Myelodysplastic Syndromes: Review of Pathophysiology and Current Novel Treatment Approaches

E.D. Warlick* and B.D. Smith

Hematologic Malignancies Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins 1650 Orleans Street, room 246 Baltimore, MD 21231, USA

Abstract: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of hematopoietic progenitors manifest by cytopenias, bleeding, infection, and potential for progression to acute myelogenous leukemia. The wide spectrum of clinical manifestations, including variability in illness severity and potential for progression, suggest that myelodysplastic syndromes encompass a multitude of disorders, likely involving numerous pathologic pathways. In fact, it is the effort to understand the underlying biology of these syndromes that has led to recent advances in treatment approaches, including the FDA approval of three new agents (5-azacitidine, decitabine, and lenalidomide) for the treatment of MDS. This review will present data supporting each of the current pathophysiologic pathways implicated in the development and progression of MDS; summarize the emerging clinical paradigms for treating patients with MDS; and offer insights into several novel approaches attempting to improve treatment options for future MDS patients.

Keywords: Myelodysplastic syndrome, pathophysiology, differentiation therapy, epigenetic therapy, bone marrow transplantation, immunomodulation.

INTRODUCTION

Myelodysplastic syndromes (MDS) are a complex and heterogeneous group of bone marrow failure disorders characterized by ineffective hematopoiesis. This dysfunctional blood cell production is manifest by peripheral cytopenias, marked morphologic dysplasia, and cellular dysfunction resulting in an increased risk of infection and need for transfusions in most MDS patients. In the general population, the incidence of MDS is approximately 2-10 cases per 100,000 people with the incidence rising to approximately 50 cases per 100,000 people over age 70 [1]. The median age at presentation approaches 70 with a slight male predominance of unknown clinical significance. Traditionally, MDS is characterized as either primary (idiopathic) or secondary with exposure to benzene [2], occupational chemicals [3], or prior treatment with radiation [4] or chemotherapy agents [5] well documented secondary causes. Additional data implicates exposure to tobacco [6], excessive alcohol [7], viral infections [8], or autoimmune disorders [9] as potential associations with MDS.

Alterations in many individual biologic pathways have been implicated in MDS pathophysiology. However, the generally accepted primary hypotheses involves an initial deleterious genetic event within a hematopoietic stem cell, subsequent development of excessive cytokines/inflammatory response leading to a pro-apoptotic/proliferative state, and resultant peripheral cytopenias despite a hypercellular bone marrow. The presence of detectable cytogenetic abnormalities in approximately 40-70% of patients with primary MDS and over 80% with secondary MDS [10] as well as the validated prognostic value of cytogenetic data in MDS supports the theory of an incident genetic event. Specifically, good prognostic cytogenetic groups (“5q minus syndrome” or deletion of the long arm of chromosome 20 (20q)) are typically associated with more indolent MDS that requires less intensive therapy while poor prognostic cytogenetic groups (loss of chromosome 5 or 7 or complex abnormalities) have a more aggressive course and are often poorly responsive even to aggressive treatments [11]. The variable response to treatment and course of disease correlating with cytogenetic findings suggests likely differential behavior of the MDS clone: differentiation arrest and a pro-apoptotic cytokine state in low risk cytogenetic cohorts versus a state of proliferative advantage, possibly due to underlying genomic instability or via altered gene expression silencing of DNA repair or tumor suppressor genes, in high risk cohorts [12-14]. Additionally, altered immune surveillance potentially creates an environment where the transformation to acute leukemia becomes possible [15]. The pathophysiology of “low” and “high” grade MDS is slowly being incorporated into the evolving classification hierarchies as well as treatment paradigms.

CLASSIFICATION SYSTEMS

Throughout the years, various classification systems have been developed to describe myelodysplastic syndromes. There is no clear “best” schema, yet the numerous revisions and expansions based on the ongoing understanding of MDS pathophysiology and clinical outcomes are notable improvements.

FAB Morphologic Classification

The initial FAB (French-American British) system was developed by an international group of clinicians and pathologists in the mid 1970’s, refined in 1982, and primarily used cellular morphology to define the MDS subgroups focusing on bone marrow blast percentage, the presence or absence of monocytosis, and the presence or absence of ringed sideroblasts. These morphologic findings, aligned with important prognostic outcomes, were eventually divided into four groups: 1) Lower-risk MDS (RA and RARS) with a median survival of 4-5 years and low risk of AML transformation; 2) Intermediate-risk MDS (RAEB) with a median survival close to 1 year; 3) Higher-risk MDS (RAEBt) with a median survival measured in months and a very high risk of AML transformation; and 4) CMML associated with a wildly variable risk [16,17]. The FAB classification served as the first framework to systematically identify and describe the different clinical entities of MDS. However, the FAB system was limited by lack of cytogenetic and molecular information now commonly available, and did not adequately accommodate hypoplastic MDS, MDS with myelofibrosis, and other overlap syndromes [18, 19].

WHO Classification

More recently, in 1999, the World Health Organization (WHO) proposed a newer classification system attempting to address some of the limitations of the FAB system [20]. The main goal of this schema was to formally incorporate biologic data, specifically cytogenetic and molecular findings, clinical outcomes, and number of dysplastic lineages involved, in order to better separate subgroups and delineate syndromes within the previous FAB classification. Notably, the WHO classification recognized the unique “5q- syndrome” and CMML within their own categories, lowered the blast percentage defining AML to 20% and eliminated the RAEBt category, assigned
patients with characteristic AML-associated chromosomal abnormalities to AML regardless of blast percentage, and used the percentage of marrow blasts to divide RAEB into two groups noting that patients with >10% blasts had a shorter survival and increased transformation to acute leukemia compared to patients with <10% [20]. The WHO system also distinguished MDS patients with <5% marrow blasts between those with multilineage dysplasia (worse outcome) and those with erythroid-only dysplasia (better outcome), and added an “unclassifiable MDS” category [19-21]. Although the WHO classification system built on the FAB foundation by including important biologic and clinical data, there remained some obvious shortcomings such as the “MDS-unclassifiable” category and controversies such as the elimination of RAEBt [18]. These concerns epitomize the larger problem that “MDS” is truly a pleomorphic “grab bag” term for multiple disorders that offer significant challenges to both physicians treating patients as well as scientists studying these disorders.

**IPSS Prognostic Classification**

In 1997, Peter Greenberg and colleagues proposed a plan to combine known prognostic variables with outcomes data from seven large, risk-based studies in an effort to determine the most important variables for assigning prognosis to MDS patients. The International Prognostic Scoring System (IPSS) emerged from the analysis of this data from nearly 900 MDS patients and represents the best effort to date for prediction of prognosis and outcome. The variables most predictive of prognosis included percentage bone marrow blasts, cytogenetic abnormalities, and number of peripheral cytopenias. Subsequent IPSS score calculation then assigns patients to one of four risk groups: Low, Intermediate-1 (INT-1), Intermediate-2 (INT-2), and High risk with decreasing survival and increasing risk of AML progression as the score increases [22]. Like each of the classification schemas, the IPSS also has limitations including failure to offer prognostic points for treatment-related MDS (t-MDS) or CMML, and lack of scoring for most of the cytogenetic abnormalities commonly found in MDS. These limitations aside, the IPSS remains a paramount tool in predicting prognosis, categorizing patients for studies, and analyzing treatment outcomes. In combination with the other classification systems, it represents the most quantitative assignment of MDS risk available.

