WHO'S NEXT?
THE WHO CLASSIFICATION OF MYELOID NEOPLASMS

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Introduction

As the focus in myeloid neoplasms turns increasingly to the genetic infrastructure of malignant cells and to molecular abnormalities that may become targets for therapy, it is reasonable to assume that genetic and molecular data will be increasingly incorporated into diagnostic algorithms and/or into the nomenclature for these disorders. The 2001 (3rd edition) of the WHO classification of myeloid neoplasms included, for the first time in any widely used classification scheme, genetic information as diagnostic criteria not only for CML but also for some subtypes of acute myeloid leukemia (AML). In the nearly 8 years that elapsed between the 3rd and 4th edition of the classification a number of genetic abnormalities were found to be associated with subgroups of myeloid neoplasms or with specific disease entities, and thus they were incorporated into the updated classification scheme. In some instances, such as the *JAK2 V617F* mutation which is often associated with the *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs), the mutated *JAK2* not only provides clues as to the pathogenesis of the disorder but is also used in diagnostic algorithms to provide evidence of the neoplastic nature of the myeloproliferation. In other instances, such as the category of neoplasms associated with rearrangements of *PDGFRA* or *PDGFRB*, the genetic defect provides an objective criterion for malignancy and also allows for selection of specific targeted therapy, and thus is a major criterion for naming the disease. However, we are at an early stage in understanding pathways of neoplastic transformation of myeloid cells. As newer techniques such as gene expression profiling and whole genome DNA sequencing become more readily accessible for studying myeloid neoplasms, they will likely reveal that the latest WHO revisions are a step forward, but that they remain imperfect and incomplete, and important genetic factors in the pathogenesis of the myeloid neoplasms remain to be discovered. What these discoveries may be and how they will affect the "next WHO" is impossible to predict, but an understanding of the basic principles of the WHO classification, the rationale for some of the changes in the 4th edition, and the recognition of controversial and unsettled issues may provide some clues.

Principles of the WHO Classification of Hematopoietic Neoplasms

The WHO classification uses all available information – morphology, cytochemistry, immunophenotype, genetics and clinical features – to define clinically significant disease entities. The relative contribution of each of these features to the final diagnosis and classification varies depending on the disease. The classification and the criteria used to define specific entities have been agreed upon by a number of experts, thus it is a consensus document. Although instigated under the auspices of the WHO, the European Association of Hematopathology and the Society of Hematopathology, a unique aspect of the WHO classification is the contribution of an advisory committee comprised of clinicians and clinical scientists from around the globe who met with the pathology committees to assure that the classification would be clinically useful. Proposals for changes in the 4th edition were based on clinical and basic research with the aim of providing a classification that can be used in daily clinical practice as well as serve as a common language for clinical trials and investigations.

Guidelines for the using the WHO Classification of myeloid neoplasms

The multidisciplinary approach that is the core of the WHO classification relies on meticulous attention to the collection and processing of diagnostic specimens and clinical information. The WHO criteria apply to initial diagnostic blood and bone marrow specimens obtained prior to any definitive therapy for the suspected myeloid neoplasm. Morphology, cytochemistry and/or immunophenotyping are used to establish the lineage of the neoplastic cells and assess their maturation. The blast count remains a practical tool for categorizing myeloid neoplasms and judging their progression. Bone marrow biopsy specimens add valuable information regarding marrow cellularity, stromal changes, and cellular maturation, and are necessary for the diagnosis and classification of MPN. A complete cytogenetic analysis is required during the initial evaluation, and additional genetic studies, such as FISH and RT-PCR, selected according to the suspected diagnosis and karyotype results. Gene mutations are increasingly being recognized as diagnostic and prognostic markers. These include, among others, mutations of *JAK2*, *MPL* and *KIT* in the MPNs, *NRAS*, *KRAS*, *NF1*, *PTPN11*, *RUNX1* and *TET2* in the MDS/MPNs, *NPM1, CEBPA, FLT3, RUNX1, KIT, WT1, IDH1*, and *MLL* in AML, and *GATA1* in myeloid neoplasms associated with Down syndrome. The most informative of these for diagnosis and risk stratification, such as *JAK2* in MPN or *FLT3* and *NPM1* in AML, should be obtained early in the diagnostic work-up.

"What's next" for recommended guidelines to use the WHO classification?

