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Recent advances in the diagnosis and classification of myeloid neoplasms – comments on the 2008 WHO classification

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SUMMARY

The fourth edition of the World Health Organization (WHO) classification of myeloid neoplasms refined the criteria for some previously described myeloid neoplasms and recognized several new entities based on recent elucidation of molecular pathogenesis, identification of new diagnostic and prognostic markers, and progress in clinical management. Protein tyrosine kinase abnormalities, including translocations or mutations involving *ABL1*, *JAK2*, *MPL*, *KIT*, *PDGFRA*, *PDGFRB*, and *FGFR1*, have been used as the basis for classifying myeloproliferative neoplasms (MPN). Two new entities - refractory cytopenia with unilineage dysplasia and refractory cytopenia of childhood have been added to the group of myelodysplastic syndromes (MDS), and 'refractory anemia with excess blasts-1' has been redefined to emphasize the prognostic significance of increased blasts in the peripheral blood. A list of cytogenetic abnormalities has been introduced as presumptive evidence of MDS in cases with refractory cytopenia but without morphologic evidence of dysplasia. The subgroup 'acute myeloid leukemia (AML) with recurrent genetic abnormalities' has been expanded to include more molecular genetic aberrations. The entity 'AML with multilineage dysplasia' specified in the 2001 WHO classification has been renamed 'AML with myelodysplasia-related changes' to include not only cases with significant multilineage dysplasia but also patients with a history of MDS or myelodysplasia-related cytogenetic abnormalities. The term 'therapy-related myeloid neoplasms' is used to cover the spectrum of disorders previously known as t-AML, t-MDS, or t-MDS/MPN occurring as complications of cytotoxic chemotherapy and/or radiation therapy. In this review, we summarize many of these important changes and discuss some of the diagnostic challenges that remain.

INTRODUCTION

In the fourth edition of the World Health Organization (WHO) classification of hematopoietic and lymphoid neoplasms, published in 2008, several important changes were made in the criteria for diagnosis and classification of myeloid neoplasms (Table 1, Vardiman *et al.*, 2008a). In this review, we highlight many of the recent advances in our understanding of the pathogenesis of myeloid neoplasms and the application of this knowledge to the diagnosis and classification of myeloid malignancies. As the current WHO classification is extensive, we have limited our comments to selected myeloid neoplasms, in which there are significant advances or changes in diagnostic criteria.

MYELOPROLIFERATIVE NEOPLASMS

In the fourth edition of the WHO classification, the term 'chronic myeloproliferative disease' is replaced by 'myeloproliferative neoplasm (MPN),' and the category now includes eight diseases. These include chronic myelogenous leukemia, BCR-ABL1+, chronic neutrophilic leukemia, polycythemia vera, primary myelofibrosis, essential thrombocythemia, chronic eosinophilic leukemia, not otherwise specified, myeloproliferative neoplasm, unclassifiable, and a new addition – mastocytosis (Table 2).

Protein tyrosine kinases and chronic myelogenous leukemia

Protein tyrosine kinases (PTKs) play an important role in the pathogenesis of MPNs. Mutations and rearrangements of genes encoding PTKs cause constitutive activation of PTKs and its downstream signal transduction pathways and provide cells with an advantage for proliferation and survival. Molecular abnormalities of PTKs have been used in the diagnosis, classification, prognosis prediction, and detection of minimal residual disease, as well as for targeted therapy in MPN. The best understood example is *ABL1*, activated as a result of t(9;22)(q34;q11)/*BCR-ABL1* in chronic myelogenous leukemia (CML). *BCR-ABL1,* has been used to refine the diagnosis of CML. *BCR-ABL1* is valuable for detection of minimal residual disease and has been successfully targeted with specific therapies. Mutational analysis of *ABL1* also has value in predicting response to therapy. The success of targeted therapy in patients with CML, changing a fatal disease into a chronic manageable disease, is a model for acquiring in-depth molecular understanding of all diseases, and thereby allowing for the rational treatment approaches to many myeloid diseases and cancer in general (Figure 1).

JAK2-mutated MPN

In 2005, several groups independently reported the presence of the *JAK2* V617F mutation in approximately 95% of patients with polycythemia vera (PV), and 40–50% of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF) (Baxter *et al.*, 2005; James *et al.*, 2005; Jones *et al.*, 2005; Kralovics *et al.*, 2005; Levine *et al.*, 2005). Subsequently, *JAK2* exon 12 mutations were identified in rare cases of PV without the *JAK2* V617F mutation (Pardanani *et al.*, 2007; Scott *et al.*, 2007), and W515L/K mutations in the thrombopoietin receptor (*MPL*) were identified in approximately 5% of patients with ET and PMF (Pardanani *et al.*, 2006; Pikman *et al.*, 2006). The overlapping clinical and biologic features of PV, ET, and PMF may now be explained by the presence of a *JAK2* mutation. JAK2 mediates activation of receptors for erythropoietin, thrombopoietin, granulocyte-macrophage colony-simulating factor, and granulocyte colony-stimulating factor. Therefore, the different clinical manifestations of PV, ET, and PMF may reflect the stage of differentiation at which the *JAK2* mutation occurs, other genetic events that evolve during disease progression and differences in the genetic background of the patient. Knowledge of *JAK2* mutation also suggests that *JAK2*-mutated PV, ET, and PMF may be better considered as a spectrum of diseases with a common molecular origin. *JAK2* and *MPL* mutations are now included as major diagnostic criteria for PV, ET, and PMF.

However, the present diagnostic distinctions between PV, ET, and PMF are still based on hematologic and clinical data, and bone marrow findings are also very helpful (Kvasnicka & Thiele, 2010). It should also be remembered that *JAK2* mutation is not an initiating event and appears to be a late event in the molecular evolution of MPNs. Clearly, additional understanding of the molecular basis of these diseases

Table 1. The 2008 WHO classification of myeloid neoplasms

- Myeloproliferative Neoplasms
  - Chronic myelogenous leukemia, *BCR-ABL1*-positive
  - Chronic neutrophilic leukemia
  - Polycythemia vera
  - Primary myelofibrosis
  - Essential thrombocythemia
  - Chronic eosinophilic leukemia, NOS
  - Mastocytosis
  - Myeloproliferative neoplasm, unclassifiable
- Myeloid and Lymphoid Neoplasms with Eosinophilia and Abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*
- Myelodyspastic/Myeloproliferative Neoplasms
  - Chronic myelomonocytic leukemia
  - Atypical chronic myeloid leukemia, *BCR-ABL1*-negative
  - Juvenile myelomonocytic leukemia
  - Myelodysplastic/myeloproliferative neoplasm, unclassifiable
    - Refractory anemia with ring sideroblasts associated with marked thrombocytosis
- Myelodysplastic Syndromes
  - Refractory cytopenia with unilineage dysplasia
  - Refractory anemia with ring sideroblasts
  - Refractory cytopenia with multilineage dysplasia
  - Refractory anemia with excess blasts
  - Myelodysplastic syndrome associated with isolated del(5q)
  - Myelodysplastic syndrome, unclassifiable
  - Childhood myelodysplastic syndrome
    - Refractory cytopenia of childhood
- Acute Myeloid Leukemia (AML) and Related Precursor Neoplasms
  - AML with recurrent genetic abnormalities
    - AML with t(8;21)(q22;q21); *RUNX1-RUNX1T1*
    - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFβ-MYH11*
    - Acute promyelocytic leukemia with t(15;17)(q22;q21); *PML-RARA*
    - AML with t(9;11)(p22;q23); *MLLT3-MLL*
    - AML with t(6;9)(p23;q34); *DEK-NUP214*
    - AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
    - AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
    - Provisional entity: AML with mutated *NPM1*
    - Provisional entity: AML with mutated *CEBPA*
  - AML with myelodysplasia-related changes
  - Therapy-related myeloid neoplasms
  - Acute myeloid leukemia, not otherwise specified
    - AML with minimal differentiation
    - AML without maturation
    - AML with maturation
    - Acute myelomonocytic leukemia
    - Acute monoblastic and monocytic leukemia
    - Acute erythroid leukemia
    - Acute megakaryoblastic leukemia
    - Acute basophilic leukemia
    - Acute panmyelosis with myelofibrosis
  - Myeloid sarcoma
  - Myeloid proliferations related to Down syndrome
  - Blastic plasmacytoid dendritic cell neoplasm

AML, acute myeloid leukemia.

is needed. The lateness of *JAK2* mutation in evolution also has implications for therapy, as drugs that inhibit JAK2 may alleviate symptoms and not eradicate the monoclonal cell population.

