded. Blasts were not increased, arguing against the typical AP. The clinical course was variable in this case series. The patient (case 324) presented in the workshop died of disease.

Leukemic Transformation in Ph-Neg MPN

Leukemic transformation of Ph-neg MPN (BP; BP-MPN) occurs less frequently than the above-described chronic manifestations of disease progression. The frequency of transformation to AML is highest in PMF (8%-10%), intermediate in PV (5%-10% after 10 years), and lowest in ET (2%-5%). The most common type of BP in patients with a history of PMF is acute megakaryoblastic leukemia, followed by erythroleukemia, AML with minimal differentiation, and AML with maturation. No cases of APL have been reported in the literature, and only rare cases of lymphoblastic transformation have been published. According to the recent series, approximately 25% of patients with Ph-neg MPN who develop BP have no history of chemoradiotherapy. Patients with BP-MPN and previous therapy typically received high-dose chemotherapy and alkylating agents. Hydroxyurea was not linked to an increased risk of leukemic transformation. The survival of patients with BP-MPN is uniformly poor, regardless of therapeutic approach, with a median survival of 3 months. The only currently available curative option is HST if a remission of BP can be achieved. Targeted therapy is highly desirable and perhaps within reach as we learn more about genetic lesions related to disease progression. There is growing evidence that the genetic landscape of AML evolving from Ph-neg MPN is different from that of de novo AML. Mutations in numerous genes such as \( TET2,\ ASXL1,\ IDH1/2,\ TP53,\ SRSF2,\ ZRSR2,\ U2AF1,\ SF3B1,\ \) and SRFS2 have been detected in BP-MPN compared with chronic phases of disease, and select mutations have shown prognostic significance. Presently, the significance of these genes as transforming events is unclear. Interestingly, as illustrated by several workshop cases, some BP-MPNs were negative for the JAK2V617F mutation, even though this mutation was present in the original CP MPN, suggesting that transformation occurs ancestral to the JAK2 mutation or represents another independent clone arising in a hematopoietic stem cell. This pattern of progression is similar to that seen in select cases of leukemic transformation in CML.
Several interesting cases of BP-MPN were submitted to the workshop. In case 367, a 68-year-old woman with PMF progressed to AML. Her PB smear showed 21% blasts, and the limited quality BM aspirate smears and touch imprint had 24% blasts with aberrant myeloid immunophenotype by flow cytometry. Molecular studies demonstrated a GNAS mutation, an unusual finding in myeloid neoplasms reported in only three MDS cases to date.48 Patients with a documented history of PV transformed to AML were reported in cases 181, 257, 331, and 332. Case 257 described an 80-year-old woman diagnosed with JAK2 mutation-positive PV and treated with phlebotomies, hydroxyurea, ruxolitinib, and recent spleen irradiation. Seven years after the original diagnosis, the patient developed massive splenomegaly. BM examination at that time showed 30% blasts with an aberrant myeloid immunophenotype and frequent dysplastic megakaryocytes with clustering. The karyotype showed monosomy 7, and sequencing detected the NRAS mutation. This patient did not have a significant exposure to cytotoxic therapy. Similarly, case 331 was a 73-year-old man with a 20-year history of PV who did not have significant exposure to therapy. He developed pancytopenia, and the BM biopsy specimen showed MF and 30% to 40% blasts. The number of blasts increased over the next 6 months to 50% to 60%. The blast population was positive for CD61, CD31, and factor VIII and partially for myeloperoxidase.

On the contrary, in case 181, the patient was a 76-year-old man who had a history of JAK2-positive PV and bronchioalveolar carcinoma treated with radiotherapy 5 years before the presentation. The patient had shortness of breath, and BM was hypercellular with 28% blasts, dyserythropoiesis, and dysgranulopoiesis. The karyotype was complex with t(9;22)(q34;q11.2) and monosomy of chromosomes 5 and 7. Rare cases of Ph-neg MPN progressing to BP with t(9;22) have been reported.49 Similarly, therapy-related AML can rarely show BCR-ABL1 rearrangement.50 These cases have to be distinguished from a controversial entity of BCR-ABL1–positive de novo AML.51,52 The existence of this entity has been questioned in the past and has recently been validated in a microarray study showing the unique immunoglobulin gene lesions, which enabled its differentiation from myeloid BP of CML.52

An interesting presentation of extramedullary blast transformation was exemplified by case 332. This 81-year-old man with a longstanding history of PV and repeated BM examinations diagnosed as post-PV MF had rapidly increasing splenomegaly Image 10. The spleen showed sheets of immature cells consistent with blasts positive for myeloperoxidase, CD117, and lysozyme and negative for CD34.