**THEORIES IN MDS PATHOPHYSIOLOGY**

Better understanding of the underlying MDS biology is crucial for the development of better treatments for individual patients. It is commonly accepted that MDS develops through a multi-step process encompassing changes within the hematopoietic stem cell, the bone marrow microenvironment, and the complex interactions between the two [1, 15, 18, 23, 24]. Below we survey specific theories and pathways implicated in the pathogenesis and progression of MDS divided into five major categories: 1) cellular damage related to toxic environmental exposures and cellular aging; 2) genetic alterations including loss or gain of function due to both cytogenetic and epigenetic abnormalities (Table I); 3) changes in the bone marrow microenvironment; 4) dysregulation of the immune system; and 5) altered cell cycle regulation and blocked differentiation. (Theories Summarized in Table II)

**Cellular Damage: Toxic Environmental Exposures and Cellular Aging**

The epidemiology of MDS has offered pivotal links between various environmental exposures, aging, and the development of disease, namely the accumulation of DNA damage. Numerous environmental exposures have been associated with the development of MDS including tobacco [6] and alcohol use [7], infections [8], or auto-immune disorders [9]; however, the classic example linking an environmental toxin exposure to MDS development is highlighted from the observational case control studies involving benzene, an aromatic hydrocarbon and organic solvent derived from petroleum-refining, used in many occupational compounds [25]. The association, first described in Turkish shoe workers who developed bone marrow failure/pancytopenia states, [26] lead to further prospective cohort studies involving 74,828 benzene exposed subjects in China and revealed a significantly increased relative risk of MDS development approaching infinity [2]. The potential mechanisms of MDS development in benzene-exposed individuals have been described in two contexts: genotoxic and non-genotoxic. Genotoxic mechanisms are characterized by the generation of oxygen free radicals from benzene metabolites with subsequent DNA damage and apoptosis [27, 28]. Support for this mechanism includes the findings of chromosomal abnormalities (trisomy (+9), deletions (-5 or -7) and translocations [t (8, 21)], oncogene mutations, and somatic mutations in benzene-exposed MDS patients [29-31]. Additionally, as increasing somatic mutations may occur with aging, exposure to benzene and other directly genotoxic agents could lead to more significant damage that progresses to clonal expansion and MDS [31]. Suggested non-genotoxic mechanisms include alteration of the bone marrow microenvironment and immune function through reduced immunoglobulin and complement levels [31].

Radiation and prior chemotherapy are other well documented exposures that can lead to the development of MDS [24, 23, 31]. Specifically, chemotherapy-associated MDS has been associated with treatment using Topoisomerase II inhibitors, anthracyclines, and alkylating agents. MDS from exposure to Topoisomerase II inhibitors and anthracyclines typically has an early onset within 1-3 years and causes balanced genetic alterations typically involving 11q23 (Mixed Lineage leukemia, MLL gene) [5] while exposure to alkylating agents results in a later onset within 5-10 years and yields unbalanced chromosomal alterations often involving chromosomes 5 and 7 [10, 32]. With regard to radiation, epidemiologic studies of atomic bomb survivors have illustrated the connection between previous radiation exposure and leukemia/MDS development, [33] and have characterized the importance of exposure dose and duration [34]. Specific effects and chromosomal abnormalities, additionally, seem to be similar to those seen with exposure to alkylating agents [4].

With a median age of newly diagnosed MDS patients nearing 70, the importance of aging in MDS is clear, yet the specific role it plays biologically remains under investigation [1]. It is theorized that aging itself may produce increased genetic mutations and alterations that result in MDS. Comparing bone marrow from normal controls and older patients is one approach to highlighting potential similarities and differences that could explain the increased incidence of MDS in the elderly. Ogawa et al. evaluated the bone marrows of 100 normal controls of a variety of ages [35] and found increased apoptosis and decreased cellularity in patients of advanced age [36] with apoptosis levels similar in degree to MDS patients. Other studies describing MDS marrows detail a skew in specific TNF-alpha receptor expression leading to increased rates of apoptosis comparable to that seen in normal elderly marrows. While the increased apoptosis in both normal elderly marrows and MDS patients may have distinct biologic causes, the finding may also suggest a reason for the preponderance of increased MDS in the elderly [37].

Another biologic link between aging, stem cell damage, and cancers such as MDS includes the study of telomerases and telomerase activity. Telomeres are simple tandem repeats at the ends of chromosomes in somatic cells that shorten with age and are also found to be shortened in many cancers [38-40]. The shortening is theorized to result from incomplete DNA replication leading to genomic instability, subsequent chromosomal abnormalities, and eventual mutations impacting disease progression once a certain threshold is breeched. Telomerase is the enzyme required for the replication of telomerases, and although not typically found in most somatic cells, has been found in over 85% of primary malignancies [41]. Telomerase may not be required for initiation of malignancy but
### Table I.  Cytogenetic Abnormalities in MDS

<table>
<thead>
<tr>
<th>Chromosomal Alteration</th>
<th>Incidence (Approximate)</th>
<th>Associations: Exposures / Other Disorders</th>
<th>Classic Clinical or Morphologic Description</th>
<th>Prognostic Impact</th>
<th>Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 5 Abnormalities</td>
<td>- 10-20% de novo MDS cases - Tx-related in 40% of cases [31]</td>
<td>-Chemical Exposure -Chemotherapy [49]</td>
<td>Clinical: -5q alone/5q syndrome = good/lower risk of AML transformation [47, 50, 51] - Complex / other abn of 5 / t-MDS = poor prognosis</td>
<td>- 5q CDS: genes for cell cycle &amp; cellular cytokine receptors - No specific gene loss yet identified [52-57]</td>
<td></td>
</tr>
<tr>
<td>Loss of Y</td>
<td>Associated w/ not diagnostic of MDS [58]</td>
<td>- Numerous cancers - Normal Aging population [11, 58]</td>
<td>Favorable prognosis if isolated abn [22]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 7 Abnormalities</td>
<td>-5% de novo MDS cases - Tx-related in 55% of cases [11]</td>
<td>Associated w/ AML, MDS, NF-1, Fanconi’s anemia, Down’s Syndrome [62]</td>
<td>Poor risk dz w/ high progression to AML [63, 58]</td>
<td>- Monosomy 7 uniformly bad regardless of dz - CDS (7q22 and 7q22-33) found but often fail 7 loss so difficult to study [64, 62]</td>
<td></td>
</tr>
<tr>
<td>17p Syndrome</td>
<td>- 5% of MDS cases [58] - Tx-related in 33% of cases [65]</td>
<td></td>
<td>Morphologic: Dyserthropoiesis, pseudo-pelger huet cells, hypogranularity [48]</td>
<td>- Often tx related = poor prognosis - Often seen w/ other abn (5 and 7) = poorer prognosis [11, 58, 65] - Loss of p53 function - Due to point mutation in p53 at 17p13 in 70% pts [58]</td>
<td></td>
</tr>
<tr>
<td>3q Abnormalities</td>
<td>-2% of MDS cases [58] - Typically tx-related [58]</td>
<td></td>
<td></td>
<td>- EVI-1 gene (3q26) alterations: - Interference w/ 3q26 transcription linked w/ abnormal differentiation: may interfere w/ GATA-1, (erythocyte differentiation) [58]</td>
<td></td>
</tr>
<tr>
<td>Complex Cytogenetics</td>
<td>- 10-20% of de novo MDS cases - Tx-related in 90% [67]</td>
<td></td>
<td>Only partial correlation w/ outcome: poor prognosis [67]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Oncogene/Tumor Suppressor Abnormalities

<table>
<thead>
<tr>
<th>Frequency of Abnormality in MDS Patients</th>
<th>Specifics of Abnormality</th>
<th>Prognostic Implications</th>
<th>Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncogene/Tumor Suppressor Function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ras</td>
<td>Family of genes encoding GTP-binding proteins: role in signal transduction</td>
<td>- Paquette Study of 220 MDS Pts – 9% w/ n-ras mutations [72]</td>
<td>- Point Mutations [73]</td>
</tr>
<tr>
<td>p53</td>
<td>Control of DNA replication repair: crucial for maintaining genome [74]</td>
<td>- In 69% of pts w/ 17p deletion [75]</td>
<td>- N-ras mutations assoc. w/ shorter survival: Shorter time to leukemia development [76, 77]</td>
</tr>
</tbody>
</table>

Legend: MDS = Myelodysplastic Syndrome; Tx = treatment; NI = normal; AML = Acute Myelogenous Leukemia; t-MDS = treatment related MDS; CDS = Commonly Deleted Segment; w/ = with; Freq. = frequency; Del = deletion; Abn = abnormality; NI-1 = neurofibromatosis 1; Dz = Disease; FAB = French American British; Assoc. = Associated; MLL = multi-lineage leukemia; Pts = patients; w/ - within
may be important in tumor propagation [42]. Interestingly, telomerase activity is found in cell populations with high renewal requirements such as hematopoietic stem cells [43]. In MDS, studies evaluating telomeres and telomerase activity show loss of the typical correlation between telomere length and age: specifically, decreasing telomere length with increasing age [44]. Further studies have found an association between shortened telomeres and abnormal cytogenetics, where progressively shortening telomeres correlated with worsening IPSS risk group [44]. Telomerase activity has also been assessed in MDS: high levels of activity have not commonly been found. The culmination of this data may suggest that the hematopoietic stem cells in MDS patients have insufficient telomerase to adequately restore cellular telomeres to their normal lengths subsequently resulting in increased genetic instability [44].