A reasonable algorithm for the diagnosis of PV, ET, and PMF is to initially test for *JAK2* V617F, followed by assessment for either a *JAK2* exon 12 mutation for patients with suspected PV, or an *MPL* W515L/K mutation for those with suspected ET or PMF. However, the appropriate specimen type and testing methodology are still subject to debate. It has been shown that *JAK2* V617F mutation can be identified with the same high degree of specificity using either DNA or RNA, when either fresh peripheral blood (PB) or bone marrow (BM) aspirate is used. The use of RNA is more sensitive and appears to be ideal for patients with low tumor burden or for detection of minimal residual disease, whereas DNA is the preferred material when archived tissue is used (Gattenlohner *et al.*, 2007). As *JAK2*-mutated progenitors are a fraction of all progenitor cells, some investigators have suggested using BM instead of PB to increase the detection rate, whereas others have shown that *JAK2* mutation can be accurately and reliably detected, with concordant results, using either PB or BM (Mirza, Sekora & Frantz, 2008). Plasma enriched with tumor-specific nucleic acid has been suggested as the sample of choice for *JAK2* mutational analysis by one group (Ma *et al.*, 2008). Subsequent studies, however, have shown that granulocyte lysis during storage can affect accurate quantification of *JAK2*. Therefore, plasma may be better used for screening rather than as a measure for allelic burden (Salama *et al.*, 2009). Granulocyte enrichment also has been proposed as a means to improve detection frequency. Sorting of

Table 2. Gene mutations in myeloproliferative neoplasms with activated receptor tyrosine kinases

Disease entity	Gene	Mechanism of activation	Main cell target
CML	*BCR-ABL1*	t(9;22)(q34;q11)	all myeloid cells
PV	*JAK2*	point mutation (V617F)	erythroid cells, granulocytes, megakaryocytes
ET	*JAK2*, *MPL*	point mutation (V617F, W515L/K)	megakaryocytes
PMF	*JAK2*, *MPL*	point mutation (V617F, W515L/K)	granulocytes, erythroid cells, megakaryocytes
Mastocytosis	*KIT*	point mutation (D816V)	mast cells
MPN with eosinophilia	*PDGFRA-FIP1L1*	interstitial deletion at 4q12	eosinophils, mast cells
MPN with eosinophilia	*ETV6-PDGFRB*	t(5;12)(q31–33;p13)	eosinophils
MPN with eosinophilia	*FGFR1-ZNF198*	t(8;13)(p11;q12)	eosinophils

CML, chronic myelogenous leukemia; PV, polycythemia vera; ET, essential thrombocythemia; PNF, primary myelofibrosis; MPN, myeloproliferative neoplasm.

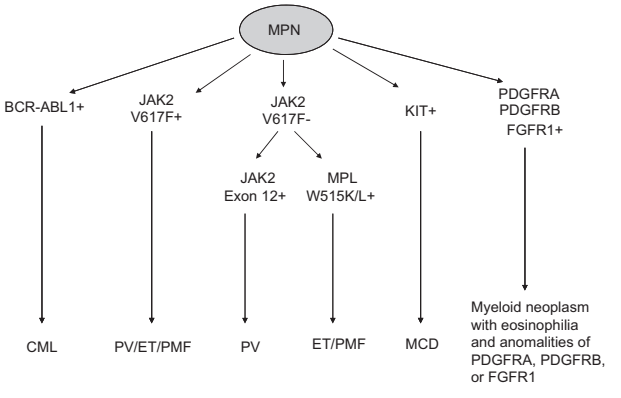

Figure 1. Diagnostic algorithm of myeloproliferative neoplasms associated with receptor tyrosine kinase activation.

granulocytes, however, is expensive and labor-intensive, with the potential for cell loss during sample procession. Testing of unfractionated samples with a sensitive assay is more practical (Stevenson *et al.*, 2006). In fact, it has been demonstrated that the choice of a detection technique and the methods of results interpretation impact the detection rate.

Several qualitative and quantitative methods are currently available to assess *JAK2* mutation. Restriction fragment length polymorphism (RFLP) and direct Sanger sequencing are relatively insensitive (lower limit of sensitivity of 20%) and nonquantitative and therefore not recommended. Pyrosequencing (lower limit 1%) or mutation-specific quantitative polymerase chain reaction (PCR) (lower limit 0.01%) provide better sensitivity and quantification to differentiate PV from ET and PMF and are methods of choice (Yin & Jones, 2010). Quantification of JAK2 mutation is of value diagnostically. High levels of allele burden (consistent with homozygous mutation) are typically observed in patients with PV. By contrast, low allelic burdens (in the range of 30–50%) are more often seen in patients with ET. Mutation level is also helpful in distinguishing prodromal phase of PMF from ET (Kvasnicka & Thiele, 2010).

Mastocytosis

Mastocytosis, an old and well-known disease, is a new addition to the MPN category. *KIT* mutations at codon 816 in exon 17 (usually D816V) have been reported in 50–95% of adults with systemic mastocytosis (SM) and in 30–50% of pediatric patients with cutaneous mastocytosis (CM) (Lim, Pardanani & Tefferi, 2008). Rare sites of *KIT* mutation include D820G, E839K, F522C, and V560G (Lim, Pardanani & Tefferi, 2008). The wide range in the reported frequency of *KIT* mutations may be explained by different diagnostic criteria, sampling issues owing to the focal nature of mast cell aggregates and BM fibrosis, whether aspirate or biopsy specimens are analyzed, and/or the differing sensitivity of detection methods. Pyrosequencing or mutation-specific quantitative PCR on DNA extracted from grossly-microdissected BM or skin biopsy specimens, or from mast cells sorted from

BM aspirates are preferred methods (Zhao *et al.*, 2007). Direct Sanger sequencing is not recommended. The detection of *KIT* mutations in nonmast cell lineages at least partially explains the increased association of SM with other hematopoietic neoplasms (i.e., SM with associated clonal hematological nonmast cell lineage disease, SM-AHNMD) (Yavuz *et al.*, 2002). It is noteworthy that although imatinib mesylate is active against the KIT kinase, the D816V mutation confers resistance, and other inhibitors (e.g. dasatinib) have been shown to be more effective (Akin, 2006).

Issues not addressed by the revised WHO classification of MPNs

Several issues remain unaddressed in the fourth edition of the WHO classification of myeloproliferative neoplasms. For example, various diagnostic criteria have been used in the literature to define the accelerated phase and blast phase of CML. Table 3 summarizes the criteria used by the WHO classification and The University of Texas M. D. Anderson Cancer Center (MDACC). The fourth edition of the WHO classification continues to use the criteria for accelerated and blast phases that were introduced in the third edition on the basis of the published literature and the collective experience of a clinical advisory committee (Vardiman, Harris & Brunning, 2002) without being tested in published trials. The criteria used at MDACC were, on the other hand, developed from a multivariate analysis of factors that independently predicted an outcome inferior to that seen in patients with chronic phase CML treated with imatinib mesylate (Cortes *et al.*, 2006). Because appropriate staging of CML affects treatment and clinical outcome, it is highly desirable to establish a uniform, well-validated system for diagnosing accelerated and blast phases of CML, especially one that is adequately evaluated in the era of PTK inhibitor therapy.

Table 3. Criteria of accelerated and blast phases of chronic myelogenous

Phase	WHO	MDACC
Accelerated		
Blasts	10–19%	15–29%
Blasts and promyelocytes	NA	≥30%
Basophils	≥20%	≥20%
Platelets (x10^9/l)	<100 or >1000*	<100*
WBC (x10^9/l)	>10*	NA
Splenomegaly	Increased	NA
Cytogenetics	CE	CE
Blast		
Blasts	≥20%	≥30%
EMT	Present	Present
Other	Large foci of blasts in BM biopsy	NA

*persistent and unrelated/unresponsive to therapy.
WHO, World Health Organization; MDACC, M. D. Anderson Cancer Center; NA, not applicable; WBC, white blood cells; CE, clonal evolution; EMT, extramedullary tumor.

Another unaddressed issue is the problematic distinction between Philadelphia chromosome (Ph)-positive acute myeloid leukemia (AML) and blast phase of CML. It has been suggested that Ph+ AML simply represents CML presenting as myeloid blast phase in a patient without a symptomatic chronic phase. However, there is evidence to support the interpretation that Ph+ AML, although rare with an incidence of <1%, is indeed a distinct entity. Soupir *et al.* compared the clinical, morphologic, immunophenotypic, and molecular cytogenetic features of Ph+ AML with those of blast phase CML in a large multi-institutional analysis (Soupir *et al.*, 2007). They found that patients with Ph+ AML, compared with patients with blast phase CML, were less likely to have splenomegaly or PB and/or BM basophilia, had lower BM cellularity and myeloid:erythroid ratios, and less commonly had major additional cytogenetic abnormalities that are typically seen in blast phase of CML. Other evidence that favors a diagnosis of Ph+ AML includes the absence of a clinical history of a hematologic disorder, lack of evidence of a chronic or accelerated phase of CML after induction chemotherapy, and return to a normal karyotype after induction chemotherapy.

