



Achievements in Understanding and Treatment of Myelodysplastic Syndromes

Eva Hellström-Lindberg (Chair), Cheryl Willman, A. John Barrett, and Yogen Saunthararajah

The myelodysplastic syndromes (MDS) constitute a challenge for the biologist as well as for the treating physician. In Section I, Dr. Willman reviews the current classifications and disease mechanisms involved in this heterogeneous clonal hematopoietic stem cell disorder. A stepwise genetic progression model is proposed in which inherited or acquired genetic lesions promote the acquisition of “secondary” genetic events mainly characterized by gains and losses of specific chromosome regions. The genetic risk to develop MDS is likely multifactorial and dependent on various constellations of risk-producing and -protecting alleles. In Section II Dr. Barrett with Dr. Saunthararajah addresses the immunologic factors that may act as important secondary events in the development of severe pancytopenia. T cells from patients with MDS may suppress autologous erythroid and granulocytic growth in vitro, and T cell suppression by antithymocyte globulin or cyclosporine may significantly improve cytopenia, especially in refractory anemia. Recent studies have also demonstrated an increased vessel density in MDS bone marrow, and a phase II trial of thalidomide showed responses in a subgroup of MDS patients espe-

cially in those with low blast counts. In Section III Dr. Hellström-Lindberg presents results of allogeneic and autologous stem cell transplantation (SCT), intensive and low-dose chemotherapy. The results of allogeneic SCT in MDS are slowly improving but are still poor for patients with unfavorable cytogenetics and/or a high score according to the International Prognostic Scoring System. A recently published study of patients between 55–65 years old showed a disease-free survival (DFS) at 3 years of 39%. Consolidation treatment with autologous SCT after intensive chemotherapy may result in long-term DFS in a proportion of patients with high-risk MDS. Low-dose treatment with 5-azacytidine has been shown to significantly prolong the time to leukemic transformation or death in patients with high-risk MSA. Erythropoietin and granulocyte colony-stimulating factor may synergistically improve hemoglobin levels, particularly in sideroblastic anemia. Recent therapeutic advances have made it clear that new biological information may lead to new treatment modalities and, in combination with statistically developed predictive models, help select patients for different therapeutic options.

I. BIOLOGIC AND GENETIC FEATURES OF THE MYELODYSPLASTIC SYNDROMES

*Cheryl L. Willman, M.D.**

Recent scientific advances have provided new insights into the etiology and pathogenesis of the myelodysplastic syndromes (MDS). Despite heterogeneous morphologic, genetic, biologic, and clinical features, all forms of MDS are clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis and peripheral cytopenias. Although a substantial proportion of MDS cases evolve to acute myeloid leukemia (AML), the natural

history of these syndromes ranges from more indolent forms of disease spanning years to those with a rapid evolution to AML. Thus, MDS is best considered a pre-leukemic disorder in which the neoplastic clone that has been established may or may not fully progress to acute leukemia. Although the relationship between MDS and de novo AML has been controversial and current disease classification systems (**Table 1**) are considered unsatisfactory, most hematologists now consider MDS and AML as part of the same continuous disease spectrum rather than as distinct disorders. This review will briefly highlight current controversies in the classification of MDS and AML, the cytogenetic and molecular genetic features of MDS, the biologic features that characterize MDS including abnormal apoptosis and an altered marrow mi-

* University of New Mexico Cancer Center, 2325 Camino de Salud NE, Room 101, Albuquerque NM 87131

