Myelodysplastic Syndrome

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I. Promise and Potential Pitfalls of Clonality Analysis in Myelodysplastic Syndrome

A. Introduction

The hallmark of neoplastic disease, including myelodysplastic syndrome (MDS), is clonal proliferation of cells. Clonal proliferation is the consequence of acquired somatic mutation that confers a proliferative advantage to cells. For the myelodysplastic syndrome, the ability to identify clonally derived cells has provided valuable information about the molecular pathogenesis of the disease, the processes that govern the transition of MDS to acute myelogenous leukemia (AML), and diagnostic and prognostic information that is useful in the clinical approach to MDS.

In this section, we will review the various methods that are available for determination of clonal derivation of cells in MDS, and briefly review the contributions that these analyses have provided to our understanding of the molecular pathogenesis of MDS. The theory behind each of the approaches will be described, as well as the advantages and potential pitfalls of each of the methods. It will be shown that no one method is adequate for evaluation of clonality, and that results of clonality assays must be interpreted with caution, especially in the context of clinical decision making.

B. Methods for Determination of Clonality

There are at least four methods for determining clonal derivation of cells in MDS: (i) cytogenetic analysis, (ii) fluorescence in-situ hybridization (FISH), (iii) characterization of point mutations, loss of heterozygosity (LOH) by microsatellite analysis and specific gene rearrangements, and (iv) X-inactivation–based clonality analysis (see **Table 1**). Other strategies that have proven useful in determination of clonality in lymphoid malignancies such as T and B cell receptor gene rearrangements are not applicable in the myeloid lineage cells that are responsible for MDS.

1. Cytogenetic Analysis

Cytogenetics have been a cornerstone for the characterization of MDS, and have provided valuable clues to the molecular pathogenesis of MDS. As many as 70–80% of patients with MDS have been reported to have clonal karyotypic abnormalities. Patients with MDS may exhibit aneuploidy, in which a whole chromosome is either lost or gained (such as trisomy 8 or monosomy 7), or may have structural abnormalities such as deletions, translocations, isochromosomes or marker chromosomes. Single chromosome aberrations are more common in primary MDS, whereas therapy-related MDS is notable for the complexity of abnormal karyotypes.[1,2]

Myelodysplasia is also notable for a predominance of chromosomal deletions, whereas AML is typified by balanced reciprocal translocations.[1,2] Consistent loss of genetic material in MDS has led to the hypothesis that MDS may be caused by so-called tumor suppressor genes. In this model, one copy of a gene is deleted by chromosomal deletion, and the other copy is inactivated either by point mutation, small deletion, or by changes in expression due to regional methylation.[3] However, thus far this paradigm for MDS has not been proven, in part due to the difficulty in cloning tumor suppressor genes located within the large chromosomal deletions.

Cytogenetic analysis has provided several clues to the molecular pathogenesis of MDS. First, clonal karyotypic markers support the notion that MDS is the consequence of acquired somatic mutation that confers a proliferative growth advantage. Second, in some cases it has been possible to clone mutant

Table 1. Utilities and hazards of various methods of clonality determination in MDS.

	Utilities/advantages	Hazards/problems
Cytogenetics	• abnormalities present in up to 70–80% of MDS patients • provides clues to genomic localization of MDS disease genes	• not diagnostic of MDS • assesses only a small fraction of BM cells
FISH	• can analyze interphase nuclei • allows for identification of the cell type that contains the mutation • can analyze populations of cells	• not diagnostic of MDS • in most cases requires advance knowledge of the mutations (e.g., +8, -7, del(5q), del(20q)
Point mutations and gene rearrangements	• PCR-based • sensitive: can assay minimal residual disease	• requires advance knowledge of mutation/ rearrangement • not quantitative: fraction of cells containing the mutation not known
LOH	• PCR-based • specific: population based analysis that identifies clonally derived cells	• does not assay minimal residual disease
X-inactivation assays	• DNA or RNA PCR-based • can analyze any affected tissue in females • informative in more than 90% of females • requires no prior knowledge of the mutation(s) that cause the MDS • X inactivation can be assessed either by methylation or by transcriptional methods	• need to use embryologically related tissue as controls • nonrandom X-inactivation primary: unequal Lyonization secondary: acquired skewing with age

genes from MDS patients based on abnormal karyotype. And third, cytogenetic analysis provides prognostic information that can be incorporated into the clinical approach to the MDS patients. An international prognostic scoring system (IPSS) has been developed in which a favorable prognosis is associated with del(5q), del(20q), -Y (as sole abnormalities), or normal cytogenetics, whereas more than 3 karyotype abnormalities or monosomy 7 confer a poor prognosis. All other abnormalities confer an intermediate prognosis.[4]

del(5q) is present in approximately 10–15% of de novo MDS patients, and in as many as 50% of therapy-related MDS. del(5q) confers a favorable prognosis, and is frequently associated with anemia that requires blood transfusion.[5] The breakpoints within a large region 5q are highly variable between individual patients, but most investigators agree that the critical region of deletion lies somewhere between 5q31 and 5q33.[6-8] del(20q) also confers a relatively favorable prognosis and is present in about 5% of primary MDS patients. Two markers that flank the deleted region have been identified (D20S174 and D20S17), and although the deletion is still quite large by molecular standards, several putative tumor suppressor genes map to this locus.[9] Monosomy 7 or del(7q) confers a poor prognosis. Although the genes on chromosome 7 that are responsible for the phenotype are not known, a critical region near 7q22.1 has been identified.[10] Finally, a number of less frequent but characteristic regions of loss have been identified in MDS, including del(13q), del(11q) and del(12p). Trisomy of chromosome 8 is the most common numerical abnormality in MDS, but trisomy 6, 13 and 21 have also been reported. The genes responsible for MDS associated with either deletions or numerical abnormalities have not yet been identified.[1]