Similarly, in some instances the term blast phase of MPN has not been used appropriately for a patient with a history of a Ph-negative MPN who subsequently develops AML. The incidence of AML at 10 years after initial presentation is 2–5% for PV, 1–2% for ET, and 5–30% for PMF (Yin & Jones, 2010). AML is known to occur more frequently in patients treated with cytotoxic agents (e.g. hydroxyurea) (Thiele *et al.*, 2008), and *JAK2* mutations are not identified in some of these AML cases

(Theocharides *et al.*, 2007). These facts support the idea that some of these AMLs are more consistent with secondary leukemia rather than MPN progression. A careful investigation into clinical history (leukocytosis, thrombocytosis, basophilia, and splenomegaly), complete morphologic evaluation (background myeloid hyperplasia, eosinophilia, and basophilia, marked megakaryocytic hyperplasia/dysplasia as highlighted by immunostain for CD61, and fibrosis and osteosclerosis as revealed by special stains for reticulum and collagen), routine cytogenetic and molecular testing (*BCR-ABL1* fusion transcript, *JAK2* V617F or exon 12 mutation, *MPL* mutation, and *FIP1LI-PDGFRA* rearrangement) may aid in the recognition of a *de novo* presentation of MPN in blast phase.

MYELOID AND LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND ABNORMALITIES OF *PDGFRA*, *PDGFRB*, OR *FGFR1*

Diseases associated with eosinophilia are numerous and diagnostically challenging. Reactive cases secondary to aberrant release of cytokines or other proteins by reactive or neoplastic T-cells are much more common than are primary hematopoietic neoplasms such as chronic eosinophilic leukemia, acute myelomonocytic leukemia, CML, chronic myelomonocytic leukemia, and mastocytosis. The revised WHO classification includes a new category 'myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1*'. This is an umbrella term that we believe includes three different diseases involving constitutive activation through chromosomal rearrangement of one of three PTKs: platelet-derived growth factor receptor (PDGFR)A PDGFRB or fibroblast growth factor receptor 1 (FGFR1). Multiple fusion gene partners have been reported, with the most common being *FIP1L1*, *ETV6/TEL,* and *ZNF198* for *PDGFRA*, *PDGFRB,* and *FGFR1*, respectively (Table 4, Cross & Reiter, 2008). However, considerable heterogeneity exists in the cell populations affected and in the clinical manifestations of these entities (Bain *et al.*, 2008; Cross & Reiter, 2008). For patients with *PDGFRA* and *PDGFRB* rearrangements, imatinib mesylate has been used for treatment, but no kinase inhibitor is currently available for patients with *FGFR1* abnormalities. The spectrum of diseases in patients with *FGFR1* abnormalities is also broader as many patients with t(8;13)(p11;q12)/*ZNF198-FGFR1* develop lymphoblastic lymphoma/leukemia, usually of T-cell lineage (Jackson, Medeiros & Miranda, 2010).

Table 4. Genes involved in the rearrangements of *PDGFRA*, *PDGFRB*, and *FGFR1*

Tyrosine kinase gene	Chromosome	Partner gene	Chromosome
PDGFRA	4q12	*FIP1L1**	4q12
		STRN	2p24
		CDK5RAP2	9q33
		KIF5B	10p11
		ETV6	12p13
		BCR	22q11
PDGFRB	5q31–33	*ETV6/TEL**	12p13
		TPM3	1q21
		PDE4DIP	1q22
		WDR48	3p22
		GOLGA4	3p22
		PRKG2	4q21
		HIP1	7q11
		CCDC6/H4	10q21
		GPIAP1	11p13
		GIT2	12q24
		NIN	14q24
		KIAA1509	14q32
		TRIP11/CEV14	14q32
		TP53BP1	15q22
		NDE1	16p13
		SPECC1	17p11
		RABEP1	17p13
FGFR1	8p11	*ZNF198/RAMP/FIM**	13q12
		FGFR1OP/FOP	6q27
		TIF1	7q34
		CEP110	9q33
		FGFR1OP2	12p11
		MYO18A	17q11
		LOC113386/HERV-K	19q13
		BCR	22q11

*Most common partner gene.

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS

There are four diseases in this category: chronic myelomonocytic leukemia, atypical CML, *BCR-ABL1* negative, juvenile myelomonocytic leukemia, and myelodysplastic/myeloproliferative neoplasm, unclassifiable.

Refractory anemia with ring sideroblasts and thrombocytosis

Refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) was initially proposed as a provisional entity by the 2001 WHO classification. The current version of the WHO classification addresses this issue in the chapter designated 'myelodysplastic/myeloproliferative neoplasm, unclassifiable' (Vardiman *et al.*, 2008c). In this chapter, the authors of the WHO classification keep RARS-T as a provisional entity, yet they also question the merits of classifying RARS-T as a distinct entity. The criteria for RARS-T are similar, except that the threshold for diagnosing thrombocytosis has been lowered to 450×10^9/l (to be consistent with ET criteria), and the presence of megakaryocytes with morphologic features similar to those seen in ET or PMF is required.

The uniqueness of RARS-T as a distinct entity has been debated in the literature. Is RARS-T simply ET with increased ring sideroblasts or RARS with thrombocytosis? It was recently reported that patients with RARS-T had *JAK2* V617F or *MPL* W515 mutation at a frequency similar to that seen in patients with ET (Boissinot *et al.*, 2006; Ceesay *et al.*, 2006; Atallah *et al.*, 2008). A recent review of RARS-T summarized the similarities between RARS-T and ET: the prognosis for these two diseases was similar, the megakaryocyte morphology was identical (by definition), and they had *JAK2* V617F and *MPL* W515 point mutations at a similar frequency (Wardrop & Steensma, 2009). These authors concluded that ring sideroblasts were a nonspecific finding and that the designation as RARS-T rather than ET gave no additional biologic insight and had no special treatment implications, other than the need to consider the effects of managing thrombocytosis and anemia. They further stated that molecular results should prompt reconsideration of RARS-T as a distinct category.

Atypical chronic myeloid leukemia, *BCR-ABL1* negative

Although the designation 'atypical chronic myeloid leukemia' has been used in the literature for many years, the term was first codified as a distinct entity under the umbrella term of 'myelodysplastic/myeloproliferative diseases' in the third edition of the WHO classification. This entity was renamed 'atypical chronic myeloid leukemia, *BCR-ABL1* negative' (aCML) in the fourth edition, while the diagnostic criteria remained similar (Vardiman *et al.*, 2008b). aCML is an extremely rare leukemic disorder characterized by both myelodysplastic and myeloproliferative features at the time of initial diagnosis. Patients with aCML present with PB leukocytosis because of increased number of neutrophils and their precursors with prominent dysgranulopoiesis. No absolute basophilia or monocytosis is present. Anemia is very common, and platelet counts are variable. The BM is typically hypercellular with granulocytic predominance and is often associated with multilineage dysplasia. Conventional cytogenetics commonly shows abnormalities, especially trisomy 8 and del(20q), but there is no evidence of the *BCR-ABL1* fusion gene, or *PDGFRA* or *PDGFRB* rearrangement. *JAK2* V617F, *NRAS*, and *KRAS* mutations have been reported.

To this point, we have simply summarized the fourth edition of the WHO classification. There is very little information on aCML in the literature (Bennett *et al.*, 1994; Hernandez *et al.*, 2000; Breccia *et al.*, 2006). Some investigators have used the term Ph-negative CML interchangeably (Kurzrock *et al.*, 2001). Although we agree that there are cases that, in part, resemble CML and yet lack *BCR-ABL1* and are associated with dysplasia, it seems clear that aCML is a heterogeneous and poorly defined 'entity', if it indeed is an entity. In one study at our institution (Kurzrock *et al.*, 2001), a retrospective review of aCML cases showed that appropriately one-third of these cases were better classified as chronic neutrophilic leukemia. We suspect that some other cases may represent CMML and detected before the monocytic component is sufficiently high.

Furthermore, the term aCML is both unimaginative and confusing as it carries little molecular resemblance to Ph+ CML. Why not use a term that is at least self-explanatory, such as 'chronic granulocytic proliferation, *BCR-ABL1* negative, associated with dysplasia'? We admit this term is not catchy, and alternative suggestions are welcome.

MYELODYSPLASTIC SYNDROMES

Several major changes have been made in the categorization of myelodysplastic syndromes (MDS) in the fourth edition of the WHO classification. These

changes include incorporation of refractory cytopenia with unilineage dysplasia (RCUD) and refractory cytopenia of childhood (RCC) as separate entities. As a result, there are now seven diseases in this category. These diseases that are not already mentioned include refractory anemia with ringed sideroblasts, refractory cytopenia with multilineage dysplasia, refractory anemia with excess blasts (RAEB-1 and 2), MDS with isolated del(5q), and MDS unclassifiable. The category of RAEB-1 also has been redefined to include cases with 2–4% blasts in the PB to emphasize the prognostic significance of increased blasts in the PB. A list of cytogenetic abnormalities that are considered presumptive evidence of MDS in the presence of a refractory cytopenia but without morphologic evidence of dysplasia also has been introduced in the current WHO classification (Brunning *et al.*, 2008a).