**Gain or Loss of Gene Expression: Cytogenetic and Epigenetic Alterations**

One of the common end results of exposure to environmental toxins is DNA damage and the loss or alteration of gene expression. Yet, chromosomal abnormalities are often detected in MDS that are not associated with such exposures. In addition, newer techniques, including comparative genomic hybridization (CGH) and single nucleotide polymorphisms (SNP) arrays, now allow the detection of even microscopic deletions of genes and continue to fuel the understanding of MDS and its pathophysiology. Finally, it is now clear that epigenetic gene silencing plays a role in loss or gain of gene expression even when the DNA sequence is completely intact.

Clonal cytogenetic abnormalities (Table 1) are found in 30-70% of patients with primary myelodysplastic syndrome and in over 80% of treatment-related MDS and remain the most important individual prognostic tool [11, 45-47]. The majority of the chromosomal abnormalities in primary MDS involve unbalanced losses: entire chromosomal loss or point mutations [11, 45]. Consequently, it is easy to invoke such abnormalities resulting in the loss or inactivation of a critical tumor suppressor protein; however, efforts to determine a culprit gene or cluster of genes have been much more difficult. Characteristic chromosomal abnormalities in MDS include alterations of chromosomes 5, 7, 8, 11, 17, 20 and Y or complex karyotypes involving multiple abnormalities within individual clonal populations [45, 46]. Several of these abnormalities are shared with AML (e.g., deletions of chromosomes 5 or 7, trisomy 8, and complex cytogenetics), while others appear almost exclusively in AML (e.g., t (15; 17), t (8; 21), and inversion 16) [22]. Although specific cytogenetic abnormalities within MDS do not typically correlate with WHO or FAB classifications, it is now clear that there are characteristic syndromes within MDS, namely 5q- and 17p- syndromes [47, 48] that have unique clinical and prognostic outcomes. Additionally, evidence suggests a correlation with increasing frequency of cytogenetic abnormalities and severity of disease.

The traditional cytogenetic alterations noted above are typically evaluated using conventional cytogenetic (CC) techniques that require adequate metaphase chromosomes for analysis. The development of newer techniques has allowed investigators to uncover more subtle genetic alterations that help to reveal further components of MDS pathophysiology.

Comparative genomic hybridization (CGH) is a useful tool that can uncover genomic imbalances without the requirement for quality mitoses or dividing cells. Specifically, it allows for detection of changes in chromosome copy number by hybridization techniques between normal and tumor cells [78]. Studies comparing information obtained through CC, FISH analysis, and CGH, showed close correlation between the three techniques with CGH providing additional information on the abnormalities identified by CC as well as uncovering unbalanced genetic alterations not described by CC [79, 80]. CGH continues to add valuable information to many common cytogenetic findings [81, 82].

Identifying alterations of single nucleotide polymorphism (SNPs) arrays have also been used to help investigators study and explain the heterogeneity of MDS. Moreover, this technique has led to insights on genetic predispositions for these diseases. SNPs are the most common form of genetic variation between individuals, and recent reports have investigated the possibility that genetic polymorphisms in genes required for proliferation and differentiation may play a role in MDS development. Specifically, a GLU785Lys polymorphism in the G-CSF receptor was described and demonstrated functional alterations in growth factor responsiveness in high risk MDS patients suggesting a potential role in MDS pathophysiology [83]. It is likely that further investigations will reveal pathways impacted by these subtle changes in gene expression. SNP techniques have also looked at the possibility that polymorphisms create a genetic predisposition to MDS. Based on evidence of defective DNA mismatch repair enzymes in patients with t-MDS and mitochondrial/genetic instability in patients with primary MDS [84], obvious implicated pathways could include those involving metabolic activation or detoxification enzymes. Utilizing both specific and non-specific probes, polymorphisms in detoxification enzymes such as glutathione S-transferase [85] as well as in quinone oxidoreductase have been found, where the presence of NQO1 quinone oxidoreductase polymorphisms correlated with an increased risk of benzene-related and treatment-related MDS [86, 87]. Additional investigation has also shown microsatellite instability (MSI) in treatment-related MDS, a hallmark of defective DNA mismatch repair [88].

In addition to these structural alternations in DNA, another mechanism for altered gene expression leading to gain or loss of function involves the silencing of genes through epigenetic modifications. The two most intensely studied alterations include DNA methylation and histone acetylation. Both of these genetic alterations can affect chromatin packing and create a DNA / histone complex that is not able to be transcribed. The result is silencing of the noted gene despite its non-mutated presence. Although any gene can be affected by these epigenetic phenomena, many of the genes implicated in cancer development involve tumor suppression, cell cycle control, cell death, cell growth, and differentiation. The silencing of gene expression secondary to DNA methylation is responsible for a variety of normal, physiologic functions including X-chromosome inactivation, facilitation of DNA imprinting, and protection against insertion of viral DNA into the genome [89]. DNA methyltransferase is the key enzyme that methylates CpG islands in the promoter region of genes leading to hypermethylation and silencing of gene transcription [90]. Many cancers appear to have extensive regional hypermethylation as well as aberrant gene expression leading researchers to explore the role of hypermethylation in tumor development and progression: AML and MDS lead the way in being the best studied diseases. There are numerous genes that are implicated in MDS/AML pathophysiology with p15INK4B being one of the most studied. Specifically, hypermethylation of p15INK4B, a known tumor suppressor and cell cycle regulatory gene, has been associated with progression of MDS to AML [91, 92]. Functionally, CDK4 and CDK6 allow cell cycle progression from G1 to S phase. p15INK4B can act as a tumor suppressor and inhibit cell cycle progression by inhibiting the function of CDK4 and 6. Hypermethylation of p15INK4B prevents this suppressive function and allows uncontrolled progression through the cell cycle and resultant uncontrolled proliferation [93]. p15INK4B hypermethylation has been seen in up to 50% of patients with MDS; specifically in higher risk MDS, where the uncontrolled proliferation has shown increased progression to AML and portends a worse prognosis [92, 94, 95]. Hypermethylation of the calcitonin gene has also been described and can be found in 65% of MDS cases. Its presence spans all morphologic subtypes and is commonly found in patients with normal cytogenetics [96]. Numerous studies now suggest that hypermethylation of the calcitonin gene may be an important marker of transformation from MDS to AML [97-99].
Other commonly reported hypermethylated genes in implicated in MDS include E-Cadherin (CDH1), HIC1, and ER [94].

Similarly, conformational alterations of the DNA histone complex also play an important role in the abnormal gene silencing seen in many cancers. Acetylation of lysine residues on the N-terminus of core histones creates an open and readily transcribable chromatin structure while deacetylation creates a tightly coiled and non-transcribable chromatin structure [100]. Acetylation status can also alter transcription factors (GATA-1), nuclear import proteins, signal transduction proteins, DNA repair enzymes, heat shock proteins, and numerous other cellular proteins [101-106]. The epigenetic modulation of these proteins leads to altered expression of pro-apoptotic and cell cycle proteins (ex. FAS/FAS ligand, Bcl2, p53, caspases, Bax, and p21/WAF1/Cip1) [106] and can result in multiple cellular effects including growth arrest in G1 or G2/M, differentiation, and preferential apoptosis of cancer cells [100, 107].