Table 1. MDS Classification Systems.¹

| FAB Classification System² | WHO Classification System³ | IPSS Risk-Based Classification System⁴ |
|--|---|---|
| Refractory Anemia (RA): Cytopenia of one PB lineage; normo- or hypercellular marrow with dysplasias; < 1% PB Blasts and < 5% BM Blasts. | Myelodysplastic Syndromes Refractory Anemia (RA) With ringed sideroblasts (RARS) Without ringed sideroblasts | Overall IPSS Risk Score Based On: Marrow Blast Percentage Blast % IPSS Score < 5 0 5-10 0.5 11-20 1.5 21-30 2.0 |
| Refractory Anemia with Ringed Sideroblasts (RARS): Cytopenia, dysplasia and the same % blast involvement in BM and PB as RA. Ringed sideroblasts account for >15% of nucleated cells in marrow. | Refractory Anemia (MDS) with Multilineage Dysplasia (RCMD) | Cytogenetic Features⁵ Karyotype IPSS Score Good prognosis 0 (-Y, 5q-,20q-) Intermediate prognosis 0.5 Poor prognosis 1.0 (abn. 7; Complex) |
| Refractory Anemia with Excess Blasts (RAEB): Cytopenia of two or more PB lineages; dysplasia involving all 3 lineages; < 5% PB blasts and 5-20% BM Blasts. | Refractory Anemia with Excess Blasts (RAEB) 5q- Syndrome Myelodysplastic syndrome, unclassifiable | Cytopenias⁶ Cytopenia IPSS Score None or 1 Type 0 2 or 3 Types 0.5 |
| Refractory Anemia with Excess Blasts in Transformation (RAEB-T): Hematologic features identical to RAEB. > 5% Blasts in PB or 21-30% Blasts in BM or the presence of Auer rods in the blasts. | Myelodysplastic/Myeloproliferative Diseases Chronic Myelomonocytic Leukemia (CMML) Atypical Chronic Myelogenous Leukemia (aCML) Leukemia (JMML) | Overall IPSS Score and Survival Overall Score Median Survival Low (0) 5.7 Yrs. Intermediate 1 (0.5 or 1.0) 3.5 Yrs. 2 (1.5 or 2.0) 1.2 Yrs. High (≥ 2.5) 0.4 Yrs. |
| Chronic Myelomonocytic Leukemia (CMML): Monocytosis in PB (>1x10 ⁹ per liter); < 5% blasts in PB and up to 20% BM blasts | | |
| Juvenile Myelomonocytic | | |

¹ Abbreviations: PB, peripheral blood; BM, bone marrow; abn, abnormality

² References 3-4.

³ Reference 21.

⁴ Reference 30.

⁵ IPSS Cytogenetic Classification²⁸: Good prognosis: -Y only, normal, del(5q) only, del(20q) only; Intermediate prognosis: +8, Single miscellaneous abnormality, double abnormalities; Poor prognosis: Complex (i.e. ≥ 3 abnormalities), any chromosome 7 abnormality.

⁶ IPSS Types of Cytopenia²⁸: Hemoglobin <10g per deciliter; Absolute neutrophil count <1500 per cubic millimeter; Platelet count < 100,000 per cubic millimeter.

croenvironment, and new and highly interesting insights into the complex genetic predisposition to MDS. Excellent, well-referenced reviews are also available.^{1,2}

MDS and AML Disease Classification Systems: Unresolved Controversies

The French-American-British (FAB) Classification, proposed in 1977, provided hematologists with the first consistent framework for morphologic classification of MDS (Table 1), the myeloproliferative disorders, and the acute leukemias.^{3,4} However, the separation of MDS as a distinct disorder from AML in the FAB classification scheme has been perceived by many to have scientifically impeded our understanding of the full spectrum of leukemic progression.¹ Indeed, the initial failure to recognize and classify MDS as a “neoplastic” pre-leukemic disorder and part of the same disease spectrum as AML resulted in the exclusion of MDS cases from virtually all US cancer registries and the NCI-sponsored Surveillance,

