The Myelodysplastic Syndromes and Myeloproliferative Disorders

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LEARNING OBJECTIVES:

1. Compare and contrast the FAB and WHO classification systems for the myelodysplastic syndromes (MDS) and the myeloproliferative disorders (MPD).
2. Discuss the rationale for the use of antiangiogenic therapy in MDS.
3. Explain epigenetic alterations of DNA, and the functional role of DNA methyltransferase and histone deacetylase (HDAC) in these changes.
4. Describe the role of molecular assays for PRV-1 and Mpl in the diagnosis of MPD.
5. Discuss the role of neoangiogenesis in idiopathic myelofibrosis.
6. Explain why Imatinib (Gleevec) (the bcr-abl tyrosine kinase inhibitor) is effective in some patients with myelofibrosis.

ABBREVIATIONS: AML = acute myelocytic leukemia; APL = acute promyelocytic leukemia; CML = chronic myelocytic leukemia; CMML = chronic myelomonocytic leukemia; CMPD = chronic myeloproliferative disorders; ET = essential thrombocythemia; FAB = French American British; FDA = Food and Drug Administration; HDAC = histone deacetylase; HDACi = HDAC inhibitor; HSCT = hematopoietic stem cell transplantation; HU = hydroxyurea; IMF = idiopathic myelofibrosis; MDS = myelodysplastic syndromes; PDGF = platelet derived growth factor; PRV-1 = polycythemia rubra vera-1; PV = polycythemia vera; RA = refractory anemia; RARS = refractory anemia with ringed sideroblasts; RCMD = refractory cytopenia with multilineage dysplasia; RAEB = refractory anemia with excess blasts; RCMD-RS = RCMD with ringed sideroblasts; TGF-β = transforming growth factor beta; TNF-α = tumor necrosis factor alpha; TPO = thrombopoietin; VEGF = vascular endothelial growth factor; VEGFR = vascular endothelial growth factor receptor; WHO = World Health Organization.

INDEX TERMS: hematopoietic stem cells; myelodysplastic syndromes; myeloproliferative disorders.

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The myelodysplastic syndromes (MDS) and the myeloproliferative disorders (MPD) are a diverse group of hematologic diseases characterized by deregulation of the $CD34^+$ hematopoietic stem cell, and with a propensity to transform to acute myeloblastic leukemia (AML). As is true for the acute leukemias, there has been intense interest in determining the molecular mechanisms underlying the cellular deregulation, and the development of more targeted therapies based on unique molecular phenotypes.

In 1997, the Clinical Advisory Committee of the World Health Organization (WHO) published a revised classification of neoplastic diseases of the hematopoietic and lymphoid tissues. This new WHO classification system incorporated morphologic, biologic, and genetic information into a working nomenclature that had clinical relevance, and replaced the previous French-American-British (FAB) classification which was predominantly a morphologic classifica-

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tion system.[1] This paper will not attempt a comprehensive discussion of the WHO classification system, but will summarize the significant changes, and then discuss selected aspects of the MDS/MPDs presented at the 45th Annual Meeting of the American Society of Hematology.

The WHO classification of the chronic myeloproliferative diseases lists seven disease entities (Table 1). The major changes from the FAB classification are:

1. Only the Philadelphia chromosome+ cases (or those with the BCR/ABL fusion gene) are called chronic myelocytic leukemia (CML) by the WHO system. The Ph- cases, which show myelodysplastic signs, and are known to have significantly worse prognosis, are called atypical CML (aCML), and belong to the newly created myelodysplastic/myeloproliferative group.
2. There are two newly recognized entities which were not included in the FAB classification, chronic neutrophilic leukemia (CNL) and chronic eosinophilic leukemia (CEL)/hypereosinophilic syndrome (HES).

The WHO classification of the myelodysplastic syndromes incorporates many of the definitions of the FAB system, but updates and refines the definition of some subtypes and thus improves their clinical relevance (Table 2). The WHO classification recognizes eight subtypes, in contrast to the five listed by the FAB system. The major changes include:

1. The definitions of the lower grade diseases, refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) have been refined, and a new category refractory cytopenia with multilineage dysplasia (RCMD) has been introduced (Table 3). In the WHO classification, RA and RARS are defined as diseases in which dysplasia is morphologically restricted to the erythroid lineage. If there is multilineage dysplasia (10% or more dysplastic cells in two or more of the myeloid lineages) the diagnosis is RCMD. In cases of RCMD with at least 15% ringed sideroblasts, the diagnosis is RCMD-RS.
2. Refractory anemia with excess blasts (RAEB) is divided into two subgroups, RAEB-1 (with 5% to 9% blasts) and RAEB-2 (with 10% to 19% blasts), reflecting a difference in median survival and rate of transformation to acute leukemia for these two groups of patients.
3. The most significant change was the lowering of the blast threshold for the diagnosis of acute myelocytic leukemia from 30% to 20% blasts in the blood or bone marrow. As a result, the FAB category RAEB-T is eliminated from the WHO classification.
4. A new subgroup, the 5q- syndrome (loss of the long arm of chromosome 5) is defined by the presence of a specific cytogenetic abnormality.
5. Chronic myelomonocytic leukemia is eliminated from the MDS category and placed in a group of myeloid disorders with features of both myelodysplasia and myeloproliferative diseases, MDS/MPD (Table 4).
6. If a myelodysplastic disease lacks findings appropriate for classification as RA, RARS, RCMD/RCMD-RS, or RAEB, it is in the category myelodysplastic syndrome, unclassifiable (MDS-U).