Altered Bone Marrow Microenvironment: Apoptosis, Cytokines and Angiogenesis

The bone marrow microenvironment has also been implicated in the development and progression of MDS with numerous studies showing altered cytokine milieu and apoptosis rates as well as changes in vascular microdensity. These alterations produce a common pathway of increased apoptosis of early, CD34+ progenitors in early-stage MDS and a relative reversal of this ratio in later-stage disease. As MDS progresses towards AML, these pathways become less well defined and additional factors, including proliferative genetic events, may play a more important role [12-14]. Each of these processes may offer scientists numerous targets upon which to focus new therapies.

Bone marrow samples from MDS patients traditionally reveal a hypercellular marrow in the context of increased cytokines, such as TNF-alpha and IFN-gamma, and persistent peripheral cytopenias. Interestingly, data support both increased and decreased programmed cell death within MDS marrows, yet careful analysis suggests that increased apoptosis is more often associated with early stage MDS (RA/RARS) while decreased apoptosis is more characteristic of advanced disease (RAEB2/AML) [13, 14]. Cytokine dynamics offer one explanation for such cycles of altered apoptosis within the malignant clones. Elevations of TNF-alpha and IFN-gamma are consistently described in the MDS bone marrow microenvironment [37, 108, 109] with additional reports suggesting TGF-beta elevations as well [110, 111] highlighting the important role cytokines play in MDS pathogenesis and maintenance. The role of increased TNF-alpha has been well studied in its involvement in promoting apoptosis in MDS, particularly early stage disease [14, 37, 108]. TNF-alpha promotes apoptosis by engaging with the tumor necrosis factor receptor II (TNFR II) and via TNFR mediates programmed cell death by direct binding with TNF Receptor 1 associated protein with a death domain (TRADD), subsequent indirect binding with FAS-associated protein with a death domain (FADD), and eventual activation of the caspase cascade. TNF-alpha can also interact with TNFRII, which lacks a death domain, but interacts directly with TNF receptor-associated factor 2 (TRAF-2), activating NF-kB and JNK, and ultimately promoting anti-apoptotic effects [37, 112, 113]. The ratio of these receptors impacts the rates of apoptosis in MDS with increased TNFRII documented in low-risk disease and increased TNFRII in high risk disease illustrating, again, the inverse correlation between rates of apoptosis and potential for progression and disease progression [37].

Raza et al. have suggested that increased levels of TNF-alpha have a dual impact on MDS development through contrary stimulatory and inhibitory effects: stimulation of primitive progenitor cells, yielding the classic hypercellularity of MDS, while exertion of apoptotic influence on more mature cells, correlating with the peripheral cytopenias [111]. The etiology of these cytokine alterations and definition of the cells from which they originate (macrophages, fibroblasts, or other) remain unanswered questions [114-117].

In addition to TNF-alpha’s impact on apoptosis in MDS, abnormalities in FAS and bcl-2 pathways have also been implicated. FAS is a transmembrane receptor belonging to the same superfamily as the TNF-alpha receptor and its stimulation follows a similar path to apoptosis that terminates with caspase cascade stimulation and degradation of “death substrates" [14]. Abnormal FAS-signaling has also been implicated in other bone marrow failure states such as aplastic anemia as well as in other malignancies with specific studies in MDS illustrating increased FAS expression and apoptosis in low-risk disease and decreased FAS expression in high-risk disease. Interestingly, although increased FAS expression has been seen, no statistically significant correlation between FAS expression and degree of apoptosis has been shown [118]. Thus, while increased apoptosis is an important component in MDS pathophysiology and is seen more in early stage disease, the mechanism for which this occurs is not explained by one pathway alone and may involve FAS signaling either via direct or indirect pathways [14, 118, 119]. While the TNF-alpha and FAS signaling has supported the increased rates of apoptosis in early stage disease, decreased apoptosis rates have also been implicated in the progression from MDS to AML and has been documented to occur via alterations in anti-apoptotic bcl-2 and bcl-x expression [14].

Changes in vascular microdensity are another component of the marrow environment identified as contributing to the development and sustenance of MDS clones [120]. Originally a focus of research in solid tumors, increased angiogenesis also appears to play a role in hematologic malignancies. In solid tumors new blood vessels are formed to sustain growth of the tumor by supplying blood and nutrients. The role of angiogenesis in hematologic malignancies is slowly being unveiled, and early findings suggest not only its importance but also its varied character among different cancers. New blood vessel growth can drive cytokine production, but may also be a byproduct of excessive cytokines. As described above, the milieu in MDS provides many cytokines capable of influencing new vessel growth. In addition to increased TNF-alpha and INF-gamma in the marrow of MDS patients, varying levels of angiogenic factors including VEGF (vascular endothelial growth factor), bFGF (fibroblast growth factor), angiogenin, HGF (hepatic growth factor), EGFR (epidermal growth factor), and TGF-beta (transforming growth factor) have been noted in the plasma of MDS patients. These findings, coupled with direct evidence of increased microvascular density (MVD) on marrow biopsies, support the development of early blood vessels in MDS [120-123]. Both plasma VEGF levels and increased MVD correlate with advanced MDS FAB class [124-126] and imply that increased VEGF may drive increased MVD eventually stimulating MDS progression to AML.

Immune System Dysregulation

Dysregulated immunity has been implicated in the development of all cancers, including MDS [127], but direct evidence in MDS has been difficult to uncover. The successful use of immunosuppressive therapies (i.e., cyclosporine-A, anti-thymocyte globulin), the potentially curative role of allogeneic stem cell transplant, and the more recent data showing improved peripheral cytopenias and elimination of certain common cytogenetic abnormalities with immunomodulatory agents (i.e., thalidomide and lenalidomide) highlight the role immune dysregulation plays in the development of MDS. Changes in both B and T cell dynamics have been implicated in MDS development. Studies have substantiated an autoimmune-type T cell attack in MDS pathogenesis. Specific in vitro work by Smith et al. investigated the possibility of auto-reactive T cells and noted the presence of inhibitory T cells in patients with MDS [128] with subsequent clinical studies showing in vivo success using anti-T-cell therapies (anti-thymocyte globulin (ATG) [129] and cyclosporine-A (CSA)) [130]. Investigation revealed an abnormal T

[545]
cell repertoire and T cell-mediated inhibition of bone marrow CFU-GM [131] as a potential mechanism to the response to ATG. Further characterization of the T cell repertoire in MDS patients demonstrated T cell expansion with recurrent patterns of V-Beta and J region skewing suggestive of unknown chronic antigenic stimulation [127] while other studies identified high percentages of cytotoxic T cells (CD8+, CD28-, CD57+) [132]. In aggregate, these findings indicate an activated immune environment [133].

Irregularities in B cell function have also been implicated in MDS based upon epidemiologic data revealing increased frequency of autoimmune disorders and immune-mediated complications in these patients [134, 135]. Studies evaluating this correlation revealed the presence of either clinical or serologic manifestation of autoimmunity in 10-12% of MDS patients with a higher prevalence in younger patients, those with treatment-related MDS, or those with cytogenetic abnormalities [135, 134]. Just as T cell repertoire skewing has been noted, clonality of B cells within MDS is also under investigation. The presence or absence of such clonality may lend insight to the cell of disease origin. Using cytogenetic abnormalities as a marker to trace lineage, various progenitor and terminally differentiated populations in MDS samples have been studied with seemingly discrepant findings. Using Trisomy 8 as the tracing abnormality in their MDS samples, Saitoh’s group found the additional 8th chromosome in granulocytes, monocytes, and erythroblasts but not in lymphocytes in the BM or in B, T, or NK cells of the peripheral blood. Additional analysis found Trisomy 8 in the level of the CFU-GEMM stem cell (CD34+, CD33+) but not in the pluripotent stem cells (CD34+, Thy1+) indicating absence of B cell clonality and instead a myeloid progenitor as the neoplastic clone [136]. Other groups have shown the presence of specific cytogenetic abnormalities in both myeloid and lymphoid lineages indicating a more pluripotent stem cell of origin. Specifically, White’s group showed the presence of deletion 20q in Epstein Barr Virus (EBV) immortalized lymphoid cell lines derived from an MDS patient [137] while Nilsson’s group has shown interesting findings with both 5q deletion and Trisomy 8 [138, 139]. Specific studies with 5q deletion documented its presence in the majority of pluripotent (CD34+, CD38-) stem cells with both myeloid and lymphoid differentiation; however, transfer of this cell population to SCID mice showed inability to reconstitute hematopoiesis indicating absence of 5q in the most primitive progenitors [138]. Interestingly, in cases with both Trisomy 8 and del 5q, it was evident that Trisomy 8 was a secondary genetic event potentially explaining the discrepancy by suggesting that the additional Trisomy 8 event developed in the progeny of the CD34+CD38- del 5q containing stem cells [139]. The quest to document the potential clonality of B cells involved in MDS pathogenesis has led to the deeper discovery that different cytogenetic abnormalities may originate in different cells of origin in MDS which could have substantial implications on treatment strategies.