Refractory cytopenia with unilineage dysplasia

Cases that are now recognized as RCUD were considered as refractory anemia (RA) or MDS, unclassifiable in the third edition of the WHO classification. Such cases encompass patients with unicytopenia or bicytopenia associated with unilineage dysplasia. The WHO classification also recommends criteria for defining cytopenia: <10 g/dl for hemoglobin, $<1.8 \times 10^9$/l for absolute neutrophils, and $<100 \times 10^9$/l for platelets. An advantage of this category is that one can further specify lineage: refractory anemia, neutropenia, or thrombocytopenia. This approach has more meaning than lumping these cases into RA as was followed previously. Blasts are absent or very rare in the PB of RCUD patients. Patients with pancytopenia and unilineage dysplasia are still classified as having MDS, unclassifiable, owing to the uncertain clinical significance of these findings (Brunning *et al.*, 2008b).

Refractory cytopenia of childhood

A second important addition to MDS is the provisional entity, refractory cytopenia of childhood (RCC). This category encompasses children with MDS that have persistent cytopenia with less than 2% blasts in the PB and less than 5% blasts in the BM (Baumann *et al.*, 2008). Unlike adult MDS, pediatric patients often present with multilineage dysplasia and/or increased myeloblasts. Single lineage dysplasia, such as RA, is less common (McKenna, 2004). Ring sideroblasts (RS) are extremely rare in children, and their presence is highly suggestive of a mitochondrial disorder or disorder of heme synthesis (Bader-Meunier *et al.*, 1994; Hasel, 2007). Chromosome abnormalities are detected more frequently in pediatric than in adult MDS cases; monosomy 7 or del(7q) is most common. Monosomy 5 and del(5q), common aberrations in adult MDS, are not usually seen in children (McKenna, 2004). The most difficult diagnostic challenge in pediatric MDS occurs when the blast count is not elevated and clonality cannot be established. These cases must be extensively worked up for secondary causes of dyspoiesis, including nutritional deficiency, medications, toxins, metabolic diseases, infections, autoimmune diseases, growth factor therapy, and congenital disorders of hematopoiesis. In fact, approximately one-third of children with MDS have a predisposing genetic condition such as Fanconi anemia or Down syndrome (McKenna, 2004). Furthermore, a greater proportion of pediatric patients with MDS have hypocellular BM; therefore, the distinction of MDS from acquired aplastic anemia (AA) and inherited BM failure syndromes is often difficult as discussed in the following paragraphs. Myeloid leukemia in children with Down syndrome has unique clinical and biological features and is considered as a separate entity in the current WHO classification.

Hypoplastic MDS

Hypoplastic MDS accounts for approximately 10% of adult and 70% of pediatric cases of MDS and is usually defined as BM less than 30% cellular at time of diagnosis in patients younger than 60 years of age, or less than 20% cellular in patients $\geq$60 years. Hypoplastic MDS represents a difficult diagnostic challenge, especially in distinguishing MDS from acquired AA. The degree of dysplasia and the blast counts appreciated on BM aspirate smears may overlap, and the hypocellularity renders interpretation difficult. Thus, an adequate core biopsy specimen with a touch imprint is necessary. The fact that some cases of AA demonstrate clonal cytogenetic abnormalities and knowledge that a subset of patients with AA progress to MDS further complicate the separation of these

two disorders (Young, 1992; Maciejewski & Selleri, 2004; Dolan *et al.*, 2006; Konoplev *et al.*, 2007). It has been reported that some pediatric patients with AA may develop MDS with marked dysmegakaryopoiesis and monosomy 7 (Dolan *et al.*, 2006). Another study showed that the use of growth factors might unmask hypoplastic MDS that mimicked AA (Konoplev *et al.*, 2007). Bennett and Orazi summarized the results of the studies by the French-American-British (FAB) Cooperative Leukemia Working Group and recommended guidelines for distinguishing hypoplastic MDS from AA (Bennett & Orazi, 2009). The presence of blasts in the PB, as well as an increased percentage of CD34-positive blasts and the tendency of these blasts to form aggregates in the abnormal central BM cavity, is indicative of MDS. Thus, an effort should be made to count at least 100 cells in the PB and 200 cells in the BM to determine the percentage of blasts in spite of marked leukopenia. Dysplasia in granulocytes or megakaryocytes is abnormal and inconsistent with a diagnosis of AA. The presence of greater than 10% hypogranular or pseudo-Pelger-Huet neutrophils is indicative of MDS; smaller numbers raise the suspicion but are not definitive. In addition, the presence of easily identifiable micromegakaryocytes (as highlighted by CD61 immunostaining) in an architecturally disorganized BM favors MDS over AA. Erythroid dysplasia, if present alone, must be moderate or severe to support a diagnosis of MDS. The presence of ring sideroblasts (rare in pediatric cases) is considered as evidence of dyserythropoiesis in this setting. The presence of reticulin fibrosis also favors MDS over AA. On the basis of these guidelines, special stains for reticulum and iron, as well as immunohistochemical stains for CD34, CD61, and CD117 are very useful tools in evaluating hypocellular BM for MDS versus AA. In addition, flow cytometry immunophenotyping, cytogenetic, and molecular analyses are valuable ancillary studies.

MDS with fibrosis

Moderate to marked BM fibrosis is observed in approximately 15% of MDS and has been identified as a distinct subgroup of MDS (MDS-F), that is, associated with multilineage dysplasia, poor-risk cytogenetics, high transfusion requirement, and poor prognosis (Della Porta *et al.*, 2009). MDS-F can be a diagnostic challenge, because BM smears are generally of poor quality for assessment of dysplasia and blast percentage. Evaluation of a touch imprint prepared from an adequate core biopsy specimen is extremely helpful in this scenario. Histologic assessment of the core biopsy specimen and immunohistochemical analysis using antibodies for CD34, CD61, and CD117 are often mandatory, and correlation with the PB smear and complete blood count (CBC) findings is necessary for the diagnosis of MDS-F. Important entities in the differential diagnoses of MDS-F include acute panmyelosis with myelofibrosis, acute megakaryocytic leukemia, MPN with fibrosis, and MDS/MPN with fibrosis, as well as nonmyeloid neoplasms, such as hairy cell leukemia and metastatic tumors. The diagnosis of MDS-F can usually be made when there are moderate to marked reticulin fibrosis, dysplasia in at least two cell lineages, and associated PB cytopenias. Organomegaly is not a prominent feature in patients with MDS-F. Unlike MPN and MDS/MPN with fibrosis, *JAK2* V617F mutation is less frequent in MDS-F (Della Porta *et al.*, 2009).

Idiopathic cytopenia of uncertain significance

Idiopathic cytopenia of uncertain significance (ICUS) is a term proposed for patients with persistent ($\geq$6 months), unexplained cytopenia in one or more myeloid lineages (hemoglobin <11 g/dl, absolute neutrophil count $<1.5 \times 10^9$/l, platelet $<100 \times 10^9$/l), and who do not fulfill diagnostic criteria for MDS (Valent *et al.*, 2007). ICUS is a diagnosis of exclusion and requires detailed history (nutritional deficiency, medications, toxin exposure, infections, etc.), CBC with differential count, careful examination of PB and BM smears, iron stain and examination of BM biopsy specimen with immunohistochemistry, as well as ancillary studies including flow cytometry immunophenotyping, chromosome analysis (Table 5), and molecular analysis. An algorithm for evaluation of a patient with cytopenia is illustrated in Figure 2. Patients with ICUS should be closely followed with frequent repeat testing to confirm or exclude MDS. A recent abstract from the Mayo Clinic reported that ICUS is rare, with only 10 cases identified over 12 years; 60% of patients went on to develop MDS within 1 to 10 years (Hanson *et al.*, 2010).