Epidemiology, and End Results (SEER) program (www.seer.cancer.gov). This has greatly impeded studies of the true incidence, natural history, and epidemiology of MDS in the US. Importantly, however, European epidemiologic studies suggest that the incidence of MDS is at least as high as that of AML, particularly AML cases that arise in older individuals.⁵ In the US, the age-specific incidence rate for AML in males aged 50 years at diagnosis is 3.5 per 100,000, increasing dramatically to 15 at age 70 and 35 at age 90.⁶ (See also www.seer.cancer.gov.) With the exponential increase in the incidence of AML with age and the aging of our populations, the median age at diagnosis of AML in the US is currently 63 years. Thus, the majority of AML cases, like MDS, occur in older individuals. Further linking MDS and AML, several studies have noted that the biologic, morphologic, and genetic features of AML arising in older individuals are similar to 1) primary MDS; 2) AML arising secondary to antecedent MDS; 3) AML arising sec-

ondary to prior therapy, particularly alkylating agent exposure; and 4) AML cases that arise from documented environmental or occupational exposures to agents such as benzene, petroleum, organic solvents, and arsenical pesticides.⁷⁻¹³

In the FAB Classification (Table 1), the two primary distinguishing features between the various MDS subtypes, chronic myelomonocytic leukemia (CMML) and AML, are blast cell percentage and the presence of dysplastic features. CMML is now considered a myeloproliferative/leukemic-like disorder and frequently associated with t(5;12)(q33;p13),^{1,14} and AML is defined as \geq 30% marrow blasts with the various MDS subtypes ranging from $<$ 5% to $<$ 30% blasts. However, the previous distinction between MDS and AML has become blurred with the recognition of several common features of the two diseases: 1) MDS is now recognized to be a clonal pre-leukemic hematopoietic stem cell disorder frequently associated with specific recurrent cytogenetic abnormalities¹⁵⁻¹⁸; 2) multi-lineage dysplasia is now recognized to occur in the majority of AML cases presenting clinically as “de novo” disease in older individuals^{7,19-21}; 3) AML arising in older individuals and primary, secondary, or therapy-induced MDS are now known to share strikingly similar biologic and genetic features⁷⁻¹³; 4) de novo AML cases such as those with t(8;21) and inv(16) may present clinically with less than 30% marrow blasts and may have dysplastic features²¹; and 5) transgenic and “knock-in” murine models of leukemia made with fusion genes from translocations associated primarily with de novo AML [t(15;17), t(8;21), inv(16)] are often characterized by hematopoietic dysplasia or an MDS-like disease antecedent to the development of AML.²²⁻²⁵ These more recent clinical and biologic studies indicate that disorders previously classified as MDS are part of the same disease continuum as AML and that MDS is best considered a pre-leukemic disorder with variable frequencies and rates of progression to AML.

As we now recognize that MDS and AML are part of the same continuous biologic and genetic spectrum of disease, the use of arbitrary “thresholds” for the distinction of AML from MDS for the purposes of disease classification and therapeutic decision making has become particularly problematic. At what blast cell percentage should a clinician institute AML-based therapies in an MDS patient progressing to RAEB-T and from RAEB-T to AML? Should AML-based therapies be instituted in a patient whose marrow has dysplastic morphologic features, a blast cell percentage $<$ 20%, and a t(8;21)-containing clonal population of cells? While the threshold of 30% blasts used by the FAB Classification to distinguish AML from MDS (Table 1) is clearly arbitrary, a reduction in this threshold to 20% blasts and the resultant elimination of RAEB-T as a distinct clinical stage in

the evolution of MDS to AML as proposed in the new WHO Classification²¹ (Table 1) has been perceived by many hematologists to be even more problematic.^{26,27} While RAEB-T and AML arising clinically as “de novo” disease in older patients share highly similar cytogenetic features,^{13,26} they have differing clinical and biologic features and therapeutic responsiveness.²⁶⁻²⁸ Although not directly tested in a randomized fashion, in several, if not all, studies RAEB-T patients appear to have a worse response to intensive chemotherapy when compared historically to AML cases with similar biologic and cytogenetic features.^{28,29} Thus, it will be particularly important to retain the distinct RAEB-T MDS subtype in order to compare future therapeutic advances in AML/MDS to historical controls. Additional concerns that have arisen with the proposed WHO Classification (Table 1) include²⁹: 1) the proposal that a diagnosis of RA and RARS be restricted to patients who have abnormalities solely involving the erythroid lineage, even though a diagnosis of MDS requires dysplasia in at least two hematopoietic lineages; 2) the creation of vague new MDS diagnostic categories (“refractory cytopenias with multilineage dysplasia (RCMD)” and “MDS, unclassifiable”) which have no biologic, clinical, or genetic basis; and 3) the general lack of clinical and prognostic relevance in the proposed WHO classification scheme. Unfortunately for clinicians and diagnosticians alike, these controversies will likely continue until our knowledge has increased to the degree that disease classification systems can be developed on clinical features, genetics/genomics, and functional biology. And as our knowledge continues to evolve, classification systems will necessarily require constant revision.