**Cell Cycle Dysregulation, Abnormal Differentiation, and Transcription Factor Alterations**

Abnormal differentiation and growth represents the most dominant morphologic feature of MDS. Although there has been an exhaustive analysis of cellular signaling pathways in an effort to define the main problems with differentiation, our best understanding of this problem is linked to studies of the cell cycle: the cell’s ability to initiate normal cellular division, speed of the cell cycle, and regulation of cell cycle checkpoints. The speed of the cycle plays an important role in assuring balanced homeostasis between self-renewal and progenitor expansion with natural aging and cellular death of each cell type required to maintain an adequate blood supply [140].

---

**Fig. (1).** Hopkins approach to MDS treatment.
Detailed molecular work has shown characteristic lineage specific cell cycle changes required for normal differentiation: 1) up-regulation of cdc2 and cyclin A in erythroid lines; 2) induction of cyclin D1 and p15 in myeloid lines; and 3) selective upregulation of cyclin D3 and induction of p21 in megakaryocyte differentiation [141]. Alterations in any of these normal differentiation pathways could thus lead to maturation arrests. Specific blocks in differentiation in MDS and AML have been linked to abnormalities such as constitutive activation of CDK inhibitor p16 leading to G0/G1 arrest and subsequent maturation arrest [141]. Additionally, such as constitutive activation of CDK inhibitor p16 leading to differentiation in MDS and AML have been linked to abnormalities could thus lead to maturation arrests. Specific blocks in differentiation from MDS to AML [93].

Resultant aberrant cell cycle function in MDS has been the common final pathway for a number of genetic, epigenetic, micronenvironmental, and common transcription factor alterations. The GATA transcription factor family (GATA-1, 2, and 3) is of interest with alterations implicated in MDS pathogenesis. Normal GATA-1 is involved in control of erythroid cells, megakaryocytes, eosinophils, and mast cells while GATA-2 is expressed in more immature progenitors including hematopoietic stem cells [142]. Abnormalities or increased levels of GATA-1 have previously been described to affect cell cycle regulation [143], proliferation, differentiation, and regulation of apoptosis [144] of both erythroid and megakaryocytic progenitors. Studies show that normal GATA-2 expression is increased in hematopoietic stem cells and its expression inhibits terminal differentiation thus playing more of a role in proliferation and self-renewal capacity of the stem cells [145]. Consequently, imbalances of GATA-1 and GATA-2 could skew the progenitor population contributing to abnormal differentiation [146], the development of MDS [147], and in the setting of an additional genetic hit may contribute to the progression to acute leukemia [142].

### Table II. Theories of Pathophysiology involved in MDS Development

<table>
<thead>
<tr>
<th>Theories of Pathophysiology involved in MDS Development</th>
<th>Potential Targets/Components Involved</th>
<th>Overall Result of Abnormality</th>
<th>Current Directed Therapies, Potential Directed Therapies, and Investigatory Treatments for MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental/Aging</td>
<td>Increased BM apoptosis</td>
<td>Decreased hematopoietic stem cell pool</td>
<td></td>
</tr>
<tr>
<td>Environmental Exposures</td>
<td>Smoking, Radiation, Benzene, Viral Infections, Chemotherapy</td>
<td>Direct Toxicity to hematopoietic stem cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genotoxic: O₂ free radicals, DNA damage</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-Genotoxic: Immune modulation (Immunoglobulin/complement alterations) and altered BM microenvironment</td>
<td></td>
</tr>
<tr>
<td>Telomere Abnormalities</td>
<td>Potential decreased telomerase and subsequent telomere shortening</td>
<td>Impaired ability to renew stem cell pool</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genetic Instability</td>
<td></td>
</tr>
<tr>
<td>Genetic Alterations</td>
<td>Common Abnormalities: 5q-, Y-, 7q/-Monosomy7, 17p Syndrome, 11q23, 3q, P53 mutations, Ras mutations, Complex Cytogenetics</td>
<td>Abnormalities: typically unbalanced genetic loss</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Numerous theories of tumor suppressor loss</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multi-Hit progression from low risk MDS to AML</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genetic Instability</td>
<td></td>
</tr>
<tr>
<td>Cytogenetic Abnormalities</td>
<td>Hypermethylation: Calcitonin gene, p15INK4B, ER, E-Cadherin</td>
<td>DNA Methylation and acetylation abnormalities lead to silencing of genes important in cell cycle, differentiation, apoptosis, angiogenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetylation Alterations: Cell cycle components: p21WAF1, Alteration of differentiation and apoptotic machinery, Alterations of angiogenesis</td>
<td>DNA Methylation Transferases Inhibitors: (DMTIs)#</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 Azacitidine*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph I Azacitidine maintenance post AlloBMT @ NCT00350818</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decitabine*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph II Decitabine + Tretinoin @ NCT00382200</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph II Decitabine post Azacitidine failure @ NCT00113321</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Histone Deacetylase Inhibitors (HDIs)</td>
<td>DNA Methylation Transferase Inhibitors: (DMTIs)#</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 Azacitidine*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph I Azacitidine maintenance post AlloBMT @ NCT00350818</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decitabine*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph II Decitabine + Tretinoin @ NCT00382200</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph II Decitabine post Azacitidine failure @ NCT00113321</td>
<td></td>
</tr>
<tr>
<td>Epigenetic Modulation</td>
<td>Comparative Genomic Hybridization: Single Nucleotide Polymorphisms (SNPs): NQO1, Glutathione S-transferase, G-CSF Receptor, Microsatellite instability</td>
<td>DNA Methylation and acetylation abnormalities lead to silencing of genes important in cell cycle, differentiation, apoptosis, angiogenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dysfunction of enzymes required for detoxification, DNA mismatch repair, or differentiation</td>
<td>DNA Methylation Transferases Inhibitors: (DMTIs)#</td>
<td></td>
</tr>
</tbody>
</table>
Given the complexity of the cell cycle control in hematopoiesis, alterations of any key component could lead to abnormal differentiation and the potential for MDS.