Table 1. Chronic myeloproliferative diseases: comparison of the FAB and WHO classifications*

FAB (1982)	WHO (1997)
Chronic Myeloproliferative Diseases (MPD)	Chronic Myeloproliferative Diseases (MPD)
Chronic myelocytic leukemia (CML)	CML Ph+: t(9;22)(q34;q11), BCR/ABL+ Chronic neutrophilic leukemia Chronic eosinophilic leukemia/hypereosinophilic syndrome
Agnogenic myeloid metaplasia with myelofibrosis (Idiopathic myelofibrosis)	Chronic idiopathic myelofibrosis
Polycythemia vera (PV)	Polycythemia vera
Essential thrombocythemia (ET)	Essential thrombocythemia

* http://www.cancer.gov/templates/doc.aspx?viewid=f3133a91-a7e0-4d6c-acf2-06fbe1f7baa6&version=1&sectionID=77

The MDS/MPD category includes myeloid disorders that have both dysplastic and proliferative features at the time of initial presentation and that are difficult to assign to either the myelodysplastic or myeloproliferative group of diseases (Table 4). The three recognized disorders in this group are chronic myelomonocytic leukemia (CMML), atypical chronic myelocytic leukemia (aCML), and juvenile myelomonocytic leukemia (JMML). Myeloid disease that shows features of both MDS and MPD but which does not meet the criteria for any of the three major MDS/MPD entities is designated as myelodysplastic/myeloproliferative disease, unclassifiable (MDS/MPD-U). The FAB classification scheme did not contain this 'overlap category'.

MYELODYSPLASTIC SYNDROMES

Myelodysplastic syndromes (MDS) are clonal stem cell disorders, characterized by dysplasia and ineffective hematopoiesis, involving one or more of the myeloid lineages. The result is a decline in one or more peripheral blood cell counts, and generally a hypercellular bone marrow, although occasionally the marrow may be normocellular or hypocellular. An increase in myeloblasts is often present, though by definition they comprise less than 20% of the differential count, in either peripheral blood or bone marrow. In contrast to the myeloproliferative disorders, organomegaly is not seen. Typically, these are disorders of older patients, a factor which may complicate therapeutic strategies.

Until recently, the mainstay of the treatment of patients with MDS has been entirely supportive, with blood and platelet transfusions as needed. The development of targeted therapies for MDS has been limited by the lack of understanding of the fundamental genetic and biologic abnormalities in MDS progenitor cells. Despite this barrier, several new classes of drugs with reasonable biochemical rationale have proved promising in early clinical development and will likely alter the current standard of care for patients with MDS. The focus of the educational program on MDS at this year's ASH meeting was the emergence of novel therapeutic strategies for this group of disorders, and the rationale for their use.

To develop effective therapies for MDS, it is first necessary to "find the engine that drives the train".[2] Unlike chronic myelocytic leukemia (CML) or acute promyelocytic leukemia (APL) in which a specific cytogenetic abnormality has been recognized to be causally associated with the disease, and for which specific targeted therapies have been developed, a variety of genetic and epigenetic components may contribute to the evolution of the MDS disorders. A variety of new therapeutic strategies are in

Table 2. Myelodysplastic syndromes: comparison of the FAB and WHO classifications*

FAB (1982) Myelodysplastic Syndromes (MDS)	WHO (1997) Myelodysplastic Syndromes (MDS)
Refractory anemia (RA)	Refractory anemia (RA) Refractory cytopenia with multilineage dysplasia (RCMD)
Refractory anemia with ringed sideroblasts (RARS)	Refractory anemia with ringed sideroblasts (RARS) Refractory cytopenia with multilineage dysplasia with ringed sideroblasts (RCMD-RS)
Refractory anemia with excess blasts (RAEB)	Refractory anemia with excess blasts (RAEB-I and RAEB-II) Myelodysplastic syndrome associated with del(5q) Myelodysplastic syndrome, unclassifiable (MDS-U)
	Reclassified from MDS to:
Refractory anemia with excess blasts in transformation (RAEB-t).	Acute myeloid leukemia (AML) as "AML with multilineage dysplasia following a myelodysplastic syndrome"
Chronic myelomonocytic leukemia (CMML)	Myelodysplastic/myeloproliferative diseases (MDS/MPD)