Table 5. Presumptive karyotypic evidence of myelodysplastic syndrome*

Type	Cytogenetic abnormalities
Balanced	t(11;16)(q23;p13.3), t(3;21)(q26.2;q22.1), t(1;3)(p36.3;q21.2), t(2;11)(p21;q23), inv(3)(q21q26.2), t(6;9)(p23;q34)
Unbalanced	−7 or del(7q), −5 or del(5q), i(17q) or t(17p), −13 or del(13q), del(11q), del(12p) or t(12p), del(9q), idic(X)(q13)

*These cytogenetic abnormalities can be used as presumptive evidence of myelodysplastic syndrome in the setting of persistent cytopenias of undertermined etiology without definite morphologic dysplasia

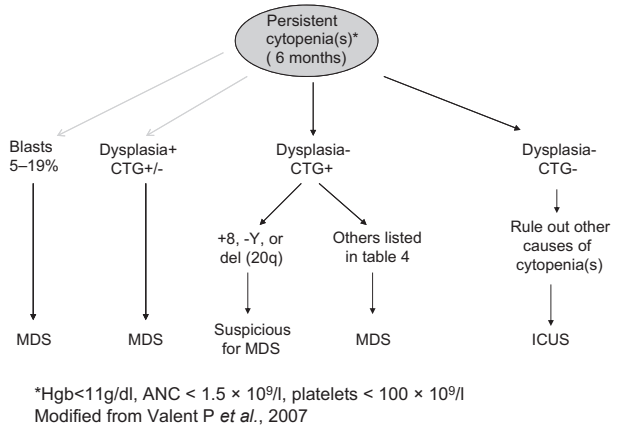

Figure 2. Diagnostic algorithm of cases with persistent cytopenia.

Refractory anemia with excess blasts in transformation – revisited

Refractory anemia with excess blasts in transformation (RAEB-T) is a high-grade MDS with increased blasts in the range of 20–29% as was defined in the FAB classification. This category was eliminated in the third edition of the WHO classification based on the fact that the survival of patients with RAEB-T was similar to those with a blast count of 30% or more. However, this decision has been somewhat controversial since the third edition was published in 2001, and the issues are not yet resolved currently. A number of studies have shown that many features of patients with RAEB-T, such as the frequency of poor-prognosis cytogenetic abnormalities and the presence of antecedent hematologic diseases, more resemble that of MDS than AML (Estey *et al.*, 1997; Albitar *et al.*, 2000; Huh *et al.*, 2002). Furthermore, MDS is a disease in which ineffective hematopoiesis is thought to be attributable for the most part to apoptosis, unlike AML in which impaired differentiation and proliferation play greater roles. It has been shown that there are no significant differences in levels of apoptosis between RAEB-T and RAEB (Huh *et al.*, 2002). Therefore, RAEB-T may be biologically closer to an advanced stage of MDS than it is to AML. To state this point another way, although the decision to change the cutoff may make sense using survival data, the historical cutoffs of 20% and 30% may reflect, in part, true biologic differences. Molecular markers are needed to further resolve this issue. Until they are developed, one should take into consideration the unique aspects of RAEB-T as we develop novel therapies that specifically target the biological and molecular abnormalities in leukemic cells. For the purpose of enrolling patients into certain treatment protocols, it may be helpful to classify such disease process as both AML based on the WHO classification and RAEB-T based on the FAB classification, as we do currently at our institution.

ACUTE MYELOID LEUKEMIAS

AML with recurrent genetic abnormalities

The group 'AML with recurrent genetic abnormalities' has been expanded to include seven neoplasms associated with specific genetic abnormalities. Recent neoplasms included in this category include AML associated with t(6;9)(p23;q34)/*DEK-NUP214*, AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)/*RPN1-EVI1*, and AML with t(1;22)(p13;q13)/*RBM15-MKL1*. Cases with mutated *NPM1* or mutated *CEBPA* are also listed as provisional entities.

In 'acute promyelocytic leukemia (APL) with t(15;17)(q22;q21)/*PML-RARA*', variant RARA translocations with other partner genes are recognized separately, because not all have typical APL features and some are resistant to all-trans retinoic acid (ATRA). The former entity 'AML with 11q23/*MLL*

abnormalities' has been redefined as 'AML with t(9;11)(p22;q23)/*MLLT3-MLL*'. Balanced translocations other than those involving *MLLT3* should be specified in the diagnosis. Other abnormalities of *MLL*, such as partial tandem duplication, should not be placed under this entity (Arber *et al.*, 2008a). As a result of the rapid advances in the understanding of molecular leukemogenesis, this group is destined to undergo additional modifications in future classification revisions. AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)/*RPN1-EVI1* also may be more appropriately listed as 'AML with *EVI1* overexpression' because cases that overexpress *EVI1* as a result of translocation with partners other than *RPN1* have also been associated with aggressive clinical course (Lin *et al.*, 2006; Yin *et al.*, 2006; Lennon *et al.*, 2007). Other molecular abnormalities such as *FLT-3* and *KIT* mutations are well-studied events that play important roles in leukemogenesis, whereas *WT1* mutation and overexpression of *BAALC*, *ERG*, and *MN1* have more recently been shown to be associated with relatively poor clinical outcome (Mrozek *et al.*, 2007).

It should also be noted that the blast percentage cutoff for most types of AML with a recurrent genetic abnormalities remains 20%, but this is not true for AML with t(8;21)(q22;q22)/*RUNX1-RUNXT1*, inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB-MYH11*, and t(15;17)(q22;q21)/*PML-RARA*. In these three entities, recognition of the molecular abnormality defines the disease, even when the blast count in PB and BM is below 20%. The scientific basis for handling the blast count in this inconsistent manner for the other neoplasms in this group is unclear. Based on our experience, we have seen patients with t(8;21)(q22;q22)/*RUNX1-RUNXT1*, or inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB-MYH11*, or t(15;17)(q22;q21)/*PML-RARA* and a blast count less than 20% in PB and BM. Follow-up showed a rapid rise in blast count supporting the diagnosis of AML based on the presence of the molecular abnormality, and therefore we agree with the WHO approach for these neoplasm. It seems reasonable, in our opinion, to extend this approach to other AMLs associated with distinctive translocations, such as t(6;9)(p23;q34)/*DEK-NUP214*, as these patients in our experience also fair poorly (Oyarzo *et al.*, 2004). We agree that the 20% blast cutoff should be retained for provisional AML categories based on gene mutations (e.g. AML with *NPM1* mutation). We believe these issues need to be addressed in future revisions of the WHO classification.

AML with myelodysplasia-related changes

Acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) is the term proposed by the fourth edition of the WHO classification to encompass all AML cases with a history of MDS or MDS/MPN, or with myelodysplasia-related cytogenetic abnormalities, or with morphologic evidence of dysplasia in 50% or more of the cells in two or more myeloid lineages (Arber *et al.*, 2008b). Even in the short time since publication, opinion differs regarding whether this category is truly a distinct entity. Earlier studies suggested that myelodysplasia-related morphologic abnormalities correlated with unfavorable cytogenetic abnormalities but had no independent impact on prognosis (Haferlach *et al.*, 2003; Wandt *et al.*, 2008). Weinberg *et al.* more recently compared patients with AML, NOS to patients with AML-MRC (Weinberg *et al.*, 2009). Patients in the latter group were significantly older, presented with a lower hemoglobin level, exhibited a decreased frequency of *CEBPA* mutations, and had a significantly worse overall survival. The authors concluded that their data supported the clinical, morphologic, and cytogenetic criteria for AML-MRC. We believe that cases of AML-MRC differ from cases of AML, NOS. However, we wonder whether the category of AML-MRC is too heterogeneous. In our opinion, patients with no morphologic evidence of MDS or history of MDS who present with a cytogenetic abnormality (e.g. del(7q) or monosomy 7) seem different from patients with overt MDS. It also seems likely that the pathogenic mechanisms attributable to the wide number of cytogenetic abnormalities in MDS are different. It is expected that AML-MRC is a term that will be substantially modified in future revisions of the WHO classification.

Therapy-related myeloid neoplasms

The fourth edition of the WHO classification uses the term 'therapy-related myeloid neoplasms' (t-MN) to cover the spectrum of disorders previously known as t-AML, t-MDS, or t-MDS/MPN occurring as late complications of cytotoxic chemotherapy and/or radiation therapy (Vardiman *et al.*, 2008a,b,c,d). Excluded from

this category is blast crisis of an underlying MPN as it is often not possible to determine whether leukemic transformation is a result of disease evolution or is therapy related. It should be noted that the term 'therapy-related' is based on the patient's history of prior exposure to cytotoxic agents and/or radiation, but the causal relationship remains to be proven, and the etiology and specific factors that predispose patients to t-MN is still largely elusive. Most cases show multilineage dysplasia, complex cytogenetic aberrations, and a poor prognosis (Yin *et al.*, 2005). Approximately 75% of cases of t-MN develop after exposure to alkylating agents and/or radiation and are characterized by a relatively long latency interval (5–10 years after exposure), presentation with t-MDS, and loss of chromosomes 5 and/or 7. The remaining patients with t-MN usually present in a relatively short interval (1–5 years) after therapy with topoisomerase II inhibitors. This subset of patients tends to develop overt AML without an antecedent MDS and is associated with balanced chromosomal translocations involving 11q23 (*MLL* gene) or 21q22 (*RUNX1* gene). The similarities in clinicopathologic and molecular genetic features between t-MDS and t-AML including the presence of multilineage dysplasia, similar chromosomal aberrations, as well as rapid progression from t-MDS to t-AML in most cases, in our opinion, justify combining t-MDS and t-AML into one category. The newly proposed name, t-MN, is reasonable although the term t-MDS/AML also seems reasonable.