There has been more success in cloning rare but recurring chromosomal translocations associated with MDS. For example, the MLL (HRX) gene localized to chromosome 11q23 has been implicated in pathogenesis of de novo AML. It has been shown that therapy-related MDS or AML (t-MDS/AML)

patients with 11q23 abnormalities also have involvement of the MLL gene, and that rearrangements of MLL in t-MDS/AML correlate with use of topoisomerase inhibitors (e.g., epipodophyllotoxins). Furthermore, the t(11;16)(q23;p13) is exclusively associated with t-MDS/AML and results in fusion of MLL to the transcriptional co-activator CBP (CREB binding protein).[11,12]

The t(3;21)(q26;q22) translocation, associated with some cases of t-MDS/AML as well as CML in blast crisis, has also been cloned. The consequence of this translocation in t-MDS/AML is fusion of AML1 with one of several fusion partners on chromosome 3, including EAP, EVI1, and MDS1.[13] AML1, also involved in the t(8;21) translocation in de novo AML, contains a highly conserved DNA-binding domain that regulates expression of myeloid-specific genes, including myeloperoxidase and neutrophil elastase. It has been suggested that the t(3;21) and t(8;21) fusions disrupt AML1 function and thereby inhibit early myeloid differentiation. inv(3)(q21;q26) associated with MDS also appears to result in overexpression of the EVI1 gene.[14]

t(5;12)(q33;p13) is a rare recurring translocation associated with the chronic myelomonocytic leukemia (CMML) subtype of MDS. The consequence of the translocation is fusion of the tyrosine kinase domain of the platelet-derived growth factor β receptor (PDGFβR) to a member of the ETS family of transcription factors, TEL. Fusion of TEL to PDGFβR constitutively activates the tyrosine kinase domain of PDGFβR, leading to abnormal myeloid proliferation.[15,16] TEL/PDGFβR causes leukemia in murine bone marrow transplant and transgenic models, and can be treated with a specific inhibitor of the PDGFβR kinase. These findings illustrate the potential for devising new approaches to therapy based on an understanding of the molecular genetic basis of disease.

Potential Pitfalls

There are several potential problems with cytogenetic analysis in MDS. (i) Cytogenetic abnormalities are not diagnostic of MDS. The diagnosis of MDS is made based on the presence of dysplastic morphology in hematopoietic progenitor cells. (ii) Standard cytogenetic banding techniques characterize only a small fraction of the cells in a bone marrow aspirate sample. The cells analyzed must be capable of mitosis to generate a suitable metaphase spread, and only 20–30 metaphases are typically assessed. In cases where all metaphases harbor the same karyotype abnormality, it is reasonable to extrapolate that finding to the remainder of the several million cells provided for cytogenetic analysis. In contrast, it is less certain how to interpret data in which only one, or a small fraction of, metaphases harbor an abnormality. This concern is highlighted by recent observations that some patients undergoing autologous bone marrow transplant for lymphoma or Hodgkin's disease can develop cytogenetic abnormalities that are characteristic of MDS, and yet show no clinical or morphologic evidence of MDS.[17] (iii) Cytogenetic abnormalities probably underestimate the number of genetic abnormalities in MDS cells. As more sophisticated genome-wide techniques have been applied to analysis of MDS cells (such as FISH with chromosome "painting probes," comparative genomic hybridization, and analysis of LOH by using microsatellite markers), it has become clear that standard cytogenetic analysis may grossly underestimate the number of structural abnormalities.

2. Fluorescence In-Situ Hybridization

FISH has provided a valuable and complementary tool for analysis of structural abnormalities of chromosomes in MDS. It is particularly useful when an underlying chromosomal abnormality has been defined (such as trisomy 8 or monosomy 7). FISH probes for aneuploidy (e.g., a FISH probe for chromosome 7) can be used to analyze interphase rather than metaphase nuclei of cells. In monosomy 7, cells will contain a single FISH signal from the remaining chromosome 7, whereas normal cells will contain two signals. Similarly, FISH probes for chromosome 8 would identify three signals in a cell with trisomy 8, compared with two signals in a normal cell. Analysis of interphase nuclei allows for identification of the cell type in which the abnormality occurs and allows for analysis of large populations of cells. FISH may improve the detection of monosomy 7 in MDS[18] and has been used to characterize lineage involvement in MDS. For example, FISH has demonstrated that myeloid cells and B cells, but not T cells, contain monosomy 7 in some patients with MDS.[19] Similarly, analysis of the 5q deletion in MDS has shown that the deletion is restricted to myeloid

cells, and that only a fraction of myeloid progenitors harbor the 5q deletion.[20] These findings, which could not have been made with standard cytogenetic banding techniques, have important implications for the pathogenesis of MDS. In addition to demonstrating lineage restriction in MDS, they suggest that there are normal hematopoietic progenitors present in some patients with MDS.

Potential Pitfalls

As for cytogenetic analysis, FISH abnormalities are not diagnostic of MDS. Perhaps the most significant limitation of the application of FISH is that in most cases it is necessary to know the chromosomal abnormality in advance to choose the appropriate FISH probe. While it may be reasonable, for example, to screen for common deletions or numerical abnormalities in MDS such as del(5q), del(20q), trisomy 8 and monosomy 7, it may not be as useful to routinely screen for less common abnormalities such as +13, +21, or del(17p). In addition, FISH alone will underestimate the number of chromosomal abnormalities since it will only detect abnormalities at the specific site of hybridization of the probe. Thus, FISH and cytogenetics are complementary and overlapping techniques that each have specific advantages and disadvantages in the analysis of MDS.

3. Point Mutations / Gene Rearrangements / LOH

There are numerous examples of both germ line and acquired point mutations that are implicated in the pathogenesis of cancer. Examples include mutations in *BRCA1, BRCA2, p53, RAS, APC, RB* to name only a few. Analysis of point mutations as clonal markers of disease in MDS has proven less fruitful. The most common known point mutations in MDS occur in the *RAS* gene family. Although initial estimates of the frequency of point mutations were quite high, most recent studies report a frequency of approximately 5–10% of cases. Mutations involving p53 are quite rare and are found most commonly in those rare MDS patients with del(17)p, the genomic locale of the p53 gene. Similarly, mutations involving the cyclin-dependent kinase inhibitors p15 and p16 are rare, though loss of function of these proteins have been convincingly implicated in the pathogenesis of other human cancers. Mutations in the *M-CSFRI (c-fms)* gene are also rare.