**STANDARD TREATMENT FOR MDS**

Until the recent past, treatment for MDS was limited to best supportive care (growth factors and transfusion support) for the...
### Table III. Modified IWG Response Criteria

<table>
<thead>
<tr>
<th>Response Category</th>
<th>Response Criteria (must last minimum of 4 weeks)</th>
</tr>
</thead>
</table>
| **Complete Remission** | BM: ≤ 5% blasts with normal trilineage maturation  
Any persistent dysplasia  
PB:  
Hgb ≥ 11 g/dl  
Neutrophils ≥ 1.0 X 10^9/L  
Blasts 0% |
| **Partial Remission** | If abnormal before treatment, all CR criteria except:  
BM blasts decreased by ≥ 50% but still greater than 5% |
| **Marrow CR** | BM: ≤ 5% blasts and greater than 50% decrease over pre-treatment  
PB: HI responses will be described in addition to marrow CR |
| **Stable Disease** | PR not achieved but no progression for > 8 weeks |
| **Failure** | Death or disease progression |
| **Relapse post CR/PR** | 1+ of the following:  
Recurrence of pre-treatment BM blast %  
≥ 50% Decrease from greatest response in neutrophils or platelets  
Hgb reduced by greater than 1.5 g/dl or transfusion dependence |
| **Cytogenetic Response** | Complete: Resolution of all cytogenetic abnormalities  
Partial: 50%+ reduction of cytogenetic abnormality |
| **Disease Progression** | For patients with:  
1) < 5% BM blasts: ≥ 50% increase in blasts to >5%  
2) 5-10% BM blasts: ≥ 50% increase in blasts to >10%  
3) 10-20% BM blasts: ≥ 50% increase in blasts to >20%  
4) 20-30% BM blasts: ≥ 50% increase in blasts to >30%  
Any of:  
1) 50%+ decrease in maximum neutrophil or platelet response  
2) Hgb reduction by ≥ 2 g/dl  
3) Transfusion Dependence |
| **Survival** | Endpoints:  
OS: Death by any cause  
EFS: Treatment failure or death by any cause  
PFS: progressive disease or death from MDS  
DFS: time to relapse  
Cause-specific death: any death related to MDS |
| **Hematologic Improvement (HI)** | Responses must be sustained for at least 8 weeks:  
Erythroid Responses (pre-treatment <11 g/dl):  
- Hgb increase by ≥ 1.5 g/dl  
- Reduced PRBC transfusions by at least 4 per 8 week period compared to pre-treatment transfusion requirements (only those given for Hgb < 9 g/dl counted)  
Platelet Responses (pre-treatment < 100 X 10^9/L):  
- Absolute increase of ≥ 30 X 10^9/L for pts starting with > 20 X 10^9/L  
- Increase from < 20 X 10^9/L to ≥ 20 X 10^9/L by ≥ 100%  
Neutrophil Response (pre-treatment < 1 X 10^9/L):  
- ≥ 100% increase and final count > 0.5 X 10^9/L  
Progression or relapse after HI:  
At least one of the following:  
- 50%+ decrease from maximum response in neutrophils or platelets  
- Hgb reduction by ≥ 1.5 g/dl  
- Transfusion dependence |

Legend: BM = Bone marrow; PB = peripheral blood; g/dl = grams per deciliter; CR = Complete Remission; PR = Partial Remission; HI = Hematologic Response; Hgb = Hemoglobin; OS = Overall Survival; EFS = Event-free survival; PFS = Progression Free Survival; DFS = Disease-Free Survival

Adapted from [148]

Majority of patients and allogeneic stem cell transplant for the small minority of patients eligible for the procedure. Of these treatments, only transplant offered an approach that changed the natural history of the disease and offered a cure. More recently, newer agents, such as azacitidine and decitabine, have been FDA approved to treat MDS based on their impact on the natural history of disease through decreasing the rate of transformation from MDS to AML. Formal evaluation of response to treatment has been described by International Working Group (IWG) criteria, which has recently been modified and described in Table III [148]. We review current standard treatments (Table IV), describe future directions and novel treatment approaches, and conclude with a treatment algorithm for MDS patients (Fig. (I)).

### Supportive Care

Based on concerns of poor tolerance to traditional cytotoxic agents in patients with advanced age, poor performance status, or medical co-morbidities, supportive care has been the longstanding approach to MDS patients. Unfortunately, these criteria of concern are common in the MDS population. Traditionally, supportive care has taken the form of close blood count monitoring, blood product transfusions, systemically administered lineage-directed growth factors, and often prophylactic antibiotics with the sole intent of improving marrow failure and minimizing the impact of the bone marrow failure on the patients’ lives. While somewhat effective, each of these approaches is relatively limited. Close monitoring of blood counts requires a compliant and diligent patient and takes time each week for testing and follow-up. Use of transfusions is ultimately limited by the development of alloimmunization and the resultant antibodies that effectively reduce the impact of both red cell and platelet transfusions. Myeloid growth factors are generally only recommended in patients plagued by recurrent neutropenic fevers, severe systemic infections, or in combination with erythropoietin. Erythropoietin is generally considered the most effective supportive approach yet yields an overall response of approximately 16% as a single agent [149, 150] and up to 36-50% in combination with G-CSF.
Allogeneic Stem Cell Transplantation

Allogeneic stem cell transplant, the only known curative treatment option for MDS patients, lies at the other treatment extreme. Unfortunately, several factors limit its application for most patients and include advanced age, poor performance status, limited access to an HLA matched sibling or matched un-related donor (MUD), and significant potential for treatment related morbidity and mortality. Despite these problems, allogeneic transplants are commonly recommended for younger patients. Transplant outcomes for more than 452 MDS patients conducted and reported by the IBMTR from 1989 through 1997 were recently presented. The median age of the group undergoing the transplant was 38 years with an overall disease free survival of 53% at 1 year and 40% at 3 years and cumulative incidences of relapse of 17% at 1 year and 23% at 3 years [158]. The most important predictor of survival was the blast percentage prior to transplant with increased relapse correlating with increasing blast percentage and higher IPSS scores. Transplant-related mortality was 32% at 1 year and 37% at 3 years. Generally, most data cite improved transplant outcomes in patients treated both early in their disease course and with low level disease [155]. Responders are typically limited to patients with significant anemia, transfusion independence, and low endogenous EPO levels [155, 149]. Newer studies using darbepoetin in MDS patients have reported erythropoietic response ranges from 26% in previous erythropoietin failures [156] to rates as high as 71% [157]. Interestingly, new reports have highlighted adverse outcomes in patients receiving high doses of erythropoietin or darbepoetin to achieve a hemoglobin level greater than 12. While tumor progression and decreased survival were the adverse outcomes in head and neck and breast cancer patients respectively, general adverse events included increased venous and arterial thrombosis, strokes, myocardial infarction, and congestive heart failure. Given the increased concern, the FDA has altered its safety information to recommend using the lowest dose of the agent needed to decrease transfusion requirements, increasing the hemoglobin gradually, and not exceeding a hemoglobin level of 12mg/dl.1 While these studies prompt caution in the extended use of erythropoietin growth factors in MDS patients, reaching a hemoglobin level greater than 12 is unusual, and consequently the degree to which these findings will affect this patient population is yet to be determined.


[168]. Efforts continue to expand the potential alternative donor source and improve upon outcomes.

**Targeting Epigenetic Changes**

Within the past 3 years, options for the medical management of MDS has evolved from the limited supportive care approach of careful count monitoring, transfusions, and growth factors to now having 3 FDA-approved agents indicated for the treatment of patients with MDS. This progress has led to enthusiasm among physicians and their patients and families. However, the availability of these drugs has raised many important questions including how to best use each of the drugs including when to institute therapy which agent to use, and how to best administer each of the drugs. There is also intensive ongoing investigation to better understand the mechanism of action of each of the drugs.

The data supporting the close relationship between epigenetic modifications and the biology of MDS grows almost daily. Importantly, 5-azacitidine and decitabine, two newly FDA approved DNA methyltransferase inhibitors, are clinically effective in high risk MDS and appear to work at least in part, by reversing these epigenetic changes. The agents cause demethylation of commonly involved promoter regions by incorporation into DNA as nucleoside analogues resulting in inhibition of the methyltransferase activity responsible for maintaining these hyper-methylated regions [169]. A phase III CALGB trial comparing 5-azacitidine versus best supportive care showed an overall response rate of 60% (7% CR, 16% PR, and 37% improved) compared to only 5% in the supportive care arm only arm [170]. The responses also appeared to confer a survival advantage and a decrease in the risk of AML transformation (21 months versus 13 months) in the 5-azacitidine treated group [170]. Retrospective analysis of several 5-azacitidine trials done in the phase III CALGB trial [171] response criteria (Table III) and confirmed its effectiveness but showed lower complete response rates (10-17%) and increased hematologic responses (23-26%) with most responses being seen by the sixth cycle [171].