The integration of morphology, cytogenetics and immunophenotyping to define unique disease entities will remain as a central principle, yet the techniques for obtaining some of the data may change in the future. Microarray-based gene expression studies have revealed that a number of myeloid neoplasms exhibit characteristic gene-expression signatures that allow rapid and accurate sub-classification in clinical settings. Just as importantly, these characteristic signatures may allow recognition of neoplasms that are difficult to distinguish from non-neoplastic myeloid proliferations by traditional techniques. Furthermore, because most of the relevant genetic mutations that contribute to leukemogenesis are still unknown and their interaction in individual patients is likely complex, whole-genome DNA sequencing may play an increasingly important role in the discovery of significant abnormalities that determine the diagnosis and prognosis of myeloid neoplasms.

WHO Classification: Myeloproliferative Neoplasms (MPN)

Revisions in the criteria for the classification of MPN in the 4th edition of the WHO classification were influenced by 2 factors: 1) the realization that abnormalities of genes encoding tyrosine kinases involved in signal transduction pathways in the MPNs can be used as diagnostic markers, and 2) better characterization of the histologic features that aid in the identification of subtypes of MPN. The discovery in 2005 of *JAK2* V617F or similar activating mutations in virtually all of the cases of polycythemia vera and nearly 50% of cases of essential thrombocythemia (ET) and primary myelofibrosis (PMF) revolutionized, yet also simplified the diagnostic criteria for these neoplasms in the WHO classification. Detection of one of these mutations identifies the case as neoplastic and eliminates a number of diagnostic procedures used to distinguish MPN from reactive hyperplasia – a not uncommon problem. For cases that lack mutations, histopathologic and clinical data are used to further subtype the proliferation.

What's next in the classification of the MPNs? There are issues that are not completely addressed or that remain controversial in the revised classification of the MPNs. These include the lack of universally accepted criteria for the accelerated phase of CML, *BCR-ABL1+*, the recognition that many cases previously diagnosed as ET using older classification schemes are more likely the pre-fibrotic stage of PMF, and the clarification of how *JAK2* V617F results in diseases with different clinical phenotypes. Furthermore, some of the genetic abnormalities in the MPNs, such as mutated *JAK2* and *MPL*, and perhaps even the *BCR-ABL1* in CML, are likely secondary genetic events, and the initiating abnormality currently remains elusive. The discovery of the seminal events in these disorders has important ramifications not only for diagnosis and classification but also for development of targeted therapy.

WHO Classification: Myeloid and Lymphoid Neoplasms with Eosinophilia and Abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*

Some cases previously diagnosed as chronic eosinophilic leukemia (CEL), as hypereosinophilic

syndrome, or as chronic myelomonocytic leukemia (CMML) with eosinophilia are now recognized to have rearrangements of *PDGFRA* or *PDGFB* that lead to their constitutive activation and to myeloproliferation and eosinophilia. Similarly, rearrangements of *FGFR1* have been implicated in myeloid neoplasms with prominent eosinophilia ("8p11.2 myeloproliferative syndrome"). However, patients with *FGFR1* abnormalities may initially present as T- or B-lymphoblastic leukemia/lymphoma associated with tissue eosinophilia and later evolve to a myeloid neoplasm with marked eosinophilia, or vice versa. Rare cases associated with rearranged *PDGFRA* have also been reported to initially have a lymphoblastic neoplasm. In order to accommodate all cases with these abnormalities under a single category rather to distribute them between CEL, CMML and lymphoblastic leukemia/lymphoma, they are combined into a new subgroup that is identified mainly by the genetic defects.

"What's next" for the subgroup associated with rearrangements of PDGFRA, PDGFRB or FGFR1? This group may be a model for future classification of diseases for which the morphologic and clinical features can be attributed to a specific genetic defect and for which molecularly-directed therapy is available. In the case of *PDGFRA* and *PDGFRB*, that therapy is imatinib, and thus this grouping is not only practical for classification purposes but has therapeutic significance as well. Unfortunately, targeted therapy is not yet available for the constitutively activated FGFR1, but the classification highlights those who would benefit from such an agent.

WHO Classification: Myelodysplastic/ Myeloproliferative Neoplasms (MDS/MPN)

This category was introduced in the 3rd edition to include myeloid neoplasms with clinical, laboratory and morphologic features that overlap MDS and MPN. The subgroup includes CMML, juvenile myelomonocytic leukemia (JMML) and atypical chronic myeloid leukemia, *BCR-ABL1* negative (aCML). A few cases of CMML and aCML do carry mutated *JAK2*, but the proliferative aspects of more cases are reported to be related to aberrancies in the RAS/MAPK signaling pathways. In JMML, nearly 75% of patients demonstrate mutually exclusive mutations of *PTPN11*, *NRAS* or *KRAS*, or *NF1*. Thus, their genetic abnormalities seem somewhat different than MDS or MPN. This category also includes the provisional entity

(a disease that needs further study to be accepted as a distinct entity), Refractory Anemia with Ring Sideroblasts and Thrombocytosis (RARS-T), which clinically and morphologically is a hybrid of RARS and ET.