Although rare, the identification of gene rearrangements that occur as a consequence of chromosomal translocations allows for PCR-based strategies to detect the resultant fusion gene. For example, it is possible to detect the t(5;12) translocation in CMML by using specific primers for the PDGFβR and TEL genes, and this strategy has been widely applied in leukemias where balanced chromosomal translocations are more common, such as the t(8;21), inv(16) and t(12;21).

LOH refers to the loss of genetic material from one allele at a specific genetic locus. LOH is often seen in association with loss of function of tumor suppressor genes[3] and can be assayed by PCR amplification of polymorphic microsatellite markers for a particular gene or locus. For example, the analysis of MDS by using microsatellite markers on 5q has identified a subset of patients who have LOH on 5q but do not have any detectable cytogenetic abnormalities. This approach has allowed investigators to delineate small genomic regions of 5q that are implicated in pathogenesis of MDS.[7] Another advantage of LOH as a clonality assay is that, like the X-inactivation assays described below, it is not an assay for minimal residual disease. Most LOH studies would not detect less than 5% of clonally derived cells against a background of normal cells. Thus, LOH at a given locus identifies a population of cells with a clonal proliferative advantage.

Potential Pitfalls

Two potential problems in the PCR analysis of point mutations or gene rearrangements are (i) the identity of the gene to be analyzed must be known, and (ii) as for any PCR based technique, there is potential for both false positive and false negative results. In addition, most methods for PCR amplification of genes are not quantitative, so they do not, similar to cytogenetic analysis, provide reliable information on the fraction of cells that contain the mutation. This concern is highlighted by recent data that demonstrate that individuals in long-term remission from t(8;21) AML have PCR detectable t(8;21), and that even normal individuals may have PCR detectable translocations such as the t(14;18) or the t(9;22). Thus, the presence of a point mutation or translocation in itself may not provide useful prognostic or diagnostic information. The LOH strategy requires the availability of other somatic tissues as controls

for homozygosity at a polymorphic locus and lacks the sensitivity of PCR for point mutations and specific gene rearrangements to detect minimal residual disease.

4. X-inactivation Clonality Analysis

X-inactivation analysis does not require any prior knowledge of the nature of the mutation that gives rise to clonal proliferation of cells. It simply assays the presence or absence of clonally derived cells. These are NOT minimal residual disease detection assays and will not detect fewer than 10% of clonally derived cells admixed in a background of polyclonal cells. On the other hand, this limitation can be viewed as a strength of the assay that obviates the problem described above for the detection of point mutations. The false positive rate for this assay is quite low, because it will only detect the presence of an acquired somatic mutation that confers clonal proliferative advantage to hematopoietic progenitors. The *sina qua non* of clonal proliferation of cells is that they should comprise the majority of cells present in the sample. If they do not, then the cells either do not have a clonal proliferative advantage or they have been assayed prior to establishing clonal dominance in the bone marrow.

The X-inactivation clonality assays are based on the process of random X-inactivation, or Lyonization, that occurs in females during embryogenesis. The pattern of X-inactivation is faithfully reproduced in all progeny, including tumor cells. Since X-inactivation is accompanied by differential methylation and gene expression between the active and the inactive X chromosome, clonal derivation of cells can be determined by simultaneously discriminating between the (i) paternal vs. maternal copy of the X-chromosome and (ii) active vs. inactive X chromosome. Assays have been developed at a number of X-linked polymorphic loci, accompanied by an analysis either of expression or methylation at that locus. Informative loci on the X-chromosome that utilize differences in methylation between the active and inactive X include the HPRT, PGK and androgen receptor loci. As well, several loci have been characterized that allow for the determination of clonality by using transcriptional analysis of coding polymorphisms. These include the use of coding polymorphisms in the G6PD gene such as C/T1311, the palmitoylated membrane protein p55 G/T 358,[21-23] the iduronate-2-sulfatase (IDS) gene C/T438[24,25] and the androgen receptor locus.[26]

An example is shown in **Figure 1** for the human androgen receptor locus. The human androgen receptor assay (HUMARA) delineates between the paternal and maternal copy of the X-chromosome by using a highly polymorphic CAG expansion repeat that is informative in more than 90% of females.[26] The state of activation of the X-chromosome is determined by using a methylation-sensitive restriction endonuclease (HpaII) that cleaves all of the active (unmethylated) X-chromosomes and prevents their amplification. The read-out for the assay is straightforward: in the presence HpaII, a polyclonal population of cells will show two bands and a clonal population of cells will show one band. Admixtures of clonal and polyclonal cells will give a variable ratio of bands.

There are several useful applications of the X-inactivation assay in MDS. First, it has been demonstrated that MDS is a clonal disorder that is the consequence of acquired somatic mutation. These data complement findings from cytogenetic analysis and FISH analysis. Second, it has been shown that a proportion of MDS patients have preserved polyclonal hematopoiesis.[27,28] Thus, it may be possible to develop therapeutic approaches that target these normal cells (e.g., harvesting of normal progenitors for autologous BMT). Third, several reports have suggested that clonal derivation of hematopoietic cells is an early event in the development of t-MDS/AML and may be predictive of this outcome.[29-32] For example, the X-inactivation clonality assay has been used to analyze female patients undergoing autologous bone marrow transplantation for lymphoma. This patient population is at risk for developing t-MDS/AML, with actuarial risks of developing this life-threatening complication that range from 4–18% at various centers.[33] Several groups have demonstrated that clonal myelopoiesis may anticipate the development of MDS[29-31] and that clonal myelopoiesis is a statistically significant predictor of outcome in MDS.[32] Of note, the X-inactivation clonality assay appears to be complementary to cytogenetics. Clonal patients by X-inactivation assays often have clonal karyotypic abnormalities, but a subset of MDS patients are clonal by X-inactivation but have normal karyotypes, and