*http://www.cancer.gov/templates/doc.aspx?viewid=f3133a91-a7e0-4d6c-acf2-06fbe1f7baa6&version=1&sectionID=77

Table 3. WHO classification and criteria for the myelodysplastic syndromes

Disease	Blood findings	Bone marrow findings
Refractory anemia (RA)	Anemia No or rare blasts	Erythroid dysplasia *only* <5% blasts <15% ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia No blasts	Erythroid dysplasia *only* 15% ringed sideroblasts <5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods <1 x 10^9/L monocytes	Dysplasia in 10% of cells in two or more myeloid cell lines <5% blasts in marrow No Auer rods <15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods <1 x 10^9/L monocytes	Dysplasia in 10% of cells in two or more myeloid cell lines 15% ringed sideroblasts <5% blasts No Auer rods
Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenias <5% blasts No Auer rods <1 x 10^9/L monocytes	Unilineage or multilineage dysplasia 5% to 9% blasts No Auer rods
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenias 5% to 19% blasts Auer rods ± <1 x 10^9/L monocytes	Unilineage or multilineage dysplasia 10% to 19% blasts Auer rods ±
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenias No or rare blasts No Auer rods	Unilineage dysplasia in granulocytes or megakaryocytes <5% blasts No Auer rods
MDS associated with isolated del(5q)	Anemia <5% blasts Platelets normal or increased	Normal to increased megakaryocytes with hypolobated nuclei <5% blasts No Auer rods Isolated del(5q)

clinical trials which may offer, for the first time, sustained benefit and possibly curtail the relentless progression of the disease.[2]

ANTIANGIOGENIC THERAPIES

Interest in antiangiogenic molecules began several years ago with the recognition that angiogenesis plays a role in MDS.[3] Evidence indicates that clonal expansion and apoptotic response in MDS arise from an interaction between the malignant clone and its microenvironment. Autocrine production of angiogenic molecules has been implicated in the self-renewal of MDS precursors, particularly vascular endothelial growth factor-A (VEGF-A). VEGF-A as well as its high affinity receptor (VEGFR-1 and/or VEGFR-2) is over-expressed by myeloblasts and monocytes derived from the abnormal clone.[3] Studies indicate an autocrine role for VEGF as a mitogenic cytokine supporting myeloblast self-renewal in MDS. Additionally, VEGF-A elaborated by the MDS clone induces the release of inflammatory cytokines, e.g., TNF-α, from $VEGFR^+$ stromal cells within the microenvironment, which potentiates ineffective hematopoiesis by suppressing proliferation of normal, VEGF receptor negative, hematopoietic progenitors. Consequently, small molecule inhibitors of angiogenic cytokines have emerged as a promising class of therapeutics for MDS.[3,4,5]

The first agent studied in MDS was thalidomide (Thalomid™ Celgene Inc). Thalidomide displays both antiangiogenic and TNF-α inhibitory properties, and has been shown to have some degree of activity in these patients, primarily a reduction in red blood cell transfusion dependence. The major problem with thalidomide was its toxicity, with 40% to 70% of patients withdrawing from the studies before the end of 12 weeks.[6,7]

The demonstration of biologic (erythropoietic) activity with thalidomide led to the search for novel, more potent thalidomide analogues with lower toxicity profiles, and several have recently entered clinical trials. CC5013 (Revimid™, Celgene Inc) is a more potent immunomodulatory derivative (IMiD) of thalidomide that lacks the neurological toxicities of the parent compound. CC5013 inhibits the trophic response to VEGF in myeloblasts and endothelial cells, while augmenting adhesion of hematopoietic progenitors to bone marrow stroma (thus promoting sustained growth arrest and extinction of the myelodysplastic clone).[8] In an early study, 62% of MDS patients with symptomatic or transfusion-dependent anemia experienced an erythroid response. Many patients maintained the response (transfusion independence and near normal hemoglobin levels) as long as one year following cessation of treatment. Not only does the drug have the capacity to promote erythropoiesis, but there is evidence that it also can suppress cytogenetic abnormality. Of the 13 patients with an abnormal karyotype at the beginning of the study, 62% had a complete cytogenetic remission. CC1088, a member of a second functional class of analogs termed selective cytokine inhibitory drugs (SelCIDs) appears significantly less active in preliminary clinical studies in MDS.