The development of t-MN is a result of a complex interplay of a number of factors including direct mutational effect of cytotoxic agents and/or radiation, the effect of an ineffective BM microenvironment because of injury to vascular supply or increasing fibrosis (caused by prior treatment), genetic instability likely driving BM dysplasia, immunosuppression from prior or current therapy, host genetic predisposition, and high frequency of unfavorable cytogenetic abnormalities. Various genetic pathways involving chromosomal rearrangements and mutations in multiple genes (e.g. *FLT-3*, *RAS*, *KIT*, *RUNX1*, *MLL*, *TP53*, *CBF*, *NPM1*, *CEBPA*) have been implicated. Based on cytogenetic abnormalities at initial presentation, Pedersen-Bjergaard *et al.* proposed eight different genetic pathways for the multistep development of t-MN (Pedersen-Bjergaard *et al.*, 1995), which have been modified subsequently with the discovery of more molecular genetic aberrations (Pedersen-Bjergaard *et al.*, 2006). These data raise the possibility that the category of t-MN is likely to be subdivided in future classification systems.

Acute erythroid leukemia

The category of acute erythroid leukemia (AEL) has substantially evolved as it is defined in the current WHO classification, in large part as a result of the new entity 'AML-MRC' as discussed earlier. Many cases of AML with blasts $\geq$20% of all BM cells and erythroid predominance that used to be classified as AEL are now classified as AML-MRC because they are associated with dysplasia. Cases with blasts <20% of all BM cells but $\geq$20% of the nonerythroid cells are classified as AEL, and cases with blasts <20% of the nonerythroid cells are classified as MDS (Figure 3). Using the criteria of the 2008 WHO classification, the distinction between AEL and AML-MRC or MDS with erythroid hyperplasia is based solely on the percentage of blasts. In fact, similarities exist among these entities, in particular, the high frequency of preceding MDS or the presence of multilineage dysplasia, as well as common cytogenetic abnormalities (Hasserjian *et al.*, 2010; Santos *et al.*, 2009). Hasserjian *et al.* studied the clinicopathologic and cytogenetic features of 124 patients with AEL as defined using the 2008 WHO classification in comparison with patients with AML-MRC or MDS associated with erythroid hyperplasia and concluded that AEL was part of a continuum with AML-MRC and MDS with erythroid hyperplasia, where karyotype rather than an arbitrary

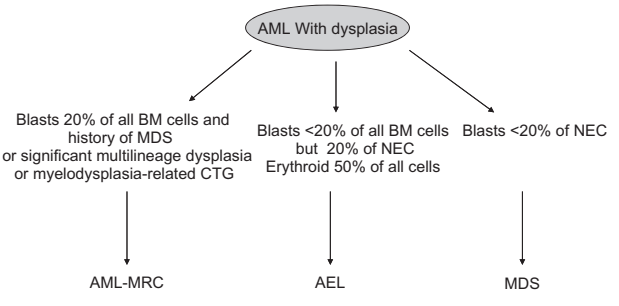

Figure 3. Diagnostic algorithm of AML with myelodysplasia and erythroid hyperplasia.

blast cutoff was the most important prognostic factor (Hasserjian *et al.*, 2010). Another recent study also showed that AEL, as currently defined in the WHO classification, is both substantially less common than the reported frequency of 5% of AML and nevertheless remains heterogeneous. One subset of AEL patients had relatively low blast count and was diploid with a relatively good prognosis. The other subset of patients had cytogenetic abnormalities similar to those seen in patients with MDS and was associated with a poor prognosis (Kasyan *et al.*, 2010).

Acute megakaryoblastic leukemia

Acute megakaryoblastic leukemia is defined as an acute leukemia with 20% or more blasts of which at least 50% are of megakaryocytic lineage. Similar to AEL, acute megakaryoblastic leukemia has become an even more rare entity using the criteria of the fourth edition of the WHO classification (Oki *et al.*, 2006). Cases previously classified in this category should now be placed in the appropriate genetic category if they are associated with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)/*RPN1-EVI1* or t(1;22)(p13;q13)/*RBM15-MKL1*. Those cases with myelodysplasia-related cytogenetic abnormalities should be re-classified as AML-MRC. Down syndrome-related cases are also excluded from this category and designated separately.

A role for the FAB classification in AML?

The FAB classification of AML, last updated in 1985, was based predominantly on traditional morphologic and cytochemical criteria. Although the current WHO classification of AML, NOS is essentially a modified FAB classification, there is little mention of the FAB, not even as synonyms for the various categories. Although we agree that it is best to define myeloid diseases on the basis of well-defined molecular alterations, the FAB classification, in our opinion, still has some value, and the clinicians at our institution continue to ask us to provide both FAB and WHO terminology for AML cases. There are some advantages to this approach. First, an FAB designation can be provided rapidly after morphological examination and assessment of cytochemical stains (mostly myeloperoxidase and butyrate esterase). We then include the WHO terminology, once the results of cytogenetics and molecular tests are available. Second, some of the FAB categories such as M3 and M4Eo highly correlate with the t(15;17)(q22;q21)/*PML-RARA* or inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB-MYH11*, respectively. Lastly, the terminology of the FAB classification is convenient. For example, M0 is much easier to say or write than AML with minimal differentiation.

CONCLUSIONS

In summary, the fourth edition of the WHO classification of myeloid neoplasms clearly manifests the progress made in deciphering the molecular pathogenesis of myeloid neoplasms, as well as the development of targeted therapy since 2001, the year the third edition was published. It is expected that additional updates and revisions to this classification will be needed as progress continues to occur rapidly. It is hoped that the time to publication of the future fifth edition will be less than the seven-year interval between the third and the fourth edition.

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REFERENCES

Akin C. (2006) Molecular diagnosis of mast cell disorders: a paper from the 2005 William Beaumont Hospital Symposium on Molecular Pathology. J Mol Diagn 8, 412–419.

Albitar M., Beran M., O'Brien S., Kantarjian H., Frieriech E., Keating M. & Estey E. (2000) Differences between refractory anemia with excess blasts in transformation and acute myeloid leukemia. Blood 96, 372–733.

Arber D.A., Brunning R.D., Le Beau M.M., Falini B., Vardiman J.W., Porwit A., Thiele J. & Bloomfield C.D. (2008a) Acute myeloid leukaemia with recurrent genetic abnormalities. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 110–123. IARC, Lyon.

Arber D.A., Brunning R.D., Orazi A., Bain B.J., Porwit A., Vardiman J.W., Le Beau M.M. & Greenberg P.L. (2008b) Acute myeloid leukaemia with myelodysplasia-related changes. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 124–126. IARC, Lyon.

Atallah E., Nussenzveig R., Yin C.C., Bueso-Ramos C., Tam C., Manshouri T., Pierce S., Kantarjian H. & Verstovsek S. (2008) Prognostic interaction between thrombocytosis and JAK2 V617F mutation in the WHO subcategories of myelodysplastic/myeloproliferative disease-unclassifiable and refractory anemia with ringed sideroblasts and marked thrombocytosis. Leukemia 22, 1295–1298.

Bader-Meunier B., Rotig A., Meilot F., Lavergne J.M., Croisille L., Rustin P., Landrieu P., Dommergues J.P., Munnich A. & Tchernia G. (1994) Refractory anaemia and mitochondrial cytopathy in childhood. British Journal Haematology 87, 381–385.

Bain B.J., Gilliland D.G., Horny H.P. & Vardiman J.W. (2008) Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGFR1. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 68–73. IARC, Lyon.

Baumann I., Niemeyer C.M., Bennett J.M. & Shannon K. (2008) Childhood myelodyspastic syndrome. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 104–107. IARC, Lyon.

Baxter E.J., Scott L.M., Campbell P.J., East C., Fourouclas N., Swanton S., Vassiliou G.S., Bench A.J., Boyd E.M., Curtin N. & Scott M.A. (2005) Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 365, 1054–1061.

Bennett J.M. & Orazi A. (2009) Diagnostic criteria to distinguish hypocellular acute myeloid leukemia from hypocellular myelodysplastic syndromes and aplastic anemia: recommendations for a standardized approach. Haematologica 94, 264–268.

Bennett J.M., Catovsky D., Daniel M.T., Flandrin G., Galton D.A., Gralnick H., Sultan C. & Cox C. (1994) The chronic myeloid leukaemias: guidelines for distinguishing chronic granulocytic, atypical chronic myeloid, and chronic myelomonocytic leukaemia. Proposals by the French-American-British Cooperative Leukaemia Group. British Journal Haematology 87, 746–754.