Similar response data also exists for a second, FDA-approve DNA methyltransferase inhibitor, decitabine. Early studies with varying treatment schedules showed hematopoietic responses nearing 50% [172, 173] and lead to further Phase III trials randomizing patients to decitabine versus best supportive care. In this Phase III study, the decitabine treatment resulted in a complete response rate of 9%, a hematologic improvement rate of 13%, and median response duration of 10 months [174]. Although no survival benefit was seen, most patients became transfusion independent and there was a trend towards a delay to AML transformation or death [174]. Retrospective subset review suggested that responses were associated with cytogenetic complete remissions in 35% of patients [174]. More recent studies have focused on improving dosing schedules of decitabine (namely lower dosing over increased days) for higher risk MDS patients and show higher CR rates (21-30%) and overall responses of up to 73% noting a median of 3 cycles needed to obtain CR [175].

Chromatin remodeling via histone acetylation status is a second epigenetic target [176] with histone deacetylase inhibitors (HDIs) another group of agents used to modify epigenetic changes. Acetylation of lysine residues on the N-terminus of core histones creates an open and readily transcribable chromatin structure. HDIs function to reverse the gene silencing caused by deacetylation and maintain the histones in an acetylated and transcribable state [100] and exist as several different classes of drugs including hydroximates (trichostatin A and SAHA), cyclic peptides (cyclic tetrapeptides), aliphatic acids (phenylbutyrate and valproic acid), and benzamides (MS-275) [177]. A variety of clinical trials have shown both single agent clinical and biologic activity. For example, the use of valproic acid either alone or in combination with all-trans-retinoic acid (ATRA) in MDS patients has shown an overall hematologic response rate of 24%. Interestingly, response rate correlated inversely with bone marrow blast percentage and those with a normal blast count had a greater than 50% response rate while those with > 10% blasts (RAEB I or II) had only a 6% response rate [178, 179]. Additional studies with various HDIs such as MS-275, valproic acid, and SAHA are ongoing.

**Use of IMiDS as Immune Therapy**

Cyclosporine and ATG were among the first immunomodulatory agents used in the treatment of MDS and showed partial success in producing transfusion independence [129, 130]. The mechanism of action of these agents remained unclear, but investigators identified a small subset of patients, typically RA patients with less than 5% blasts, with the following associations that responded with transfusion independence: HLA-DR15 or HLA-DR2 association, younger age, and shorter duration of red cell transfusion dependence [180]. Efforts to study agents that had immunomodulatory impact in MDS were undertaken and thalidomide emerged as having mild clinical activity with very interesting potential mechanisms of action, including: 1) immunomodulatory effects related to a shift of T cell response from Th1 to a Th2 [181], partial T cell activation via alteration of secondary T cell signals, and increased NK cell number and cellular lytic activity [182]; 2) anti-angiogenic properties through possible mechanisms such as nitric oxide inhibition and blockage of endothelial cell migration [183], inhibition of VEG-F and bFGF [120], inhibition of TNF-alpha-induced upregulation of cell adhesion markers, [184] and additional TNF-alpha independent mechanisms [185]; 3) alterations of the bone marrow microenvironment through the impact of cytokines and augmentation of stromal cell growth [120]; 4) alterations of apoptosis [182, 186]; and 5) alterations of adhesion molecule expression [187, 188].

Early studies of thalidomide’s effectiveness in patients with MDS reported 31% hematologic responses after twelve weeks of therapy [188]. Subsequent studies revealed response rates from minimal to nearly 50% with partial success inducing transfusion independence seen in a subset of patients [189-192]. Unfortunately, the side effect profile of hypotension, neuropathy, constipation, and drowsiness makes the drug poorly tolerated, especially in elderly patients [190]. Consequently, based on the clinical activity of thalidomide, newer analogues have been developed to try to minimize side effects. Lenalidomide is the only currently clinically available thalidomide analogue for treatment of patients with MDS. It is reported to share many of the same mechanisms of action with thalidomide; however, lenalidomide has not shown the same effect on endothelial cell proliferation [193-195]. Given the better toxicity profile and similar mechanism of action, clinical investigation by List et al. began with early phase I/II trials showing extraordinary clinical response specifically in a subset of MDS patients who either hadn’t responded to EPO therapy or had high endogenous levels, had low risk IPSS, or had cytogenetic abnormalities involving chromosome 5 [196]. The study boasted a 56% overall hematologic response rate across all cytogenetic abnormalities; in predominantly low risk IPSS, with 63% of previously transfusion-dependant patients achieving transfusion independence. Median time to hematologic response ranged from 9 to 11.5 weeks depending on the dose of lenalidomide. Hematologic effects, primarily erythroid, were the most notable in patients with deletion 5q31. Additionally, complete cytogenetic responses were seen in 50% with a median time to response of 8 weeks. Toxicities included significant neutropenia and thrombocytopenia in greater than half the patients [196]. Further larger scale trials are ongoing to study the effects of lenalidomide in specific subsets of MDS and have substantiated the early success [197]. Additionally, trials investigating the combination of lenalidomide with other agents are ongoing.

**NEW THERAPEUTIC APPROACHES UNDER DEVELOPMENT FOR MDS**

The incredibly complex pathophysiology intrinsic to MDS provides many targets and fertile ground for investigators to develop
Table IV. Standard Treatments for MDS

<table>
<thead>
<tr>
<th>Treatment Categories</th>
<th>Drugs</th>
<th>Dose</th>
<th>Expected Response</th>
<th>Studies</th>
</tr>
</thead>
</table>
| **Growth Factors**   | Erythropoetin | Standard Starting Dose:  
  - 150 IU/kg SQ 3 times weekly (Micromedix)  
  - 40,000-60,000 IU 2-3 times a week for 2-3 months (NCCN Guidelines)  
  - Prolonged EPO administration (minimum 26 weeks) using 150 IU/kg 3 times weekly dose | Overall HR: 16% [149]  
  RR by certain characteristics;  
  Trans. Indep. vs. Dep. (44% vs. 10%)  
  Mode using FAB group (RARS vs. other)  
  + Epo level + transf. status predicts response to EPO  
  MDS (not RARS) + Trans. Indep. RR >50%  
  + RARS + EPO=200U/1RR<0%  
  Overall RR: 45%  
  Better RR if:  
  - low blast %  
  - Good Cyto genetics  
  - EPO=250U/L | Hellstrom-Linberg et al. [149]  
Terpos et al. [155] |
| **Darbepoetin** | * under investigation for MDS  
  - 300mcg SQ weekly then 300mcg SQ q2weeks maintenance  
  - Transition from EPO to Dar: Darbepoetin dose 200mcg q2weeks | Tx for 12 wks: ER: 71%  
  - 8/13 patients previous EPO non-responders  
  Tx. Naive Patients:  
  - Epo: MR 35%  
  - Dar: MR 46%  
  Prev EPO:  
  - Epo: MR 17%  
  - Dar: MR 26% | Mannone et al. [157]  
Patton et al. [156] |
| **Epigenetic Modulators**  
**DNA Methyltransferase Inhibitors** (DMTIs) | 5-Azacytidine | 75mg/m2 SQ qd X 7 days q4 weeks | Response Typically Seen after 4-cycles:  
  Phase III CALGB:  
  OR: 60%  
  CR: 7% + PR: 16% + Improved: 37%  
CALGB Reanalysis:  
  CR: 10%  
  OR: 5%  
  HR: 23-26%  
  Majority of Responses seen by 6th cycle | Silverman et al. [170]  
Silverman et al. [171] |
| **Decitabine** | 15mg/m2 IV q8hours times 3 days q6 wks | Phase III Trial:  
  OR: 30%  
  CR: 9% + PR: 8% + HR: 13%  
  All responders gained trans. indep.  
  Majority of Responders: cytogenetic response | Kantarjian et al. [174] |
| **Bone Marrow Transplant** | Standard  
**Myeloablative Conditioning** | Per specific regimens | 3 yr DFS: 40%  
3 yr Relapse Rate: 21%  
3 yr TRM: 37% | IBMTR Data/Sierra et al. [158] |
| Reduced Intensity  
**Conditioning** | Per specific Regimens | 3 yr DFS: 33%  
3 yr Relapse Rates: 45%  
3 year NRM: 22% | Martino et al. [161] |
| **T Cell Depletion** (TCD) | Per specific regimen | Entire Group:  
  2 yr DFS: 39%  
  2 yr Relapse: 34%  
  RA Pts:  
  DFS: 73%  
  Relapse: 11%  
  Advanced MDS: (in CR)  
  DFS: 42%  
  Relapse: 34% | Mattijsen et al. [166]  
Sierra et al. [158] |
| **Immunotherapy** | Thalidomide | 100mg titrated to 400mg/day for 12 wks | ER: 31%  
  Varied Response:  
  - 0 – 50% | Raza et al. [188]  
Numerous Studies [189-192] |
| Lenalidomide | 10mg po qday | 10mg/day for 21 days | ER: 54%  
  Improved Response:  
  Low IPSS/FAB class and highest in del5q>83% | List et al. [196] |