A second class of antiangiogenic agents being investigated are small molecule inhibitors of the VEGF receptor tyrosine kinases. These agents impair ligand-induced activation of the VEGFR. Clinical trials are underway, but to date, no data are available on their effectiveness.[2]

Arsenic trioxide (ATO) (Trisenox™, Cell Therapeutics) has been approved by the Food and Drug Administration (FDA) for treatment of relapsed APL. ATO has broad biological properties derived from its ability to bind to and deplete sulfhydryl-rich proteins such as glutathione. ATO inhibits glutathione peroxidase, thus potentiating peroxide generation, disrupting mitochondrial membrane integrity and respiration, repressing anti-apoptotic proteins, and initiating caspase-mediated apoptotic responses. In MDS, the antiproliferative effects of ATO relate in part to its ability to suppress myeloblast elaboration of VEGF-A. The results of several clinical trials indicate that ATO has activity in MDS, with approximately a third of patients having experienced hematological improvement.[9,10]

FARNESYL TRANSFERASE INHIBITORS

The second class of targeted therapeutics being investigated in MDS are farnesyl transferase inhibitors. While activating mutations of the RAS proto-oncogene are detected in only about 20% of patients with MDS, they are common in CMML (40% to 70% of patients). The RAS gene superfamily encodes guanosine triphosphate hydrolases (GTPase) that function as critical regulatory elements in signal trans-

Table 4. WHO classification of the myelodysplastic/myeloproliferative diseases

Chronic myelomonocytic leukemia (CMML)
Atypical chronic myeloid leukemia (aCML)
Juvenile myelomonocytic leukemia (JMML)
Myelodysplastic/myeloproliferative disease, unclassifiable

duction, cellular proliferation and maintenance of the malignant phenotype. The protein products of the 3 RAS proto-oncogenes (H-, N-, and K-RAS) are post-translationally modified before incorporation into the inner leaflet of the plasma membrane. Farnesyl protein transferase (FPT) catalyzes the transfer of a 15-carbon farnesyl group to the C-terminal region of the RAS protein. Farnesylation of RAS is required for membrane association and activation.[11] The farnesyl protein transferase inhibitors (FTI) represent a novel class of potent, orally bioavailable inhibitors of RAS activation and are able to modulate multiple signaling pathways that have been implicated in the pathobiology or progression of CMML and MDS. Preliminary results of PhaseI/II studies in MDS and CMML indicate promising hematopoietic promoting activity.[12-14]

The constitutive activation of RAS can result from either mutations within RAS alleles or from reciprocal translocations deregulating receptor tyrosine kinases. A subset of CMML patients have a characteristic translocation involving the platelet-derived growth factor receptor beta chain (PDGFRβ) gene on the long arm of chromosome 5 (5q33).[15] The partner chromosome in this reciprocal translocation may vary, although the most common is chromosome 12 (the TEL gene locus, 12p12-13). An exciting recent discovery is that patients harboring the 5q33 translocation respond to imatinib (Gleevec). Imatinib binds to the PDGFβ receptor analogous to its interaction with BCR/ABL, to act as a potent inhibitor of receptor kinase activity. Among five patients reported to date, each achieved rapid hematological control and sustained complete cytogenetic remission with imatinib therapy.[16]

THERAPIES TARGETED AT EPIGENETIC ALTERATIONS

Recent interest in the treatment of neoplastic cells by targeting epigenetic changes to restore normal gene transcription has been intense. Unlike genetic changes (mutations, deletions) which are irreversible outside of the introduction of new genetic information, epigenetic changes represent potentially reversible modifications to DNA. The potential reversibility of epigenetic changes makes them attractive targets for cancer therapeutics.[17]

Histones (DNA packaging proteins) have lysine tails, which can be acetylated in a post-translational modification step. When acetylated, histones interact loosely with DNA so that the chromatin is open and genes downstream from the acetylated histone can be transcribed. Hypoacetylation is associated with heterochromatin formation and gene silencing.
DNA methyltransferase is the enzyme responsible for imparting a parent cell's methylation pattern to daughter cells. Abnormalities of cytosine methylation constitute some of the best characterized and most common epigenetic changes in cancer. The DNA of neoplastic cells may be characterized by global hypomethylation, dysregulation of DNA methyltransferase, and regional methylation of CpG dinucleotide clusters in gene promoter regions. These CpG clusters (CpG islands) are normally protected from methylation in normal cells. In cancer cells, CpG islands are heavily methylated.[17] Methylated CpG islands attract transcriptional inhibitory complexes which include histone deacetylases (HDAC). The enzyme HDAC deacetylates the lysine tail of histones, resulting in the tight association of the histone with DNA, forming a transcriptionally repressive conformation and inhibiting gene transcription.