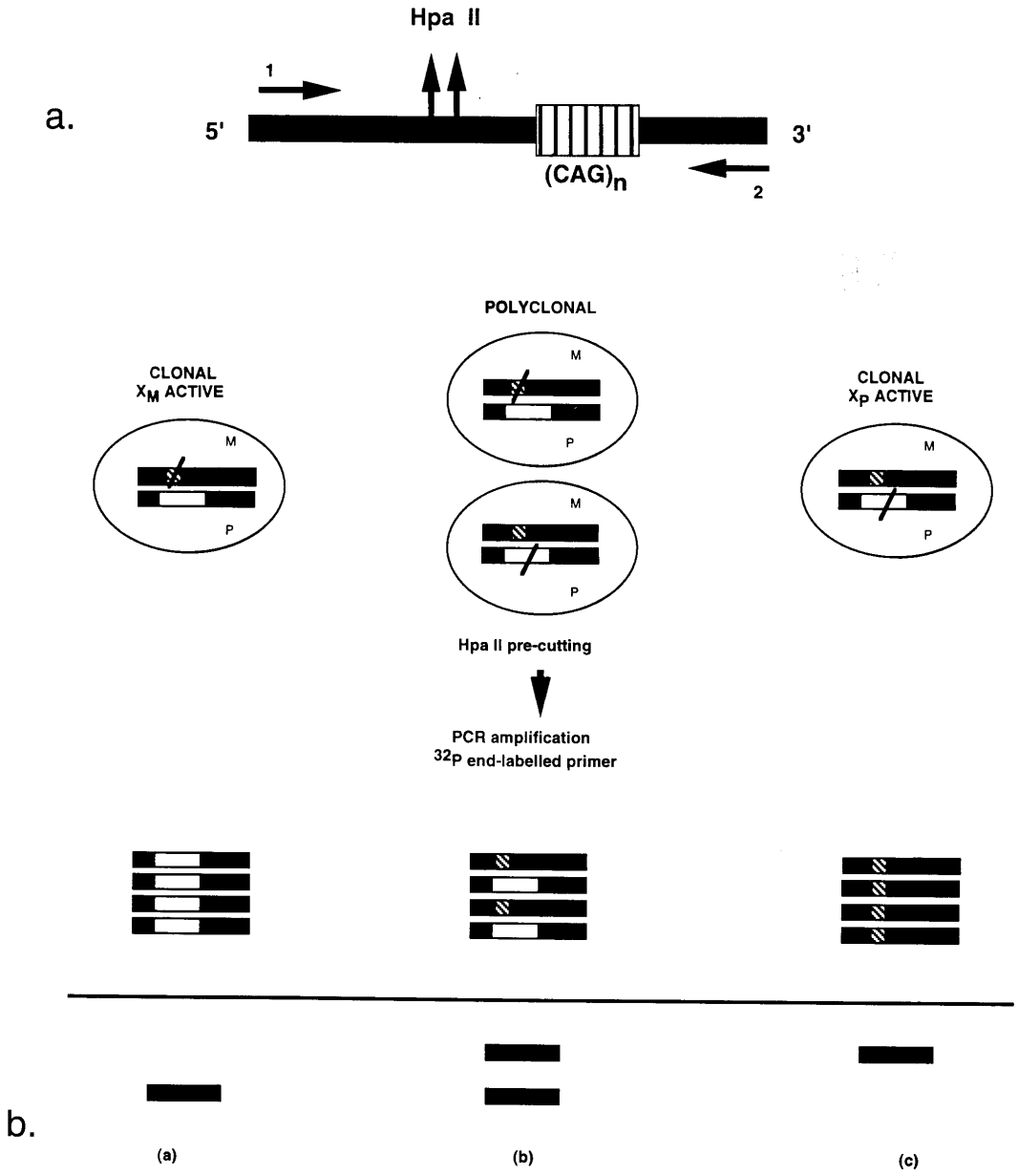

Figure 1. Schematic for analysis of clonality using an X-inactivation assay at the human androgen receptor locus.

From Mach-Pascual et al. Blood 91:4496, 1998.[32] Reprinted with permission.

a. Human androgen receptor locus on X chromosome. The first exon of the human androgen receptor gene contains a highly polymorphic CAG repeat with more than 20 different alleles and a heterozygosity frequency of 90%. One-hundred base pairs 5' of the (CAG)n lies a site of differential methylation that is invariably unmethylated on active X alleles and invariably methylated on inactive alleles. Methylation restriction enzymes such as HpaII will cleave active alleles (indicated by the diagonal through the gene), precluding their amplification with flanking PCR primers 1 and 2.

b. The HUMARA clonality analysis. DNA is digested with HpaII and amplified by PCR using ^{32}P end-labeled or fluorescent primers. In a polyclonal populations of cells, (b), both the maternal (M) and paternal (P) inactive X alleles will be amplified and discriminated as two bands of different molecular weight on a denaturing polyacrylamide gel by the variablilty in length of the polymorphic CAG repeat. In contrast, for a clonal population of cells (a and c), in which all cells are derived from a single common progenitor, only the maternal *or* paternal inactive X allele will be amplified, giving a single band.

some have abnormal karyotypes in a fraction of metaphases but do not have clonal hematopoiesis by X-inactivation assays.[32]

Potential Pitfalls

There are several potential problems and hazards in the application of X-inactivation assays for clonality analysis. First, the assay is only applicable in informative females. Second, X-inactivation assays determine clonal derivation of cells but provide absolutely no clues to the nature of the mutation that causes the clonal proliferation. In contrast, we now recognize that clonal karyotypic abnormalities serve as flags for the genomic localization of genes that cause MDS. A third problem is non-random X-inactivation (NRXI), which can mimic clonal derivation of cells.[34] Primary NRXI, or unequal Lyonization, refers to the proportion of females at birth who have skewed allelic ratios. This could result from the stochastic process of X-inactivation occurring in a limited number of cells early in embryogenesis, but recent evidence suggests that there may also be a genetic cause for primary NRXI. In addition, X-inactivation ratios may vary from tissue to tissue.[35] For this reason, it is important to compare X-inactivation ratios in unaffected but embryologically related tissues. For example, in MDS there is no evidence for T cell involvement in the disease. One can thus use T cells as controls for unequal Lyonization. A second cause of NRXI is the acquired skewing of allelic ratios that occurs with age. The molecular basis for acquired skewing is not yet known, but experimental evidence in cat models of hematopoiesis suggests that one of the X-chromosomes may confer a selective growth advantage to blood cells.[36] Acquired skewing is present in as many as 30% of females over the age of 30, and 40% of females over the age of 60.[37,38] The problem can be compensated for in part by the use of embryologically related Lyonization controls, such as T cells, but this approach assumes that acquired skewing affects T cells at the same rate as myeloid lineage cells.