Taking an alternative approach, others have worked to develop risk-based classification systems for MDS in order to facilitate clinical decision-making.³⁰ The International Scoring System for Evaluating Prognosis (IPSS) in MDS assigns IPSS scores to varying degrees of those clinical and biologic features that today provide the most prognostic significance in MDS: marrow blast cell percentage, karyotype, and degree of cytopenia (Table 1).²⁹ An overall IPSS score developed using these variables has a strong correlation with predicted median survival.²⁹ The IPSS system has proven to be a highly useful method for evaluating prognosis in MDS patients, has achieved international acceptance, and is being used to design clinical trials.

Genetic Features of MDS: Models for Genetic Progression and Clues to Etiology

MDS is a clonal hematopoietic stem cell disorder characterized by step-wise genetic progression

Initially demonstrated by studies of expression of the various isoforms of the X-linked gene G6PD and more re-

cently by molecular methods that detect non-random patterns of X-inactivation, evidence for clonality has been found in all forms of MDS, even in their very earliest stages.^{17,18} Interestingly, cytogenetic and molecular data provide evidence, in some MDS patients, for the existence of a clonal phase of disease prior to the acquisition of the characteristic cytogenetic abnormalities associated with MDS.¹⁸ Similarly, MDS patients who evolve to acute leukemia may after therapy revert to a cytogenetically normal but persistently clonal remission. Such findings have led to the hypothesis that the recurrent cytogenetic abnormalities associated with MDS, previously considered the “primary” cause of disease, are actually “secondary” cytogenetic abnormalities that arise due to cytogenetically undetectable initiating lesions in a clonal hematopoietic stem cell population.³⁰ Such initiating events are likely to be heterogeneous and could either be inherited or result from acquired somatic DNA damage, genomic instability, defective DNA repair, or perturbations in cell signal transduction pathways that give rise to stem cell clones that have a growth or survival advantage. In contrast to the de novo acute leukemias that occur primarily in younger patients (particularly those associated with balanced translocations and inversions such as t(8;21), t(15;17), inv(16), t(9;11), etc. lacking dysplastic features), MDS and AML arising in older individuals appear to have a different model of genetic progression (Figure 1).³¹⁻³² In this proposed model, initiating genetic lesions (which may be inherited or acquired) promote the acquisition of “secondary” genetic events that are

primarily characterized by stepwise gains and losses of specific chromosomal regions (particularly chromosome 3p-, 3q-, 5q-, 7q-, 12p-, -17, -18, 20q11-12, +8). Such gross chromosomal changes are ultimately accompanied by sub-microscopic DNA mutations of genes such as *p53*, *FLT3*, or *RAS*, methylation of specific gene promoters, and in some cases by the reciprocal translocations and inversions more frequently associated with AML. This model of step-wise genetic progression for MDS and related AML is strikingly reminiscent of those proposed for human solid tumors, such as colon cancer. Three lines of evidence support this model and the existence of a genetic predisposition to MDS: 1) the occurrence of AML and MDS in families with inherited defects in DNA repair or neurofibromatosis-type I³³⁻³⁷; 2) genetic mapping studies in the rare families with “familial” MDS and AML³⁸⁻⁴¹; and 3) studies of the association of various genetic polymorphisms with AML and MDS.⁴²⁻⁴⁸