Extensive studies have demonstrated promoter methylation, associated with transcriptional silencing, of a wide variety of cell regulatory genes in many cancers. These epigenetic changes are associated with phenotypic abnormalities in the malignant cells. Because promoter methylation is relatively "cancer-specific", DNA methylation is an attractive therapeutic target. In malignant myeloid cells, the most widely reported methylated gene is the cyclin-dependant kinase inhibitor $p15^{INK4B}$.[18,19] Methylation of the p15 promoter has been demonstrated in 68% of primary AML samples[20], and 35% of MDS[21]; the frequency of methylation increases with MDS disease progression. A variety of other genes are methylated in myeloid neoplasms, including E-cadherin, p73, and RARβ.[22]

The DNA methyltransferase inhibitors which have been most extensively characterized for the treatment of AML and MDS are 5-azacytidine (5AC) and 5-aza-2'-deoxycytidine (decitabine, DAC). A Phase III trial of 5AC demonstrated an overall hematologic response rate of 60%, including a 7% complete remission rate and a 16% partial remission rate.[23] Similar response rates have been reported utilizing DAC.[24] Current data suggest that the two drugs as currently studied are equivalently effective in the treatment of MDS.[25] The relationship of DNA methyltransferase inhibition to the clinical activity of 5AC and DAC in the treatment of MDS remains a critical question. Although a subset of patients treated with DAC did exhibit reversal of P15 methylation, associated with re-expression of p15 protein, a link between changes in methylation and clinical response could not be ascertained.[26]

A variety of HDAC inhibitors (HDACi) are under clinical investigation, including sodium butyrate, sodium phenylbutyrate, valproic acid, suberoylanilide hydroxamic

acid (SAHA), and FK228 (depsipeptide).[25] Once it was recognized that one of the primary mechanisms by which methylated DNA repressed transcription was through recruitment of HDAC, investigators became interested in whether it was possible to augment gene re-expression by combining or sequencing a methyltransferase inhibitor with a histone deacetylase inhibitor. Currently, the only data available is a Phase I dose-finding study of 5AC followed by phenylbutyrate.[27] The combined therapy was well tolerated, and significant sustained clinical responses were achieved.

NOVEL DIFFERENTIATION APPROACHES

Although growth factors are often potent differentiation inducers, the proliferation-inducing effects of these cytokines often outweigh their differentiation effects. Most of the agents that have been used to induce terminal differentiation in vitro are antiproliferative; they induce the cell cycle inhibitor p21, and thus cell cycle arrest.[28] In vitro data suggested that the pairing of such cytostatic agents with myeloid growth factors might lead to the predominance of differentiation activity of the growth factor over the proliferative effects. The protein kinase C activator bryostatin has been paired with GM-CSF in a Phase I trial, with promising results.[25]

TRANSPLANTATION AND IMMUNOSUPPRESSIVE THERAPY IN MDS

There has been debate on whether hematopoietic stem cell transplantation (HSCT) was a curative option for patients with MDS. Autologous HSCT in MDS is theoretically feasible only in a small proportion of patients who achieve a complete remission following induction chemotherapy and in whom a suitable autologous harvest can be collected. However, there is a high relapse risk of up to 72%, and enthusiasm for this approach is limited.[29]

Conventional myeloablative allogeneic HSCT has a significantly lower relapse rate than autografts, but the transplant-related complications increase in frequency and severity with advancing age. However, allogeneic HSCT is the only therapeutic modality at present that is potentially curative in MDS. Allogeneic HSCT replaces recipient dysplastic hemopoiesis with healthy donor hemopoiesis. Its applicability, however, is limited by the availability of a suitable HLA-matched donor and by the toxicity of the conditioning regimen, which is directly proportional to the age of the recipient.[30] As the majority of patients with MDS are of advanced age, often with concurrent medical conditions that effectively preclude standard conditioning for allogeneic HSCT, various strategies have been adopted in order to attempt to reduce the toxicities associated with the transplant procedure. Recently there has been significant interest in a reduced-intensity or 'nonmyeloablative' conditioning which can result in stable donor hemopoietic engraftment, without the toxicity associated with conventional HSCT.[31]

Even in a clonal disease like MDS, there is a subgroup of patients in whom, for whatever reasons, there is evidence of immune dysfunction.[32] Such evidence may include abnormal CD4:CD8 ratios and increased activated cytotoxic T-cells. Immunotherapeutic agents that inhibit these immune mechanisms play an important role in the management of the immune-mediated marrow failure syndrome in MDS. Antithymocyte globulin has been shown to produce clinically meaningful responses in patients with MDS, with 34% to 64% of patients becoming transfusion independent.[29] The administration of cyclosporine in patients with hypoplastic MDS has also resulted in prolonged partial hematologic improvements.[33]

Levels of the cytokine tumor necrosis factor alpha (TNFα) have been demonstrated to be elevated in patients with MDS and have been shown to play a major role in the apoptosis of hemopoietic cells in MDS.[34] The inhibition of TNFα therefore appears to be a legitimate target for directed therapy. At present, there are 2 anti-TNFα agents available for clinical use, with limited data available in MDS.[29]

Chronic myeloproliferative disorders

The chronic myeloproliferative disorders (CMPD) are characterized by proliferation within the bone marrow of granulocytes, erythrocytes and/or megakaryocytes. Under the new WHO classification, the CMPD include Philadelphia chromosome positive chronic myelocytic leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (IMF), chronic neutrophilic leukemia (CNL), and chronic eosinophilic leukemia/hypereosinophilic syndrome (CEL/HES) (Table 1). This article will focus on the Philadelphia chromosome-negative CMPD, primarily PV, ET, and IMF. CML is to be covered by a separate article in another issue of the journal.