Fourth, as noted above, there is now evidence that many patients with MDS have at least some preserved polyclonal hematopoiesis.[27,28] This finding may provide an opportunity for various therapeutic interventions but may also confound the interpretation of the assay. That is, a patient may meet all diagnostic criteria for MDS, including the presence of cytogenetic abnormalities in 2 out of 20 metaphases, but have polyclonal hematopoiesis as defined by X-inactivation assays. For example, two of the patients in the aforementioned study of MDS after ABMT for lymphoma developed MDS despite having polyclonal hematopoiesis by this assay.[32] As for any of these assays, the results of X-inactivation clonality assays must be interpreted with caution.

C. Summary

Analysis of clonality has provided valuable insights into the pathogenesis and molecular genetics of MDS. In addition, clonality analysis provides useful clinical prognostic information. X-inactivation clonality assays may predict patients at risk for developing MDS even when no other abnormalities are present, and cytogenetic analysis, in combination with other clinical parameters, provides information on predicted median survival and likelihood of progression to AML. Although each of these approaches to analysis of clonality has strengths and weaknesses, taken together they provide complementary and overlapping data that will provide further insight into molecular pathogenesis of disease, diagnosis, prognosis, and novel therapeutic approaches. *~D.G.G.*

II. Novel Therapeutic Strategies for Myelodysplastic Syndrome

The MDS are relatively common clonal stem cell disorders, the incidence of which exceeds that for AML. Despite their prevalence, progress in the management of these disorders has been limited by their biological and clinical diversity. Morphologic categorization by the French, American and British (FAB) classification provides a reproducible nomenclature for comparison of clinical studies and has demonstrated prognostic utility.[1] Heterogeneity in the hematologic expression of disease and overall survival persists within each of these FAB categories, necessitating further refinement of prognosis risk by the application of scoring systems such as the International Prognostic Scoring System (IPSS), which incorporates variables unique to an individual patient such as bone marrow blast percentage, karyotype, and the number and severity of peripheral

cytopenias.[2] The clinical heterogeneity of the MDS reflects their underlying biological diversity and has yielded new avenues for therapeutic intervention. This review will highlight the current understanding of the pathogenesis of these disorders and recent developments in treatment including the use of trophic or anti-apoptotic agents, pharmacologic differentiating agents, novel chemotherapeutics, and immunosuppressive therapy.

Biologic Features

The ineffective hematopoiesis characteristic of MDS reflects a profound shift in the normal balance of cellular homeostasis, which results in an excessive rate of progenitor loss that exceeds effective blood cell production. Despite often exuberant bone marrow cellularity, the clonogenic growth of multipotent and primitive erythroid progenitors is generally deficient.[3,4] Accumulating evidence indicates that features inherent to the malignant clone and the microenvironment contribute to a net decrease in trophic signals essential for progenitor survival. With the exception of CMML and its unique myeloproliferative features, myelodysplastic bone marrow progenitors are characterized by impaired response to growth factor stimulation despite normal cytokine receptor number and intact ligand binding capacity.[4-7] Phosphorylation of the signal transducer and activator of transcription (STAT5) in response to erythropoietin stimulation and induction of the erythroid-specific transcription factor GATA-1 is deficient in MDS erythroid progenitors.[8] Progenitor growth is augmented by exposure to super-saturating concentrations of recombinant cytokines, supporting an intrinsic disturbance in receptor-signal transduction.[4,9] Although normal erythroid progenitors constitutively express the fas ligand, the fas receptor (CD95) is aberrantly expressed in high density in myelodysplastic CD34+ cells permitting autocrine activation of the cell death program.[10,11] Bone marrow macrophages responsible for engulfment of apoptotic corpses are increased in MDS and offer a paracrine source of fas ligand presentation that may accelerate the activation of programmed death of proximate hematopoietic progenitors.[11,12] A recent report that the intensity of CD95 expression on CD34+ cells inversely correlates with blast number in MDS indicates that myeloblast populations more resistant to fas-induced cell death emerge with disease progression.[13] Overproduction of growth inhibitory cytokines including TNFα, TGFβ, interleukin-1β (IL-1β) and gamma interferon demonstrable in the marrow microenvironment and plasma of patients with MDS may induce CD95 expression in erythroid colony forming cells and further compromise blood cell production.[14-18] Observations that elevations in plasma TNFα correlate with oxidation of nucleotide pyrimidines and depletion of cellular glutathione in CD34+ bone marrow mononuclear cells supports an effector role for these cytokines in the pathogenesis of ineffective hematopoiesis in these disorders.[19] In hypocellular MDS, suppression of hematopoiesis may be compounded by an aberrant immune response. Laboratory studies indicate that T-cell depletion and incubation with cyclosporin-A enhance progenitor recovery in this morphologic variant, confirming an effector role of lymphocytes in the pathogenesis disease.[20] These findings illustrate the biologic diversity presented by MDS and the challenges for therapeutic intervention.