An inherited genetic predisposition to MDS

Support for an inherited predisposition to MDS and related AML has long been evident from studies of inherited constitutional genetic defects (such as Schwachman-Diamond syndrome, the defective DNA repair of Fanconi anemia, or deregulation of the RAS signal transduction pathway in neurofibromatosis-type 1) that are present in a large proportion of children who develop MDS and AML.³³⁻³⁷ Indeed, recent studies indicate that up to 30% of children affected by MDS and related myeloproliferative disorders have an inherited constitutional genetic

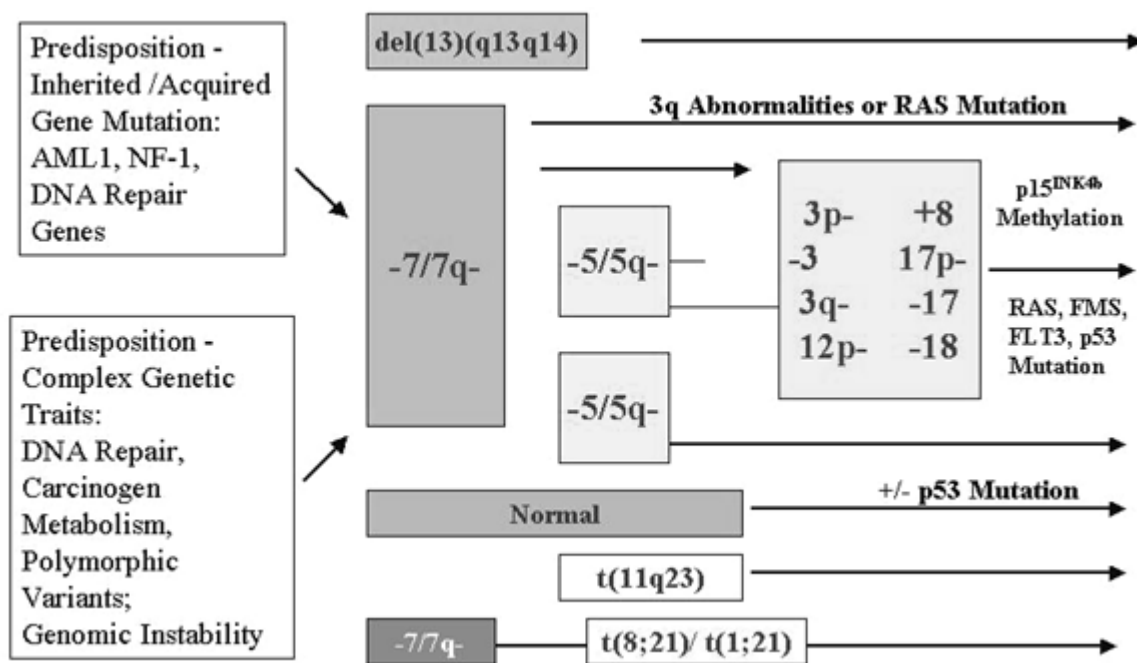


Figure 1. Proposed model for genetic progression of MDS to AML.