Cases 66, 89, 380, and 413 demonstrated various aspects of a BP evolving in the background of ET. Case 89 was a 73-year-old man with ET who developed leukemic transformation and a complex karyotype, including del(12)(p12p13) and i(17)(q10). BM procured after induction therapy showed features of persistent CP ET. Case 413 illustrated erythroid-predominant transformation in a 77-year-old woman with a 1-year history of ET treated with anagrelide, hydroxyurea, and busulfan. The patient developed progressive cytopenias, with BM showing 82% dysplastic erythroid precursors and more than 20% blasts Image 11. The karyotype was complex with partial loss of the long arm of
chromosome 5, trisomy 8, and TP53 and JAK2-V617F mutation. The remaining two cases represent a rare occurrence of B-lymphoblastic leukemia/lymphoma following ET. Case 66 described a 65-year-old man with a 16-year history of JAK2-mutated ET who developed anemia and neutropenia due to B-lymphoblastic transformation. Blasts were positive for JAK2 mutation and showed an additional cytogenetic abnormality, del(9)(p13). Case 380 was a 59-year-old man with a 10-year history of ET who developed MF and was treated with hydroxyurea. One year later, the patient developed JAK2-mutated B-lymphoblastic leukemia/lymphoma with a karyotype identical to that of one of the clones seen in previous BM (deletion of 13q and 20q).

The remaining cases demonstrated various aspects of leukemic transformation of possible preexistent MPN. Not surprisingly, the review of these cases emphasizes that in advanced stage disease with no detailed clinical history or review of prior pathology slides, the definitive diagnosis of prior MPN is challenging. Case 364 was a 71-year-old man who carried a previous diagnosis of Ph-neg MPN. He showed unintentional weight loss and abdominal pain due to splenomegaly. Both PB smear and BM biopsy specimen showed a marked increase in blasts diagnostic of leukemic transformation. In addition, an increased number of small, hypolobated, and hyperchromatic megakaryocytes were present in association with decreased erythropoiesis. The cytogenetics showed del(20)(q11.2) and t(3;21)(q26.2;q22). The latter cytogenetic abnormality is typically seen in cases of therapy-related MDS/AML. However, in the discussed case, previous exposure to chemo- or radiotherapy was unknown. Case 240 was a 23-year-old man with a clonal myeloid neoplasm with t(7;9)(q22;p13), not easily classifiable within the framework of the 2008 WHO classification of myeloid neoplasms. Within a year from the original diagnosis, he developed B-lymphoblastic transformation with acquired t(8;22)(q24.1;q11.2) and showed persistent disease despite aggressive chemotherapy.

Mastocytosis

Mastocytosis is a hematopoietic neoplasm that most commonly involves skin. Aggressive SM and mast cell leukemias are rare and show a rapidly progressive clinical course. Several unusual cases of aggressive mastocytosis and mast cell leukemia were submitted to the workshop.

Case 263 was a 73-year-old man with a history of MDS who presented with weight loss, dysphagia, thrombocytopenia, macrocytic anemia, and monocytosis. Imaging suggested generalized metastatic disease and retroperitoneal lymphadenopathy. BM examination showed an extensive mast cell infiltrate (approximately 50% of BM cells), trilineage dysplasia, and 8% blasts. Liver biopsy specimen confirmed SM. Interestingly, despite extensive involvement and coexistent high-risk MDS, the patient responded to nilotinib therapy.

Case 135 described a 69-year-old man with a history of MDS and leukocytosis with left shift and monocytosis, anemia, and thrombocytopenia. Mast cells constituted 3% of the differential count. The BM showed decreased maturing hematopoiesis, 30% mast cells with morphology of...
promastocytes, and 41% blasts. Dysplastic changes were seen in the neutrophilic series. This case represents aleukemic mast cell leukemia accompanied by AML with myelodysplasia-related changes.

The patient described in case 244 was a 61-year-old man who developed symptoms of acute cholecystitis and urticarial rash. There was leukocytosis with left shift, monocytosis, and 6% primitive cells with purple-red cytoplasmic granules. The latter cells constituted 80% of a hypercellular BM and were positive for CD117 and mast cell tryptase. Megakaryopoiesis was increased with significant dysplasia. The karyotype was highly complex with losses of chromosomes 5 and 7q.

**Summary**

Disease progression in MPN is defined by a combination of morphologic, laboratory, clinical, and genetic/molecular features. Unusual presentations and overlapping morphologic features of progression underscore the importance of a complete BM evaluation. Baseline BM examination is necessary to establish diagnosis and exclude early signs of progression.

In CML, close attention to combined morphologic and clinical characteristics allows one to accurately diagnose unusual cases with overlapping AP and BP features or those presenting as medullary BP or myeloid sarcoma at the
onset of the disease. The recurrent cytogenetic abnormalities, such as t(15;17), inv(16), or t(8;21), are seen rarely in patients with BP-CML and are not associated with a favorable prognosis. TKI therapy may lead to genomic instability and acquisition of cytogenetic abnormalities in a Ph-neg clone, which underscores a need for continued cytogenetic monitoring. Finally, rare cases of MDS or Ph-neg AML have been reported in patients receiving TKI therapy, which demonstrates the importance of morphologic examination of BM in this clinical setting.