Trophic Agents

The recombinant growth factors are the first therapeutic agents to successfully impact progenitor survival and establish a role in the management of selected patients with MDS. The risk of promoting leukemia transformation, excessive cost, and lineage-restricted activity of the majority of these cytokines have limited their routine application. Amifostine (Ethyol®) is a phosphorylated organic thiol that has hematopoietic trophic effects on myelodysplastic progenitors and has demonstrated activity in preliminary clinical trials. Amifostine is a prodrug that is dephosphorylated by membrane alkaline phosphatase to the intracellular aminothiol, WR1065.[21] The latter agent is recognized for its ability to protect cells from cellular stresses induced by chemotherapy and/or radiation, which results in part from its action as an antioxidant. In cultures of myelodysplastic bone marrow mononuclear cells (MNC), amifostine potentiates the growth of multipotent progenitors and erythroid bursts following brief preincubation exposure.[22] Enhanced progenitor recovery occurs in association with recruitment of CD34+ cells and a corresponding reduc-

tion in the proportion of apoptotic CD34+ MNC.[23] Maturation of myeloid elements appears conserved in amifostine-treated colonies, and cytogenetic or FISH analysis of stimulated clonogenic cells confirms that this agent acts on the myelodysplastic clone. The trophic effects of amifostine and its metabolites relate in large part to their structural similarity to polyamines and their apparent ability to favorably alter the milieu for progenitor growth. WR1065 and its disulfide generated following intracellular oxidation are polycations with structural homology to spermidine and spermine and exhibit overlapping biological effects, which include the suppression of inflammatory cytokine release and alteration of the profile of *bcl*-2 family proteins.[23]

The hematologic effects of amifostine were evaluated in a phase I/II trial in 18 patients with MDS other than CMML.[24] Amifostine was administered intravenously in a three times per week or weekly dose schedule. Single- or multi-lineage hematologic effects were observed in 15 of the patients who received the more frequent dose schedule, with a corresponding improvement in bone marrow progenitor recovery in the majority of responders. Hematologic responses occurred in 43% of patients with thrombocytopenia and 78% of patients with neutropenia. A meaningful reduction in red blood cell transfusions was observed in 33% of transfusion-dependent patients. Treatment was well tolerated at doses ≤200 mg/m^2, whereas nausea was limiting at higher doses. The potential of this agent to accelerate leukemic transformation in patients with relatively high leukemia burden remains of concern. Among six patients with refractory anemia with excess blasts (RAEB) or RAEB in transformation (RAEBt), three experienced a rise in blast percentage or evolution to acute leukemia during study treatment. A multicenter phase II study is in progress to further define the biologic activity of amifostine in MDS, the optimal dose and schedule, and response durability. Although approximately 70% of the drug is bioavailable when administered subcutaneously, a recent phase II trial in patients with MDS suggests that biologic activity with this route of administration may be lower.[25] The capacity of amifostine to alter an otherwise unfavorable microenvironment for progenitor survival makes it an attractive agent to incorporate into combination therapy. Several trials now underway are evaluating its potential application with novel cytotoxic agents, hematopoietic growth factors, and pharmacologic agents.

Pharmacologic Differentiating Agents

Pharmacologic induction of differentiation of neoplastic progenitors is a conceptually appealing therapeutic strategy in MDS. Agents such as hexamethylene bisacetamide (HMBA) and butyrate, induce a sustained activation of STAT5, cell cycle arrest in G_1, and erythroid differentiation of leukemia cell lines.[26-28] In clinical trials, however, HMBA yielded largely myelosuppression owing to nonselective inhibition of progenitor growth.[29] Investigations of sodium phenylbutyrate are ongoing, and preliminary results indicate hematologic activity in MDS.[30] The pyrimidine analog, 5-azacytidine (5-Aza), inhibits DNA methyltransferase activity at low concentrations, which leads to hypomethylation of cytosine residues.[31] Aberrant methylation of promoter CpG islands contributes to repression of gene transcription in human malignancies. Demethylation of these regulatory elements results in activation of previously silenced genes and differentiation of leukemia cell lines. In two phase II studies performed by the Cancer and Leukemia Group B (CALGB), 5-Aza yielded hematologic improvement in over 40% of MDS patients treated with continuous intravenous infusion or subcutaneous drug administration.[32,33] The CALGB recently reported the results of a prospective randomized trial comparing treatment with 5-Aza to supportive care in 191 patients with MDS.[34] Patients were prospectively stratified according to FAB category and symptomatic cytopenias. Patients randomized to the observation arm were eligible for crossover to treatment with 5-Aza upon disease progression. Treatment with subcutaneous 5-Aza at a dosage of 75 mg/m^2/d for seven consecutive days each month experienced more frequent hematologic responses (66% versus 7%; $p < 0.0001$), a corresponding reduction in the frequency of transformation to AML, and a prolongation in the interval to leukemia transformation or death (22 vs 12 months). Quality of life as measured by physical function, symptoms, and psychologic state were improved in patients treated with 5-Aza.[35] The impact of 5-Aza treatment on the natural history of disease and overall survival, although difficult to

assess because of the crossover design of the study, is encouraging. This study confirmed that 5-Aza has significant hematologic activity in MDS and can be administered to the majority of patients with limited toxicity, and therefore merits consideration in patients with high-risk disease who are not candidates for alternate clinical trials. Whether 5-Aza promotes differentiation in vivo remains in question. Myelosuppression and transfusion frequency were increased in patients treated with 5-Aza, suggesting that this agent, like those that preceded it, exerts it hematologic benefit principally by clonal suppression.

Low-Intensity Cytoreduction

Effective and sustained suppression of the myelodysplastic clone represents a treatment strategy with the greatest potential to alter the natural history of the disease. This type of intervention is particularly attractive for FAB subtypes with either high leukemia burden, such as RAEBt, or myeloproliferative features, such as CMML. Because of the advanced age of patients with MDS, a number of low-intensity treatments have been investigated. Low-dose cytarabine represents the prototype of this approach and yielded response rates ranging from 10–25% in phase II trials, but offered marginal benefit over supportive care in a National Cancer Institute-sponsored intergroup study.[36] Based on promising activity in small case studies, oral etoposide was compared to treatment with hydroxyurea in a randomized crossover trial in patients with CMML in a French cooperative group study.[37] Patients received treatment with oral etoposide at a dosage of 50 mg 3 times/week or hydroxyurea 1 g/day, with allowance for hydroxyurea dose adjustment as necessary. Patients treated with oral etoposide had an inferior response rate (36%) compared to hydroxyurea (60%), with a corresponding lower overall survival (20 months vs 9 months). Although a recent study suggests that an extended schedule of etoposide administration might improve its remitting activity,[38] newer agents have demonstrated greater clinical promise.