The diagnosis, and management, of patients with PV, ET, and IMF has been difficult for a number of reasons. They share many overlapping clinical features. In addition to their phenotypic mimicry, there is a lack of specific molecular diagnostic markers, a lack of understanding of their molecular basis, and a paucity of controlled, prospective therapeutic trials for treatment decisions. As a result, currently there are

still significant unresolved issues concerning the CMPD, including whether these disorders are truly different, and/or how they are related.

There are a number of features which are common to the CMPD. There is involvement of a multipotential hematopoietic progenitor cell, and usually clonal dominance of the abnormal clone over normal hematopoietic progenitor cells. The disorders share abnormalities of chromosomes 1, 8, 9, 13, and 20, although a consistent cytogenetic abnormality as described for CML or APL is not found.[35] In general, they result in a hypercellular bone marrow, and the various cell lines involved generally show relatively normal maturation, although the megakaryocytic lineage may display dysplastic features. Hematopoiesis is generally considered 'effective', as elevated cell counts in the peripheral blood are observed. The CMPD are often associated with organomegaly (enlarged spleen and/or liver), as a result of either cell sequestration, extramedullary hematopoiesis, or leukemic infiltration. Thrombotic and hemorrhagic diatheses are common. The CMPD disorders have a varying tendency to progress either to a stage of bone marrow failure (decline of peripheral blood cell counts and evolving marrow fibrosis) or to undergo transformation to acute leukemia.

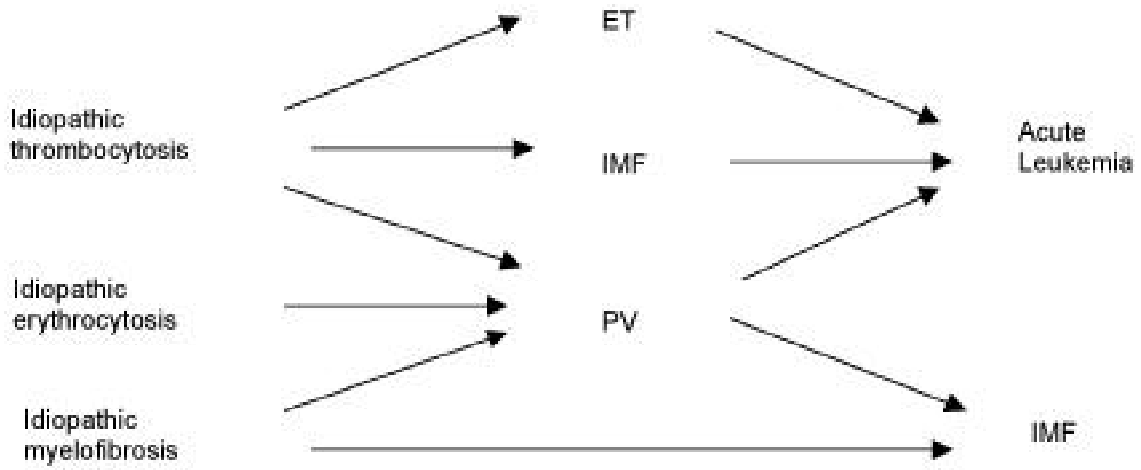
In addition to the phenotypic mimicry among these disorders, there is also overlap with nonclonal hematopoietic disorders. Many patients with idiopathic thrombocytosis go on to develop ET, IMF, or PV, and many patients with idiopathic erythrocytosis go on to develop PV (Figure 1).

Despite the extensive overlap in characteristics for these disorders, it is important to appreciate that they are not monolithic, but actually show both clinical and genetic heterogeneity.[35] For example, the increase in bone marrow reticulum (myelofibrosis) occasionally seen as a complication in PV must be distinguished from the bone marrow failure state associated with IMF. Myelofibrosis is a histologic condition, and the increase in marrow fibrosis in PV generally has minimal effect on erythropoiesis (most patients continue to have increased hemoglobin and packed cell volume).[35]

Recently, two new molecular assays have been described which may prove useful for the diagnosis of CMPD. Polycythemia rubra vera-1 (PRV-1), a novel member of the urokinase-type plasminogen activator receptor (uPAR) superfamily, has been reported to be overexpressed in mature peripheral blood granulocytes from patients with PV, but not in a variety of controls including healthy individuals, patients with secondary erythrocytosis, and patients with CML.[36] CD177, the current designation for the gene that encodes PRV-1, was found to be the only gene strongly overexpressed in polycythemia neutrophils. While CD177 is almost universally overexpressed in polycythemia vera, the mechanisms of overexpression are not known. No abnormalities of the CD177 gene have been found.[37] Also, although the expression of CD177 mRNA is markedly elevated in patients with PV, the neutrophil protein encoded by CD177 (NB1 glycoprotein) is similar to that of health subjects.[38]

Decreased expression of Mpl, the receptor for thrombopoietin, has been described in platelets and mega-

Figure 1. Overlap and interconversion among hematopoietic disorders



karyocytes in patients with PV, ET, and IMF. The biologic basis appears to be either alternative splicing of Mpl mRNA, or a single nucleotide polymorphism. The number of patients expressing increased quantities of CD177 mRNA, or decreased Mpl, varies among the disease type and among studies (Table 5).[35,37] However, both have become important molecular markers of myeloproliferative disorders.