Boissinot M., Garand R., Hamidou M. & Hermouet S. (2006) The JAK2-V617F mutation and essential thrombocythemia features in a subset of patients with refractory anaemia with ring sideroblasts (RARS). Blood 108, 1781–1782.

Breccia M., Biondo F., Latagliata R., Carmosino I., Mandelli F. & Alimena G. (2006) Identification of risk factors in atypical chronic myeloid leukemia. Hematologica 91, 1568–1570.

Brunning R.D., Orazi A., Germing U., Le Beau M.M., Porwit A., Baumann I., Vardiman J.W. & Hellstrom-Lindberg E. (2008a) Myelodysplastic syndromes/neoplasms, overview. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 88–93. IARC, Lyon.

Brunning R.D., Hasserjian R.P., Porwit A., Bennett J.M., Orazi A., Thiele J. & Hellstrom-Lindberg E. (2008b) Refractory cytopenia with unilineage dysplasia. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 94–95. IARC, Lyon.

Ceesay M.M., Lea N.C., Ingram W., Westwood N.B., Gaken J., Mohamedali A., Cervera J., Germing U., Gattermann N., Giagounidis A., Garcia-Casado Z., Sanz G. & Mufti G.J. (2006) The JAK2 V617F mutation is rare in RARS but common in RARS-T. Leukemia 20, 2060–2061.

Cortes J.E., Talpaz M., O'Brien S., Faderl S., Garcia-Manero G., Ferrajoli A., Verstovsek S., Rios M.B., Shan J. & Kantarjian H.M. (2006) Staging of chronic myeloid leukemia in the imatinib era. An evaluation of the World Health Organization proposal. Cancer 106, 1306–1315.

Cross N.C. & Reiter A. (2008) Fibroblast growth factor receptor and platelet-derived growth factor receptor abnormalities in eosinophilic myeloproliferative disorders. Acta Haematologica 119, 199–206.

Della Porta M.G., Malcovati L., Boveri E., Travaglino E., Pietra D., Pascutto C., Passamonti F., Invernizzi R., Castello A., Magrini U., Lazzarino M. & Cazzola M. (2009) Clinical relevance of bone marrow fibrosis and CD34-positive cell clusters in primary myelodysplastic syndromes. Journal of Clinical Oncology 27, 754–762.

Dolan M.M., Singleton T.P., Neglia J. & Cioc A. (2006) Aplastic anemia and monosomy 7-associated dysmegakaryocytopoiesis. American Journal of Clinical Pathology 126, 925–930.

Estey E., Thall P., Beran M., Kantarjian H., Pierce S. & Keating M. (1997) Effect of diagnosis (refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, or acute myeloid leukemia [AML]) on outcome of AML-type chemotherapy. Blood 90, 2969–2977.

Gattenlohner S., Peter C., Bonengel M., Einsele H., Bargou R., Muller-Hermelink H.K. & Marx A. (2007) Detecting the JAK2 V167F mutation in fresh and 'historic' blood and bone marrow. Leukemia 21, 1599–1602.

Haferlach T., Schoch C., Löffler H., Gassmann W., Kern W., Schnittger S., Fonatsch C., Ludwig W.D., Wuchter C., Schlegelberger B., Staib P., Reichle A., Kubica U., Eimermacher H., Balleisen L., Grüneisen A., Haase D., Aul C., Karow J., Lengfelder E., Wörmann B., Heinecke A., Sauerland M.C., Büchner T. & Hiddemann W. (2003) Morphologic dysplasia in de novo acute myeloid leukemia (AML) is related to unfavorable cytogenetics but has no independent prognostic relevance under the conditions of intensive induction therapy: results of a multiparameter analysis from the German AML Cooperative Group studies. Journal of Clinical Oncology 21, 256–265.

Hanson C.A., Durnick D.K., Hoyer J.D., Hodnefield J.M., Ketterling R.P. & Steensma D.P. (2010) Idiopathic cytopenia of undetermined significance (ICUS): the importance of clinical, morphologic, and cytogenetic evaluation. Modern Pathology 23(Suppl 1), 300A.

Hasel H. (2007) Myelodysplastic and myeloproliferative disorders in children. Current Opinion in Pediatrics 19, 1–8.

Hasserjian R.P., Zuo Z., Garcia C., Tang G., Kasyan A., Luthra R., Abruzzo L.V., Kantarjian H.M., Medeiros L.J. & Wang S.A. (2010) Acute erythroid leukemia: a reassessment using criteria refined in the 2008 WHO classification. Blood 115, 1985–1992. [Epub ahead of print].

Hernandez J.M., del Canizo M.C., Cuneo A., Garcia J.L., Gutierrez N.C., Gonzalez M., Castoldi G. & San Miguel J.F. (2000) Clinical hematological and cytogenetic characteristics of atypical chronic myeloid leukemia. Annals of Oncology 11, 441–444.

Huh Y.O., Jilani I., Estey E., Giles F., Kantarjian H., Freireich E. & Albitar M. (2002) More cell death in refractory anemia with excess blasts in transformation than in acute myeloid leukemia. Leukemia 16, 2249–2252.

Jackson C.C., Medeiros L.J. & Miranda R.N. (2010) 8p11 myeloproliferative syndrome: a review. Human Pathology 41, 461–476.

James C., Ugo V., Le Couedic J.P., Staerk J., Delhommeau F., Lacout C., Garcon L., Raslova H., Berger R., Bennaceur-Griscelli A., Villeval J.L., Constantinescu S.N., Casadevall N. & Vainchenker W. (2005) A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 434, 1144–1148.

Jones A.V., Kreil S., Zoi K., Waghorn K., Curtis C., Zhang L., Score J., Seear R., Chase A.J., Grand F.H., White H., Zoi C., Loukopoulos D., Terpos E., Vervessou E.C., Schultheis B., Emig M., Emst T., Lengfelder E., Hehlmann R., Hochhaus A., Oscier D., Silver R.T., Reiter A. & Cross N.C. (2005) Widespread occur-

rence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood 106, 2162–2168.

Kasyan A., Medeiros L.J., Zuo Z., Santos F.P., Ravandi-Kashani F., Miranda R., Vadhan-Raj S., Koeppen H. & Bueso-Ramos C.E. (2010) Acute erythroid leukemia as defined in the World Health Organization Classification is a rare and pathogenetically heterogeneous disease. Modern Pathology. [Epub ahead of print].

Konoplev S., Medeiros L.J., Lennon P.A., Prajapati S., Kanungo A. & Lin P. (2007) Therapy may unmask hypoplastic myelodysplastic syndrome that mimics aplastic anemia. Cancer 110, 1520–1526.

Kralovics R., Passamonti F., Buser A.S., Teo S.S., Tiedt R., Passweg J.R., Tichelli A., Cazzola M. & Skoda R.C. (2005) A gain-of-function mutation of JAK2 in myeloproliferative disorders. New England Journal of Medicine 352, 1779–1790.

Kurzrock R., Bueso-Ramos C.E., Kantarjian H., Freireich E., Tucker S.L., Siciliano M., Pilat S. & Talpaz M. (2001) *BCR* rearrangement-negative chronic myelogenous leukemia revisited. Journal of Clinical Oncology 19, 2915–2926.

Kvasnicka H.M. & Thiele J. (2010) Prodromal myeloproliferative neoplasms: the 2008 WHO classification. American Journal of Hematology 85, 62–69.

Lennon P.A., Abruzzo L.V., Medeiros L.J., Cromwell C., Zhang X., Yin C.C., Kornblau S.M., Konopieva M. & Lin P. (2007) Aberrant EVI1 expression in acute myeloid leukemias associated with the t(3;8)(q26;q24). Cancer Genetics and Cytogenetics 177, 37–42.

Levine R.L., Wadleigh M., Cools J., Ebert B.L., Wernig G., Huntly B.J., Boggon T.J., Wlodarska I., Clark J.J., Moore S., Adelsperger J., Koo S., Lee J.C., Gabriel S., Mercher T., D'Andrea A., Frohling S., Dohner K., Marynen P., Vandenberghe P., Mesa R.A., Tefferi A., Griffin J.D., Eck M.J., Sellers W.R., Meyerson M., Golub T.R., Lee S.J. & Gilliland D.G. (2005) Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 7, 387–397.

Lim K.H., Pardanani A. & Tefferi A. (2008) KIT and mastocytosis. Acta Haematologica 119, 194–198.

Lin P., Medeiros L.J., Yin C.C. & Abruzzo L.V. (2006) Translocation (3;8)(q26;q24): a recurrent chromosomal abnormality in myelodysplastic syndrome and acute myeloid leukemia. Cancer Genetics and Cytogenetics 166, 82–85.

Ma W., Kantarjian H., Zhang X., Sun W., Buller A.M., Jilani I., Schwartz J.G., Giles F. & Albitar M. (2008) Higher detection rate of JAK2 mutation using plasma. Blood 111, 3906–3907.