Topotecan is a topoisomerase I inhibitor with broad antitumor activity that was recently approved by the Food and Drug Administration. In an initial trial reported from the MD Anderson Cancer Center, treatment with topotecan at a dosage of 2 mg/m^2/day by continuous 5-day intravenous infusion yielded a high rate of complete hematologic and cytogenetic remissions in patients with CMML and RAEB.[39] Overall, 13 (28%) of the 47 patients enrolled in the trial achieved a complete response, with an additional six patients (13%) experiencing hematologic improvement. Remission rates were not influenced by FAB type, and remitting patients with cytogenetic abnormalities were restored to a normal karyotype. Toxicities were significant with mucositis occurring in 64% of patients, documented infections in 47%, and a 19% treatment-related mortality. Although the median remission duration was 7.5 months, survival at one year was 38%, suggesting that this agent may have sustained remitting activity in selected patients. Based upon in vitro synergy with cytarabine, topotecan (1.25 mg/m^2/day) was combined with cytarabine (1 g/m^2/d for 5 days) in a subsequent study.[40] Among the initial 35 patients enrolled in the study, 22 (63%) achieved a complete hematologic remission, 19 occurring after one course of therapy. The remission rate was higher in patients with RAEB or RAEBt (75%) compared to patients with CMML (43%), but response was not impacted by cytogenetic pattern. Although documented infections were noted in 48% of patients, hypoplastic deaths occurred in only 2 (6%) patients. Although the intensity of this type of treatment approximates that of conventional AML induction regimens, the preliminary results of this study suggest that the remitting potential of this combination may be greater with possibly lower treatment-related mortality compared to conventional regimens.

Immunosuppressive Therapy

Recognition that immunologic suppression of progenitor growth may contribute to ineffective hematopoiesis in hypocellular MDS provided impetus for recent trials testing the benefit of immunosuppressive therapy. Sustained hematologic responses were reported in 40–60% of patients with the hypocellular variant treated with either cyclosporin-A or antithymocyte globulin (ATG) in limited case series.[20,42] The benefit of this treatment approach, however, is not restricted to patients with reduced bone marrow cellularity. Investigators at the National Institutes

of Health treated 25 transfusion-dependent patients with refractory anemia or RAEB with ATG at a dosage of 40 mg/kg/d for four consecutive days.[41] Eleven (44%) patients achieved transfusion independence, 3 patients achieved a complete or trilineage hematologic response, and 8 patients had significant hematologic improvement. Although the median duration of response was 10 months, overall survival at 38 months follow-up was 84%. Hematologic response to ATG was associated with loss of MHC-restricted inhibition of myeloid progenitors mediated by CD8+ T-lymphocytes.[44] These studies indicate that immunosuppressive therapy with agents such as ATG or cyclosporin-A may eliminate T-cell clones contributing to ineffective hematopoiesis and yield sustained hematologic responses in selected patients with MDS, and may warrant a therapeutic trial in patients with severe cytopenias.

Future Directions

The advanced age of patients with MDS and the heterogeneity in the pathogenetic and clinical features of the disease mandate that therapeutic options be individualized. In candidates selected by prognostic risk and disease features, treatment with remitting agents may impact the natural history of disease. The survival benefit of higher intensity regimens awaits evaluation of randomized clinical trials. Newer strategies that exploit inherent biological features of the disease offer the prospect for improved disease-specific therapy. These include agents such as the farnesyl transferase inhibitors, which offer the prospect to block oncogenic *ras* mutations, and interleukin-10, which suppresses cytokine elaboration and the spontaneous growth of CMML myeloid progenitors.[43] We are approaching a new era in hematology in which patients with MDS may be offered a growing number of treatment alternatives with established clinical benefit. *~A.F.L.*

III. Autologous Stem Cell Transplantation for Patients with Myelodysplastic Syndrome and Acute Myeloid Leukemia after MDS

The myelodysplastic syndromes are a heterogeneous group of disorders with a variable prognosis. The outlook of patients with RAEB, RAEBt, therapy-related myelodysplastic syndromes, or secondary AML (sAML) is poor, if untreated, with median survival durations of less than 12 months.[1,2] If a histocompatible sibling donor is available, the treatment of choice for young patients (e.g., under 55 years of age) with MDS is allogeneic bone marrow transplantation. This treatment results in a long-term disease-free survival rate of approximately 40%.[3-6] Patients who undergo allogeneic transplantation in the phase of refractory anemia or RAEB have a better outcome, due to a lower relapse rate, than do patients with RAEBt or sAML. For patients who are not eligible for allogeneic stem cell transplantation, intensive chemotherapy followed by autologous stem cell transplantation (ASCT) may provide an alternative therapy.[7-11]

Intensive Chemotherpy

Patients with poor risk features who are below the age of 65 years may be candidates for treatment with combination chemotherapy. Chemotherapy, such as that applied to induce complete remission in de-novo AML, showed complete remission rates varying from 15% to 64%.[1,2,7,12,13] The overall complete remission rates of patients with MDS or secondary leukemia are usually lower than those of patients with de-novo AML treated with similar chemotherapy regimens. However, patients with RAEB, RAEBt, and de-novo AML showed no difference in treatment outcome, when matched for prognostic factors such as a history of cytopenias and cytogenetics.[14,15] This result may reflect a conflict in defining the distinction between MDS and de-novo AML. A patient with the morphological picture of RAEBt but without cytogenetic abnormalities and a short history of cytopenias may respond to intensive antileukemic therapy as an average patient with de-novo AML. On the other hand, a patient with de-novo AML, trilineage dysplasia and a monosomy 7 may respond as an average patient with MDS.[15]

Maintaining remission after remission induction-chemotherapy is a difficult issue. Patients who are not eligible for allogeneic bone marrow transplantation may be treated with post-remission chemotherapy. Some patients may achieve prolonged, disease-free survival with this approach,[16,17] but overall median remission duration is usually less

than 12 months.[3,18,19] Patients without cytogenetic abnormalities appear to have a better outcome after antileukemic chemotherapy compared to patients with cytogenetic abnormalities.[1]