"What's next" for the subgroup, MDS/MPN? The most problematic issue regarding CMML is the marked heterogeneity of the morphologic, clinical and even genetic findings encompassed within this entity. However, to date there are no features that reliably separate CMML into clinically relevant subgroups, other than the percentage of blasts (i.e., CMML-1 vs. CMML-2). Recently, mutations of *TET2*, *RUNX1*, and *CBL* have been reported as genetic abnormalities other than mutated *NRAS* and *KRAS* that are important in the pathogenesis of CMML, although there are conflicting reports regarding their influence on specific disease characteristics. Whether these or additional mutations may be associated with unique subgroups of CMML remain to be proved. RARS-T is another entity in this subgroup that remains to be clarified. Whether it is a unique entity, ET that has acquired ring sideroblasts secondarily, or RARS that has acquired megakaryocytic proliferation secondarily is controversial. A possible new entity for this group is the leukemia associated with isolated isochromosome 17, an MDS/MPN-like disorder with hyposegmentation of neutrophils nuclei and a high rate of transformation of acute leukemia.

WHO Classification: Myelodysplastic Syndrome (MDS)

MDS remains the most challenging of the myeloid neoplasms to diagnose, particularly when the clinical and laboratory findings suggest MDS but the morphologic findings are inconclusive, or when there is secondary dysplasia caused by nutritional deficiencies, medications, toxins, infections, etc., or when marrow hypocellularity or myelofibrosis obscure the marrow findings. The 4th edition attempted to clarify minimal criteria for the diagnosis of MDS. In the appropriate clinical setting, at least 10% of cells of at least one myeloid lineage must show unequivocal dysplasia for that lineage to be considered dysplastic. Causes of secondary dysplasia should be excluded before making the diagnosis of MDS. On the other hand, if the patient has clinical and laboratory features consistent with MDS but inconclusive morphologic features, a presumptive diagnosis of MDS can be made if specific MDS-related cytogenetic abnormalities are discov-

ered. Further changes in MDS in the 4th edition included the addition of an over-arching category, refractory cytopenia with unilineage dysplasia to incorporate patients who exhibit unilineage dysplasia associated with refractory anemia, refractory neutropenia or refractory thrombocytopenia and who have less than 1% blasts in the blood and fewer than 5% in the bone marrow. The latter two groups would have been "unclassifiable" according to previous criteria. To address concerns that the previous WHO classification did not pay sufficient attention to the significance of blasts in the blood, patients with 2-4% blasts in the blood but less than 5% in the marrow are now categorized as RAEB-1 if other clinical and laboratory findings of MDS are present.

"What's next" for the classification of MDS? The most urgent issue in MDS remains the identification of early stages of MDS when morphologic and genetic findings are inconclusive, yet the patient has refractory anemia, thrombocytopenia and/or neutropenia that cannot be explained by any other disease. This is a particularly problematic and common scenario in older patients. Clearly, additional techniques to substantiate or disprove the diagnosis are needed, and the WHO classification should strive to improve the diagnostic criteria for such cases. Phenotypic abnormalities by flow cytometry, particularly the asynchronous expression of maturation associated antigens on myeloid cells, have been well-reported in MDS. It was the consensus of the clinical advisory group that they should be considered as "suggestive" of MDS in the appropriate clinical setting, but currently, too few patients with secondary myelodysplasia have been studied to assure that the changes are specific for MDS. Such data would be important and would add yet another diagnostic tool. Recently, differential gene expression profiling has been advocated as an additional method to distinguish between MDS and normal individuals.

WHO Classification: Acute Myeloid Leukemia (AML)

After the publication of the 3rd edition of the classification in 2001, it became more widely appreciated that multiple genetic lesions, including not only chromosomal rearrangement or numerical abnormalities but also mutated genes, cooperate to establish the leukemic process and influence its morphologic and clinical features. Importantly the discovery of the significance of gene muta-