disorder.³⁴ The original studies by Shannon and colleagues^{35,36} of the genetic basis of familial MDS with chromosome 7q abnormalities (the “monosomy 7 syndrome”) are important in that they first revealed that abnormalities of chromosome 7q were not the “primary” cause of the syndrome; indeed, these investigators concluded that the predisposition locus mapped to some other as yet unidentified chromosomal location. Thus, the foundation was laid for the putative existence of genetic loci that could “predispose” to chromosomal instability, secondary loss of specific chromosomal regions (such as 5q, 7q, and 20q), and the ultimate development of MDS and AML in adults and children. This original hypothesis was recently supported the findings of Gilliland and colleagues who determined the genetic basis of familial platelet disorder with leukemia (FPD/AML), an autosomal-dominant congenital thrombocytopenia characterized by platelet aggregation abnormalities.³⁸⁻³⁹ Affected individuals in the seven pedigrees studied to date all have a striking propensity to develop MDS, AML, and more rarely, chronic myelogenous leukemia (CML). Interestingly, the MDS and AML cases that develop in these pedigrees have the cytogenetic abnormalities classically associated with MDS, particularly abnormalities of chromosomes 5q and 7q and complex abnormalities. After mapping the FDP/AML predisposition locus to chromosome 21q22 in 1998, Gilliland and colleagues went on to determine that the causative gene for this disorder was CBFA2 (AML1), the gene whose function is most frequently disrupted in the acute leukemias by various reciprocal translocations and inversions, including the t(8;21), inv(16), t(3;21), and t(12;21).⁴⁹ Heterogeneous point mutations and small deletions of a single AML1 allele were found in these different pedigrees.³⁹ Despite this molecular heterogeneity, each mutation characteristic of each pedigree affected the DNA-binding domain of one AML1 allele, particularly targeting the two arginine residues at positions 166 and 201 that bind to DNA. The change of arginine to glutamine resulted in a loss of DNA-binding activity.³⁸ These data thus support the hypothesis that AML1 may surprisingly function as a tumor suppressor gene and that loss of one AML1 allele (hemizygous loss) is sufficient to initiate tumorigenesis and establish a neoplastic clone in affected individuals. This loss of function of a single AML1 allele appears to also confer a susceptibility to the acquisition of secondary mutations and the gain and/or loss of the chromosomal regions frequently associated with AML and MDS. This discovery has led to a particularly attractive model for MDS/AML whereby AML1 mutations predispose to chromosome instability leading to the eventual loss of chromosomes 5q, 7q; such models are currently being developed in mice in which the mutated AML1 allele has been introduced (Downing and Gilliland, personal communication). These pivotal

studies also further cement a genetic and etiologic link between MDS and AML (and even CML). Not unexpectedly, a low percentage of sporadic AML, ALL, and CML cases (5–8%) have recently been reported to have similar AML1 mutations⁵⁰; whether such AML1 mutations can be observed in primary “sporadic” MDS cases is currently under investigation. Whether AML and MDS cases with AML1 mutations are indeed “sporadic” or represent AML and MDS cases that have arisen in individuals with inherited AML1 mutations is as yet unknown. Given the functional role and association of CBF β (mapping to chromosome 16q22) with AML1 in normal and neoplastic hematopoiesis,⁵¹ it is tempting to further speculate that CBF β might be the causative gene for the second predisposition locus in AML and MDS in those familial cases where the predisposition has been mapped to chromosome 16q21-23.2.⁴⁰

Models for the development of sporadic MDS: Cumulative environmental exposures in genetically predisposed individuals

While genetic and familial mapping studies have clearly demonstrated that mutations in a specific gene, such as AML1, NF1, or genes mediating DNA repair, can predispose to the acquisition of secondary cytogenetic abnormalities and MDS, it is likely that such inherited genetic mutations will account for only a minority of MDS cases. How the majority of “sporadic” MDS cases arise is as yet undetermined. However, epidemiologic case-control studies of MDS (and related AML) have demonstrated associations between MDS and smoking, exposure to chemical compounds (particularly petroleum products and diesel derivatives, exhausts, organic solvents, fertilizers, and nitro-organic explosives), semi-metals (arsenic and thallium), stone dusts (such as silica), and cereal dusts.⁵²⁻⁵³ In light of these epidemiologic studies, it is interesting that evidence is increasing for a complex genetic predisposition to MDS involving naturally occurring DNA polymorphisms in genes that mediate DNA repair and metabolize environmental carcinogens.⁴²⁻⁴⁸ These studies are leading to a model, also diagrammed in Figure 1, in which MDS arises as a result of cumulative environmental exposures in genetically predisposed individuals. In this case, the genetic predisposition is a more complex genetic trait: a constellation of genetic variants in critical polymorphic genes. The initial attempts to dissect this complex genetic predisposition have focused on the association of MDS with naturally occurring polymorphisms in genes that mediate carcinogen metabolism.⁴²⁻⁴⁸ Following initial reports of the association of a non-functioning ⁶⁰⁹C→T polymorphic allele of the NAD(P)H:Quinone Oxidoreductase (NQO1) gene that plays a critical role in detoxifying benzene metabolites with an increased incidence of hematologic malignan-

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