Maciejewski J.P. & Selleri C. (2004) Evolution of clonal cytogenetic abnormalities in aplastic anemia. Leuk Lymph 45, 433–440.

McKenna R.W. (2004) Myelodysplasia and myeloproliferative disorders in children. American Journal of Clinical Pathology 122(Suppl. 1), S58–S69.

Mirza I., Sekora D. & Frantz C. (2008) Testing for JAK2 V617F mutation across specimen types yields concordant results. Journal of Clinical Pathology 61, 975.

Mrozek K., Marcucci G., Paschka P., Whitman S.P. & Bloomfield C.D. (2007) Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood 109, 431–448.

Oki Y., Kantarjian H.M., Zhou X., Cortes J., Faderl S., Verstovsek S., O'Brien S., Koller C., Beran M., Bekele B.N., Pierce S., Thomas D., Ravandi F., Wierda W.G., Giles F., Ferrajoli A., Jabbour E., Keating M.J., Bueso-Ramos C.E., Estey E. & Garcia-Manero G. (2006) Adult acute megakaryocytic leukemia: an analysis of 37 patients treated at M.D. Anderson Cancer Center. Blood 107, 880–884.

Oyarzo M.P., Lin P., Glassman A., Bueso-Ramos C.E., Luthra R. & Medeiros L.J. (2004) Acute myeloid leukemia with t(6;9)(p23;q34) is associated with dysplasia and a high frequency of flt3 gene mutations. American Journal of Clinical Pathology 122, 348–358.

Pardanani A.D., Levine R.L., Lasho T., Pikman Y., Mesa R.A., Wadleigh M., Steensma D.P., Elliott M.A., Wolanskyj A.P., Hogan W.J., McClure R.F., Litxow M.R., Gilliland D.G. & Tefferi A. (2006) MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood 108, 3472–3476.

Pardanani A., Lasho T.L., Finke C., Hanson C.A. & Tefferi A. (2007) Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. Leukemia 21, 1960–1963.

Pedersen-Bjergaard J., Pedersen M., Roulston D. & Philip P. (1995) Different genetic pathways in leukemogenesis for patients presenting with therapy-related myelodysplasia and therapy-related acute myeloid leukemia. Blood 86, 3542–3552.

Pedersen-Bjergaard J., Christiansen D.H., Desta F. & Andersen M.K. (2006) Alternative genetic pathways and cooperating genetic abnormalities in the pathogenesis of therapy-related myelodysplasia and acute myeloid leukemia. Leukemia 20, 1943–1949.

Pikman Y., Lee B.H., Mercher T., McDowell E., Ebert B.L., Gozo M., Cuker A., Wernig G., Moore S., Galinsky I., DeAngelo D.J., Clark J.J., Lee S.J., Golub T.R., Wadleigh M., Gilliland D.G. & Levine R.L. (2006) MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med 3, e270.

Salama M.E., Swierczek S.I., Hickman K., Wilson A. & Prchal J.T. (2009) Plasma quantitation of JAK2 mutation is not suitable as a clinical test: an artifact of storage. Blood 114, 223–224.

Santos F.P., Faderl S., Garcia-Manero G., Koller C., Beran M., O'Brien S., Pierce S., Freireich E.J., Huang X., Borthakur G., Bueso-Ramos C., de Lima M., Keating M., Cortes J., Kantarjian H. & Ravandi F. (2009) Adult acute erythroleukemia: an analysis of 91 patients treated at a single institution. Leukemia 23, 2275–2280.

Scott L.M., Tong W., Levine R.L., Scott M.A., Beer P.A., Stratton M.R., Futreal P.A., Erber W.N., McMullin M.F., Harrison C.N., Warren A.J., Gilliland D.G., Lodish H.F. & Green A.R. (2007) JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. New England Journal of Medicine 356, 459–468.

Soupir C.P., Vergilio J.A., Dal Cin P., Muzikansky A., Karntarjian H., Jones D. & Hasserjian R.P. (2007) Philadelphia chromosome-positive acute myeloid leukemia: a rare aggressive leukemia with clinicopathologic features distinct from chronic myeloid leukemia in myeloid blast crisis. American Journal of Clinical Pathology 127, 642–650.

Stevenson W.S., Hoyt R., Bell A., Guipponi M., Juneja S., Grigg A.P., Curtis D.J., Scott H.S., Szer J., Alexander W.S., Tuckfield A. & Roberts A.W. (2006) Genetic heterogeneity of granulocytes for the JAK2 V617F mutation in essential thrombocythaemia: implications for mutation detection in peripheral blood. Pathology 38, 336–342.

Theocharides A., Boissinot M., Girodon F., Garand R., Teo S.S., Lippert E., Talmant P., Tichelli A., Hermouet S. & Skoda R.C. (2007) Leukemic blasts in transformed JAK2-V617F-positive myeloproliferative disorders are frequently negative for the JAK2-V617F mutation. Blood 110, 375–379.

Thiele J., Kvasnicka H.M., Tefferi A., Barosi G., Orazi A. & Vardiman J.W. (2008) Primary myelofibrosis. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 44–47. IARC, Lyon.

Valent P., Horny H.P., Bennett J.M., Fonatsch C., Germing U., Greenberg P., Haferlach T., Haase D., Kolb H.J., Krieger O., Loken M., van de Loosdrecht A., Ogata K., Orfao A., Pfeilstocker M., Ruter B., Sperr W.R., Stauder R. & Wells D.A. (2007) Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: Consensus statements and report from a working conference. Leukemia Res 31, 727–736.

Vardiman J.W., Harris N.L. & Brunning R.D. (2002) The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 100, 2292–2302.

Vardiman J.W., Brunning R.D., Arber D.A., Le Beau M.M., Porwit A., Tefferi A., Bloodfield C.D. & Thiele J. (2008a) Introduction and overview of the classification of the myeloid neoplasms. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 18–30. IARC, Lyon.

Vardiman J.W., Bennett J.M., Bain B.J., Brunning R.D. & Thiele J. (2008b) Atypical chronic myeloid leukaemia, *BCR-ABL1* negative. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 80–81. IARC, Lyon.

Vardiman J.W., Bennett J.M., Bain B.J., Baumann I., Thiele J. & Orazi A. (2008c) Myelodysplastic/myeloproliferative neoplasm, unclassifiable. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 85–86. IARC, Lyon.

Vardiman J.W., Arber D.A., Brunning R.D., Larson R.A., Matutes E., Baumann I. & Thiele J. (2008d) Therapy-related myeloid neoplasms. In: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele & J.W. Vardiman), pp. 127–129. IARC, Lyon.

Wandt H., Schäkel U., Kroschinsky F., Prange-Krex G., Mohr B., Thiede C., Pascheberg U., Soucek S., Schaich M. & Ehninger G. (2008) MLD according to the WHO classification in AML has no correlation with age and no independent prognostic relevance as analyzed in 1766 patients. Blood 111, 1855–1861.

Wardrop D. & Steensma D.P. (2009) Is refractory anaemia with ring sideroblasts and thrombocytosis (RARS-T) a necessary or useful diagnostic category? British Journal Haematology 144, 411–419.

Weinberg O.K., Seetharam M., Ren L., Seo K., Ma L., Merker J.D., Gotlib J., Zehnder J.L. & Arber D.A. (2009) Clinical characterization of acute myeloid leukemia with myelodysplasia-related changes as defined by the 2008 WHO classification system. Blood 113, 1906–1908.

Yavuz A.S., Lipsky P.E., Yavuz S., Metcalfe D.D. & Akin C. (2002) Evidence for the involvement of a hematopoietic progenitor cell in systemic mastocytosis from single-cell analysis of mutations in the c-kit gene. Blood 100, 661–665.

Yin C.C. & Jones D. (2010) Myeloproliferative neoplasms. In: Neoplastic Hematology (eds D. Jones), pp. 177–192. Humana Press, USA.

Yin C.C., Glassman A.B., Lin P., Valbuena J.R., Jones D., Luthra R. & Medeiros L.J. (2005) Morphologic, cytogenetic, and molecular abnormalities in therapy-related acute promyelocytic leukemia. American Journal of Clinical Pathology 123, 840–848.

Yin C.C., Cortes J., Barkoh B., Hayes K., Kantarjian H. & Jones D. (2006) t(3;21)(q26;q22) in myeloid leukemia: an aggressive syndrome of blast transformation associated with hydroxyurea or antimetabolite therapy. Cancer 106, 1730–1738.

Young N.S. (1992) The problem of clonality in aplastic anemia: Dr. Dameshek's riddle, restated. Blood 79, 1385–1392.

Zhao W., Bueso-Ramos C.E., Verstovsek S., Barkoh B.A., Khitamy A.A. & Jones D. (2007) Quantitative profiling of codon 816 KIT mutations can aid in the classification of systemic mast cell disease. Leukemia 21, 1574–1576.