tions in leukemogenesis paved the way for the genetic characterization of many cases of cytogenetically normal AML. In some cases, the newly discovered genetic abnormalities are associated with clinical, morphologic and/ or phenotypic features that allow identification of a new leukemic entity, whereas in other cases, they have proved to be powerful prognostic indicators. One of the major challenges in the revision of the AML classification was how to incorporate important and/or recently described genetic aberrations, yet adhere to the WHO principle of defining homogeneous, biologically relevant and mutually exclusive entities based not only on their prognostic value but on their correlation with morphologic, phenotypic and/or other unique properties. This proved particularly difficult for some of the most frequent and important mutations in AML, particularly for *FLT3*, *NPM1* and *CEBPA*, which are associated with few, if any, mutually exclusive morphologic or clinical features, and which are not even entirely exclusive of each other. However, because of their frequency and importance in leukemogenesis, mutated *NPM1* and *CEBPA* were incorporated as defining "provisional entities" in the classification scheme, awaiting further study before being considered as distinct entities. Other additions and refinements were made to the AML classification are too numerous to list here, but include, among others, three new cytogenetically defined entities [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(megakaryoblastic) associated with t(1;22)(p13;q13);*RBM15-MKL1*], refinement to the AML with 11q23 (*MLL*) category and the APL variant category. Specific myelodysplastic-related cytogenetic abnormalities were added to allow for assigning a case to the category, AML with myelodysplastic related changes. Myeloid neoplasms related to Down syndrome were placed in a separate category, and Blastic Plasmacytic Dendritic Neoplasm is added to the AML related grouping.

"What's next" for AML? As new genetic information regarding AML accumulates almost every day, it is nearly impossible to predict what changes will occur in the next edition. It is important to keep in mind that many of the genetic abnormalities reported are cooperating lesions or abnormalities that affect prognosis, but are not related to specific morphologic or clinical entities. On the other hand, if they define an actionable target for therapy, they may well be worthy of being recognized as a unique disorder. One area to watch for

change is in the AML with myelodysplasia related changes; recent data suggest that when categorized by dysplastic morphology alone, this group may not clinical relevance. Lastly, the leukemia associated with t(8;16)(p11;p13); *MYST3-CREBBP*, will likely be recognized as a distinct entity.

Conclusion: The 4th edition of the WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues represents the cooperative effort of over 130 pathologists and clinicians from around the world. This project has led to an exciting international exchange of ideas and to a commitment by the pathology societies and the WHO to periodically review and update the classification. That's "What's Next"!

WHO CLASSIFICATION OF MYELOID NEOPLASMS (4th Edition, 2008)

MYELOPROLIFERATIVE NEOPLASMS (MPN)

Chronic myelogenous leukemia, BCR-ABL1 positive

Chronic neutrophilic leukemia

Polycythemia vera

Primary myelofibrosis

Essential thrombocythemia

Chronic eosinophilic leukemia, not otherwise specified

Mastocytosis

Myeloproliferative neoplasms, unclassifiable

MYELOID AND LYMPHOID NEOPLASMS ASSOCIATED WITH EOSINOPHILIA AND ABNORMALITIES OF PDGFRA, PDGFRB, or FGFR1

Myeloid and lymphoid neoplasms associated with PDGFRA rearrangement

Myeloid neoplasms associated with PDGFRB rearrangement

Myeloid and lymphoid neoplasms associated with FGFR1 abnormalities

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS (MDS/MPN)

Chronic myelomonocytic leukemia

Atypical chronic myeloid leukemia, BCR-ABL1 negative

Juvenile myelomonocytic leukemia

Myelodysplastic/Myeloproliferative neoplasm, unclassifiable

Provisional entity: Refractory anemia with ring sideroblasts and thrombocytosis

MYELODYSPLASTIC SYNDROME (MDS)

Refractory cytopenia with unilineage dysplasia

Refractory anemia

Refractory neutropenia

Refractory thrombocytopenia

Refractory anemia with ring sideroblasts

Refractory cytopenia with multilineage dysplasia

Refractory anemia with excess blasts

Myelodysplastic syndrome with isolated del(5q)

Myelodysplastic syndrome, unclassifiable

Childhood myelodysplastic syndrome

Provisional entity: Refractory cytopenia of childhood

ACUTE MYELOID LEUKEMIA AND RELATED NEOPLASMS

Acute myeloid leukemia with recurrent genetic abnormalities

AML with t(8;21)(q22;q22); RUNX1-RUNX1T1

AML with inv(16)(p13.1q22) or t((16;16)(p13.1;q22); CBFB-MYH11

APL with t(15;17)(q22;q12); 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 cont...

Acute myeloid leukemia 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/monocytic leukemia

Acute erythroid leukemia

Pure erythroid leukemia

Erythroleukemia, erythroid/myeloid

Acute megakaryoblastic leukemia

Acute basophilic leukemia

Acute panmyelosis with myelofibrosis

Myeloid Sarcoma

Myeloid Proliferations related to Down Syndrome

Transient abnormal myelopoiesis

Myeloid leukemia associated with Down syndrome

Blastic Plasmacytoid Dendritic Cell Neoplasm

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