Autologous Stem Cell Transplantation

Until recently, the experience with autologous stem cell transplantation in patients with myelodysplastic syndromes or leukemia secondary to MDS was limited to case reports.[8-10] Laporte reported the results of autologous bone marrow transplantation with mafosfamide-treated marrow in patients with AML following MDS. The hematopoietic engraftment was slow in these seven patients, but all patients engrafted except for one, who died of treatment-related causes before engrafting.[11] The leukemia cooperative groups of the EORTC, EBMT, GIMEMA, and SAKK initiated a clinical trial to assess the efficacy of intensive remission-induction and consolidation chemotherapy, followed by ASCT or allogeneic bone marrow transplantation in a prospective study. All patients with a donor were candidates for allogeneic bone marrow transplantation either in first complete remission (CR) after one intensive consolidation course or as salvage therapy if no CR was achieved. The remaining patients in CR were candidates for ASCT. Entry into the study finished in March 1997 with 197 patients registered. Sufficient follow-up is available from 177 patients. Ninety-seven patients achieved complete remission (55%). Thirty-three of the 59 CR patients (56%) without a donor received an autograft. This interim analysis shows that the majority of patients without a donor who achieve CR may be treated by autologous stem cell transplantation following one intensive consolidation course.[20]

The EBMT reported the results for 79 patients with MDS/sAML transplanted with autologous marrow in first CR.[21] The two-year survival, disease-free survival (DFS) and relapse risk were 39%, 34% and 64%, respectively. Within the group of 79 patients, a cohort of 55 patients for whom the duration of first CR was known were compared with a matched control group of 110 patients with de-novo AML. The DFS at 2 years was 28% for the cohort of 55 patients transplanted for MDS/sAML and 51% for those transplanted for de-novo AML (p=0.025). The relapse rates were 69% for patients with MDS/sAML and 40% for those with de-novo AML (p=0.007). Autologous bone marrow transplantation for MDS and secondary leukemia resulted in a lower DFS than autologous bone marrow transplantation for de-novo AML. This difference was mainly due to a higher relapse rate in the MDS/sAML group since the treatment-related mortality rate was low in both patient groups. The higher relapse rate in patients treated for MDS or secondary leukemia suggests a higher burden of residual disease in these patients. For that reason it is important to monitor carefully residual disease in future studies both by cytogenetic techniques and by molecular techniques.

An update of this analysis[21] was performed on 173 recipients of autologous stem cell grafts with MDS or sAML who have been reported to the registries of the EBMT. The three-year DFS of the 126 patients transplanted in first CR was 33% and the actuarial relapse rate was 55%. Patients younger than 40 years had a better DFS compared to patients older than 40. This difference could be explained by the higher treatment-related mortality of 39% in the older age group compared to a treatment-related mortality of 17% in patients younger than 40 years (**Table 2**). The number of patients is too small and the follow-up is too short to assess the impact of autologous stem cell transplantation on subgroups of patients with MDS stratified according to prognostic factors, such as cytogenetics and FAB classification.

Because myelodysplastic syndromes are clonal stem cell disorders, there may be concern about the presence of sufficient numbers of residual normal stem cells to perform autologous stem cell transplantation. However, chemotherapy induces cytogenetically normal complete remissions in the majority of patients with cytogenetic markers of the malignant clone.[1] In addition, Delforge et al. demonstrated that the peripheral stem cell harvests of three patients with MDS were polyclonal when assessed by PCR techniques based on X-chromosome inactivation patterns.[22] Demuynck et al. investigated the ability to collect peripheral blood stem cells in 11 patients with myelodysplasia in complete remission after chemotherapy. Seven patients yielded adequate numbers ($>1 \times 10^6$/kg b.w.) of CD34+ cells.[23] Six patients have received autologous peripheral blood stem cells in the above-mentioned prospective study.[20,24] Preliminary data in-

Table 2: Three-year actuarial probability of disease-free survival (DFS), survival, treatment-related mortality (TRM), and relapse of MDS or sAML patients treated with autologous transplantation reported to EBMT registries. The influence of age and remission status on treatment outcome.

Autologous Transplants	Number	DFS %	Survival %	TRM %	Relapse %
All patients	173	30	32	29	58
CR-1	126	33	38	25	55
no CR-1	47	18	14	51	64
p-value		0.06	0.01	0.07	0.32
< 20 years*	12	46	58	17	44
20–40*	48	36	41	15	58
> 40 years*	66	29	29	39	51
p-value		0.08	0.05	0.22	0.27

* only patients in first complete remission

dicate that repopulation after autologous peripheral blood stem cell transplantation was much faster compared to autologous bone marrow transplantation.[23,24] In a recent study,[25] peripheral blood stem cells were mobilized and collected during the recovery phase of the remission-induction therapy in nine patients with MDS or sAML. All patients had a cytogenetic marker of the malignant clone. In six out of nine patients the stem cell product was entirely karyotypically normal. Three patients were autografted. The engraftment was relatively fast for this category of patients but follow-up was too short to evaluate relapse-free survival.

Autologous stem cell transplantation has emerged as a treatment option for patients with MDS or AML evolved from MDS. Only patients who are in complete remission after intensive chemotherapy are considered as candidates for autologous stem cell transplantation. About one-quarter of the patients who undergo autologous stem cell transplantation may be free of disease for three years or longer. The high relapse rate contributes substantially to this result, which is inferior to the results obtained by transplantation for de-novo AML. Prospective, multicenter studies may reveal which categories of patients with MDS will benefit from intensive chemotherapy followed by autologous stem cell transplantation. This treatment approach has to be assessed against the merits and disadvantages of allogeneic stem cell transplantation with donors other than HLA-identical siblings.~*T.M.deW.*

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III. Autologous Stem Cell Transplantation for Patients with Myelodysplastic Syndrome and Acute Myeloid Leukemia after MDS

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