CYTOREDUCTIVE THERAPY IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

PV and ET are chronic MPDs in which there is a thrombohemorrhagic diathesis, and a variable incidence of progression to myelofibrosis or acute myeloid leukemia (AML). The main cause of morbidity and mortality in these disorders is thrombosis, which occurs more frequently in older patients or in those with previous vascular complications. Severe bleeding is relatively rare, and limited to patients with a very high platelet count and to those taking antiplatelet drugs. It has been suggested that propensity for vascular complications can be reduced by cytoreductive treatment. While cytoreductive therapy is effective in preventing thrombosis, there is concern that some myelosuppressive agents may increase the rate of transformation to acute leukemia. Thus the aim of treatment of both PV and ET must be to reduce/prevent thrombosis and hemorrhage without increasing the risk of acute leukemia.[39] It has been suggested that patients with ET or PV be stratified into low, intermediate, and high risk groups (in terms of their relative risk for thrombohemorrhagic events), and cytoreductive therapy be given only to high risk patients.

Major risk factors for thrombosis are age (greater than 60 years), a previous thrombotic event, and long duration of thrombocytosis (platelet count higher than 1500 x 10^9/L).[40]

Table 5. Molecular markers for Philadelphia chromosome negative CMPD

PRV-1 Overexpression in CMPD:	
Polycythemia vera	69% to 100%
Idiopathic myelofibrosis	50% to 100%
Essential thrombocythemia	33% to 67%
Impaired Mpl Expression in CMPD:	
Polycythemia vera	30% to 95%
Idiopathic myelofibrosis	67% to 75%
Essential thrombocythemia	35% to 68%

In addition, patients with impaired expression of Mpl in bone marrow megakaryocytes or with over expression of PRV-1 were at higher risk for vascular complications.[39,41] Both prospective and retrospective studies confirmed that thrombotic deaths are rare in low-risk ET patients.[39] Thus, because of the low risk of thrombotic complications, and the potential leukemogenicity of cytotoxic drugs, it has been suggested that chemotherapy be withheld for young (<60 years), asymptomatic ET patients with a platelet count below 1500 x 10^9/L and with no additional risk factors for thrombosis.

Several platelet count-lowering agents have been investigated. Hydroxyurea (HU) has emerged as the treatment of choice in high risk patients with ET because of its efficacy in preventing thrombosis and rare acute toxicity. However, the incidence of acute leukemic transformation is higher in patients with ET who have cytogenetic abnormalities or who are receiving multiple cytotoxic drugs.[42] Alternatives to HU include interferon and anagrelide. Both are effective in reducing platelet counts without leukemic transformation, but the high cost and side effects reduce their attractiveness for some patients. Generally, HU is recommended for older high-risk ET patients, while anagrelide or interferon is recommended for younger patients because of possible leukemogenicity associated with long-term use of HU.

The approach to treatment of patients with PV follows a similar stratification based on probability of developing thrombotic complications. The mainstay of treatment for all patients has not changed in several decades – phlebotomy is recommended for all patients to keep the hematocrit below 0.45. Stable patients at low risk for thrombosis (age <60 years, no history of thrombosis) might not require additional cytoreductive therapy. In patients at high risk of thrombosis or with a very high phlebotomy requirement, myelosuppressive therapy is indicated.[43] As with ET, the choice of cytoreductive agent is age-adapted. Typically, ^{32}P or busulfan is used for older patients (>70 years), HU is the agent of choice in middle-aged patients (50 to 70 years of age), and interferon is preferred in younger patients (<50 years).[39]

An ongoing question has been the decision whether or not to treat PV patients with aspirin in an attempt to reduce the incidence of major thrombotic events. Although thrombotic complications in patients receiving cytoreductive treatment are far less frequent than in untreated patients, they still remain a major cause of morbidity and mortality.[43] The efficacy and safety of antithrombotic drugs is unclear. An initial study suggested that aspirin increased the risk of bleeding

with no reduction of thrombotic complications, and resulted in the avoidance of the use of aspirin in treating patients with PV.[44] Subsequent studies using low-dose aspirin did document an improved clinical outcome (decrease of vascular deaths) and aspirin has become an accepted treatment modality in patients with PV.[45]

IDIOPATHIC MYELOFIBROSIS

Idiopathic myelofibrosis (myelofibrosis with myeloid metaplasia) has remained one of the least understood of the MPDs. Aspects of the disease which remain inadequately explained include the cause of the blunting of hematopoiesis with the development of anemia and thrombocytopenia, and the cause of the displacement of hematopoiesis from the bone marrow to extramedullary organs.