The careful evaluation of morphologic features and correlation with clinical history may be helpful in distinguishing PMF and advanced stages of other Ph-neg MPNs. However, when follow-up BM shows a prominent MF, a review of an original diagnostic BM is often critical for a definitive classification. The CD34 immunohistochemical stain facilitates blast enumeration in fibrotic BM and is especially helpful in cases with hemodilute aspirate smears. For the accurate determination of disease progression, it is also crucial to distinguish megakaryoblasts from dysplastic monolobate megakaryocytes in BM biopsy specimens. The unusual presentations of advanced disease that have to be distinguished from other myeloid neoplasms include MDS-like progression of PV, PMF with monocytosis resembling chronic myelomonocytic leukemia, PV with prominent neutrophilia, and blast transformation at extramedullary sites. At transformation, the most common immunophenotype is that of AML, but lymphoblastic transformation has been reported. Rarely, BP-MPN includes unusual GNAS and KRAS mutations or t(9;22). The latter must be distinguished from BP-CML and BCR-ABL1–positive de novo AML.

Finally, cases of mast cell leukemia coexisting with MDS or with AML with myelodysplasia-related changes were presented in the workshop, and the former case responded to nilotinib therapy.

**References**


21. Yin CC, Medeiros LJ, Glassman AB, et al. t(8;21)(q22;q22)


15. Bujassoum S, Rifkind J, Lipton JH. Isolated central nervous


10. Johansson B, Fioretos T, Mittelman F. Cytogenetic and

molecular genetic evolution of chronic myeloid leukemia. 

9. Kussick SJ, Wood BL. Using 4-color flow cytometry to

detect abnormal myeloid populations. Arch Pathol Lab Med.
2003;127:1140-1147.


7. Fu R, Zhang L, Yang R. Paediatric essential


4. Dingli D, Grand FH, Mahray V, et al. Der(6)t(1;6)


2. Bjorkholm M, Derolf AR, Hultcrantz M, et al. Treatment-


Kucine N, Chastain KM, Mahler MB, et al. Primary 

Fu R, Zhang L, Yang R. Paediatric essential 
thrombocytopenia: clinical and molecular features, diagnosis 

DeLario MR, Sheehan AM, Ataya R, et al. Clinical, 
histopathologic, and genetic features of pediatric primary 
myelofibrosis—an entity different from adults. Am J Hematol. 
2012;87:461-464.

Kralovics R, passamonti F, Buser AS, et al. A gain-of-
function mutation of JAK2 in myeloproliferative disorders. 

of the tyrosine kinase JAK2 in human myeloproliferative 

Nangalia J, Massie CE, Baxter EJ. Somatic CALR mutations 
in myeloproliferative neoplasms with nonmutated JAK2. N 

mutations of calreticulin in myeloproliferative neoplasms. 

Tefferi A, Thiele J, Vannucchi AM, et al. An overview on 
CALR and CSF3R mutations and a proposal for revision of 
WHO diagnostic criteria for myeloproliferative neoplasms. 

Rumi E, Pietra D, Ferretti V, et al. JAK2 or CALR mutation 
status defines subtypes of essential thrombocytopenia with 
substantially different clinical course and outcomes. 

Spivak JL. Polycythemia vera: myths, mechanisms, and 

Kreft A, Buche G, Ghalibafian M, et al. The incidence of 
myelofibrosis in essential thrombocytemia, polycythemia 
vera and chronic idiopathic myelofibrosis: a retrospective 
evaluation of sequential bone marrow biopsies. Acta 

examinations including sequential bone marrow biopsies 
in essential thrombocytemia (ET): a retrospective 
clinicopathological study of 120 patients. Am J Hematol. 
2002;70:283-291.

Barbui T, Thiele J, Passamonti F, et al. Survival and disease 
progression in essential thrombocytemia are significantly 
influenced by accurate morphologic diagnosis: an 

Boiocchi L, Mathew S, Gianelli U, et al. Morphologic 
and cytogenetic differences between post-polycythemic 
myelofibrosis and primary myelofibrosis in fibrotic stage. Mod 

evolution and clinical correlates of somatic mutations in 

Dingli D, Grand FH, Mahray V, et al. Der(6)t(1;6)
(q21-23;p21.3): a specific cytogenetic abnormality in 
2005;130:229-232.

Bjorkholm M, Derolf AR, Hultcrantz M, et al. Treatment-
related risk factors for transformation to acute myeloid 
leukemia and myelodysplastic syndromes in myeloproliferative 

monocytosis in patients with primary myelofibrosis indicates an 