Legend: Trans. = Transduction; Indep. = Independence; Dep. = Dependence; kg = kilogram; mcg = microgram; OR = Overall response; RR = Major response; Epo = Erythropoietin; CR = Complete response; ER = Erythroid Response; Dar = Darbepoetin; PR = Partial response; RARS = Refractory Anemia w/ Ringed Sideroblasts; HR = Hematologic Response; RR = Response Rate; mg/m2 = milligrams per meter squared; CALGB = The Cancer and Leukemia Group B; NRM = Non-relapse mortality; TRM = Treatment-related mortality; DFS = Disease Free Survival; PFS = Progression Free Survival; Pts = patients; TCD = T Cell Depletion.

new treatment approaches. A very well done recent review captured many of these approaches [199]. We focus our new therapeutic discussion on three areas under development at our own institution: epigenetics, immune modulation, and differentiation-based approaches.
Epigenetic Treatments – Expanding Targets

Epigenetic modulation through a combination of DMTIs and HDIs seemed to be a logical approach noting the responses seen with these individual classes of agents. A pilot study combining phenylbutyrate (HDI) with 5-azacitidine showed that both complete and partial remissions could be achieved in nearly half of the MDS and AML patients and showed a strong association between the clinical responses and changes in hypermethylation and expression of p15 [200]. The clinical responses were supported with findings from a second pilot study of the same combination [201]. Gore et al. combined MS-275 (HDI) with 5-azacitidine (DMTI) in a follow-up pilot study recently presented in abstract form at ASH 2006. Thirty one patients (13 MDS, 4CMMol, and 14 AML 4 of which had multi-lineage dysplasia) were treated with the combination. Azacitidine was given in 3 different doses subcutaneously for 10 days and MS-275 was given orally in 4 different doses on day 3 and 10 of a 28 day cycle. The treatment regimen was based on in vitro studies illustrating the optimal timing for reversal of epigenetic silencing. Of patients with MDS or AML w/ MLD, 50% responded with a median time to best hematologic response of 4 cycles. Of the entire cohort, 6 clinical responses were seen in the 20 patients with cytogenetic abnormalities and included 4 with complete cytogenetic responses. Best cytogenetic responses were seen in a median of 4 cycles. Correlative studies showed increased H3 or H4 acetylation after 5 azacitidine alone in 30% of patients with increase in H3 and H4 acetylation after combination therapy in 25/29 and 28/29 patients respectively. Gore’s study provided additional excitement for the successful combination of DMTIs and HDIs in treating MDS and AML w/ MDL and prompted the current ECOG randomized trial comparing MS-275 + 5-azacitidine to 5-azacitidine alone.

Immune Strategies – Immune System Modulation through Vaccines

Vaccine strategies have been employed in many solid tumor malignancies and more recently, hematologic malignancies. Despite the enthusiasm from many clinical immunologists, there is little published clinical efficacy data for these approaches to date. Some strategies are based on targeting tumor antigens preferentially expressed by the cancer cells. Specifically in MDS and AML, much of the vaccines work has been based on overexpressed antigens (WT-1, survivin, and proteinase 3). A peptide vaccine against WT-1 was developed and studied in a phase 1 trial in Japan [202]. The study treated 12 patients with AML and considered 7 responders based on a decrease in WT-1 serum levels following the vaccines. Of interest, 2 MDS patients receiving the vaccine became markedly cytopenic. Based on concerns that the vaccine may in fact be targeting both normal and MDS stem cells, no further patients with MDS were enrolled [202]. However, this may be the stronger piece of correlative data supporting the activity of such approaches. Currently at the Sidney Kimmel Cancer Center at Johns Hopkins, we have created a pilot study evaluating the use of an institutionally-developed, K562/GM-CSF secreting vaccine in patients with MDS. Given that K562 expresses a number of defined myeloid antigens, such as WT-1, proteinase 3, and survivin, found overexpressed in MDS as well as other myeloid malignancies, it has the potential to generate specific anti-tumor responses to these antigens. Open to 15 MDS patients, our trial will evaluate treatment success based on clinical parameters including resolution of cytogenetic abnormalities or improvement of transfusion requirements with additional investigation of immune correlates.

Differentiation Therapy – Combinations that Improve Responses

The outstanding success of ATRA in the treatment of acute promyelocytic leukemia (APL) is proof of the principle that differentiation therapy has potential to be an important therapy [203]. MDS has also been the target of attempts at utilizing differentiation therapy with the use of agents such as interferon, polynuclear compounds, homoharringtonine, 5-azacitidine, ara-c, butyrates, amifostine, retinoids, vitamin D derivatives, growth factors, and/or combinations of the above drugs [204]. Each of these agents has shown glimpses of differentiating activity, including single agent interferon [205-209], demethylating agents [170, 171, 173, 174], vitamin D derivatives [210-212], and retinoids in combination with other compounds [179, 213, 214].

Interestingly, each of these agents appears to have differing mechanisms of action raising the question of how pharmacologic differentiating agents actually work. In vitro studies have shown that lineage-specific growth factors can enhance the activity of many pharmacologic differentiation agents noted above in both AML and MDS cell lines and primary bone marrow cells [215-217]. Further investigation noted that this synergy only occurred with cytostatic doses of the pharmacologic differentiating agents. This supported the hypothesis that pharmacologic differentiating agents functioned primarily through inhibition of cell cycle and could preferentially augment the differentiating capacity of growth factors indicating that the agents were not sufficient in and of themselves for terminal differentiation of malignant cells [217]. Matsu’s group went on to show that growth factors were actually required for substantial in vitro differentiation [215-217]. While growth factors alone are sufficient to induce differentiation, their use as single agents is limited due to their pleiotropic effects of stimulating proliferation and enhancing survival via inhibition of apoptosis [218, 219]. Thus, in theory, preferential enhancement of self-renewal and survival could cause tumor progression while selective induction of differentiation could exhaust the neoplastic clone. This hypothesis was recently tested in a phase I study with the differentiating agent bryostatin in combination with GM-CSF at the Sidney Kimmel Cancer Center at Johns Hopkins. Bryostatin, a protein kinase C agonist, was administered via continuous infusion in a dose escalation fashion. Smith and colleagues found that Bryostatin, in conjunction with GM-CSF, had remarkable clinical activity with 1 poor risk AML patient achieving a CR following a single course of the combination therapy and a second MDS patient having a morphologic CR and partial cytogenetics response following 2 cycles. Unfortunately, further development was limited due to an intolerable side effect profile at clinically active doses. Future directions that build upon these principles include agents with in vitro activity in combination with growth factors including the HDI, MS-275, and novel retinoids, like bexarotene.

SUMMARY

The myelodyplastic syndromes are an incredibly complex spectrum of hematopoietic stem cell disorders. Their range of severity along with variations in response to multiple treatment approaches suggests that the pathophysiology underpinning these disorders is just as complex. We are only beginning to unravel the biology of MDS. This review hopes to not only provide a broad introduction to the complex pathways involved in the development and progression of these disorders but also to intrigue and challenge both basic and clinical scientists to continue to work towards unlocking the mysteries of MDS.


Stein, R. S. The role of erythropoietin in the anemia of myelodysplastic syndrome. Clin. Lymphoma 2005, 6(Suppl. 1), S36-S40.