IMF has been reported to be a clonal disorder involving erythroblasts, megakaryocytes, granulocytes, monocytes, and B and T lymphocytes, originating from a pluripotent progenitor. The number of $CD34^+$ progenitor cells in the bone marrow of patients with IMF are increased in the early hypercellular stages of the disease, indicating a higher proliferative activity of the precursor cell pool. When the disease evolves into an overt fibrosclerotic stage, bone marrow progenitor cells are usually reduced in number.[46] Simultaneously, hematopoietic stem cells mobilize and exit the bone marrow, traveling via the peripheral blood to colonize the spleen and other organs. The clonal cells excessively produce hematopoietic, fibrogenic, and angiogenic growth factors.[47]

Megakaryocytes have been linked to the induction of an abnormal cytokine environment that is critical for the stimulation of fibroblasts, causing collagen fibrosis. The megakaryocyte lineage has been shown to have a proliferative advantage, demonstrated by the elevated growth of progenitor cells in vitro, by their enhanced sensitivity to thrombopoietin (TPO), or by their autonomous growth. Platelets and megakaryocytes of patients with IMF express an abnormal TPO-receptor (Mpl) isoform that is paradoxically poorly expressed on the cell surface, yet associated with an enhanced response to TPO and a proliferative advantage.

Mouse models of IMF have documented that TGF-β and osteoprotegerin (OPG) are essential for the development of myelofibrosis and osteosclerosis, respectively. Murine hematopoietic cells in which the TGF-β gene has been 'knocked-out' ($TGF\text{-}\beta^{-/-}$) cannot produce myelofibrosis. OPG is a molecule secreted by TGF-β activated osteoblasts, which blocks a cytokine signaling pathway for the activation of osteoclast precursors to osteoclasts. In IMF, there is increased production of OPG, in response to elevated TGF-β which is directly responsible for the osteosclerosis seen in this disorder.[48]

An interesting finding in patients with IMF is the observation of excessive and pathologic emperipolesis of polymorphonuclear leukocytes (PMN) into the megakaryocyte. It has been proposed that PMN entering the megakaryocyte release proteases which cause cell lysis and leaking of TGF-β and other α-granular proteins.[49]

Neoangiogenesis, or formation of new vessels, has emerged as a hallmark of IMF. Immunohistochemical staining demonstrates that 70% of patients with IMF had a substantial increase in bone marrow microvessel density.[50] Neoangiogenesis has now been documented as an integral component of medullary and extramedullary hematopoiesis. There is also a correlation between angiogenesis and both bone marrow cellularity and the amount of spleen and blood $CD34^+$ hemopoietic stem cells.[48,50] Recently, increased serum levels of VEGF have been demonstrated in most patients with IMF, suggesting a cytokine-mediated stromal reaction inducing angiogenesis in IMF.[51]

Therapeutic options in IMF

The conventional therapeutic options for IMF included supportive care, chemotherapy, or biologic-modifying agents. Allogeneic stem cell transplantation has usually been used in the setting of advanced and refractory disease, often after the failure of standard therapy. New approaches being investigated in the treatment of IMF include some of the same ones being used for other MPD and MDS. Reduced-intensity conditioning stem cell transplantation appears to reduce transplantation-related mortality while maintaining a graft-versus-leukemia reaction potentially sufficient to obtain disease eradication.[48] Thalidomide is being studied for its antiangiogenic action and modulation of cytokines, particularly TNF-α. In preliminary studies, thalidomide has been reported to ameliorate anemia, thrombocytopenia and splenomegaly in a subset of patients.[48]

Imatinib mesylate (STI571, Gleevec) is a potent and selective tyrosine kinase inhibitor with significant in vitro activity against c-abl and bcr-abl. Imatinib also inhibits two other tyrosine kinases: c-kit (CD117), which is highly expressed on $CD34^+$ cells in IMF, and the platelet derived growth factor receptor, believed to play a role in the pathogenesis of the fibrosis. Imatinib is being investigated as a potential therapy in IMF, with mixed early results.[48]

SUMMARY

Our understanding of the exact molecular and genetic alterations responsible for the evolution of the diverse diseases included under the myelodysplastic and myeloproliferative disorders lags behind that of the acute leukemias and CML. However, progress is being made, and new tests for molecular markers (increased PRV-1/CD177, decreased Mpl expression) are being developed. While in the past, treatment for both groups of diseases was primarily supportive, an improved understanding of the underlying pathobiology has led to new treatments with promising preliminary results. The laboratory's role in the diagnosis, prognosis, and determination of efficacy of treatment will continue to expand as new tests become available.

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