

Genome sequencing in the management of myelodysplastic syndromes and related disorders

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Abstract

Myeloid neoplasms originate from the clonal proliferation of hematopoietic stem cells, which is driven by the acquisition of somatic genetic mutations. Within these disorders, myelodysplastic syndromes (MDS) are specifically characterized by morphological abnormalities (dysplasia) and impaired maturation of myeloid precursors (ineffective hematopoiesis), resulting in peripheral blood cytopenia. Several studies have advanced the field of MDS, with a few landmark papers leading to a paradigm shift, opening new avenues of research and enabling a molecular revolution. These seminal papers include the first description of the 5q- syndrome, the identification of somatic mutations of *TET2* in myeloid neoplasms, the detection of common pathway mutations in the splicing machinery, and the discovery of clonal hematopoiesis. The somatic genomic landscape of MDS is now well defined. Genes that are recurrently mutated include epigenetic regulators, as well as genes of RNA splicing machinery, transcription regulation, DNA repair control, cohesin complex, and signal transduction. Furthermore, several disorders with a germline genetic predisposition to MDS have been identified, collectively accounting for up to 15% of all MDS cases. Genomic profiling can significantly improve the diagnostic approach to MDS, allowing the identification of distinct nosological entities such as *SF3B1*-mutant or *TP53*-mutant MDS. The Molecular International Prognostic Scoring System for MDS has already proven to be a valuable tool for individualized risk assessment and treatment decisions. In addition, the recently developed molecular taxonomy of MDS will likely facilitate the implementation of precision medicine approaches for these disorders. This will necessitate the establishment of specialized infrastructures within public health systems, involving close collaboration between healthcare institutions, academia, and the life-sciences industry.

Introduction

In 1982, the French-American-British (FAB) Co-operative Group defined myelodysplastic syndromes (MDS) as hematopoietic disorders characterized by morphological defects in the bone marrow (dysplasia) and peripheral blood cytopenia, with a variable risk of progression to acute myeloid leukemia (AML).^{1,2} Subsequently, the World Health Organization (WHO) recognized the malignant nature of MDS and included these disorders in the classification of tumors of hematopoietic and lymphoid tissues, defining MDS with isolated del(5q) as a distinct MDS subtype.³ Although the WHO authors explicitly introduced the concept of MDS as a clonal disorder, the

identification of the clonal nature of hematopoiesis was not considered a prerequisite for clinical diagnosis.⁴ Cytogenetic abnormalities, present in approximately half of the patients, have been utilized for years to stratify the risk of MDS patients but have not been employed primarily to demonstrate the clonal nature of the disease.^{5,6} Even the revision of the WHO classification of MDS in 2016 was based to a large extent on morphological criteria.^{7,8} The genomic era in MDS began with the advent of massively parallel DNA sequencing techniques, commonly referred to as next-generation sequencing.⁹ These methods have been employed in well-characterized patient populations to elucidate the genetic basis of MDS and to develop novel approaches for the diagnosis, classifica-

tion, and risk stratification of these disorders.¹ The most recent classifications of MDS, namely the International Consensus Classification (ICC) and the 5th edition of the WHO classification of myeloid neoplasms and acute leukemias, now include genetically defined subtypes of these disorders.^{10,11} In this article, we discuss the role of genome sequencing in managing patients with MDS and related disorders. Figure 1 depicts the key pathophysiological processes underlying MDS and the genomic features that may inform clinical decision-making.

The discoveries that have enabled the molecular revolution in myelodysplastic syndromes

Several studies have contributed to the field of MDS over the years. Still, a few groundbreaking papers have led to

a paradigm shift, opening new avenues of research and enabling the molecular revolution in MDS.

1974: the 5q- syndrome, the first myelodysplastic syndrome subtype found to be associated with a somatic genetic lesion

The molecular revolution in MDS began in 1974, when Herman van den Berghe and colleagues reported in *Nature* a distinct hematologic disorder associated with an acquired deletion of the long arm of chromosome 5, that is, the del(5q).¹² This was the year after Janet Rowley had reported in *Nature* that the minute chromosome previously described by Nowell and Hungerford in patients with chronic myeloid leukemia resulted from a translocation between the long arm of chromosome 22 and the long arm of chromosome 9.^{13,14} In a follow-up paper, van den Berghe and co-workers reported that patients with del(5q) had macrocytic anemia with oval macrocytes, normal to slightly decreased white blood cell counts, and normal to increased platelet counts. The

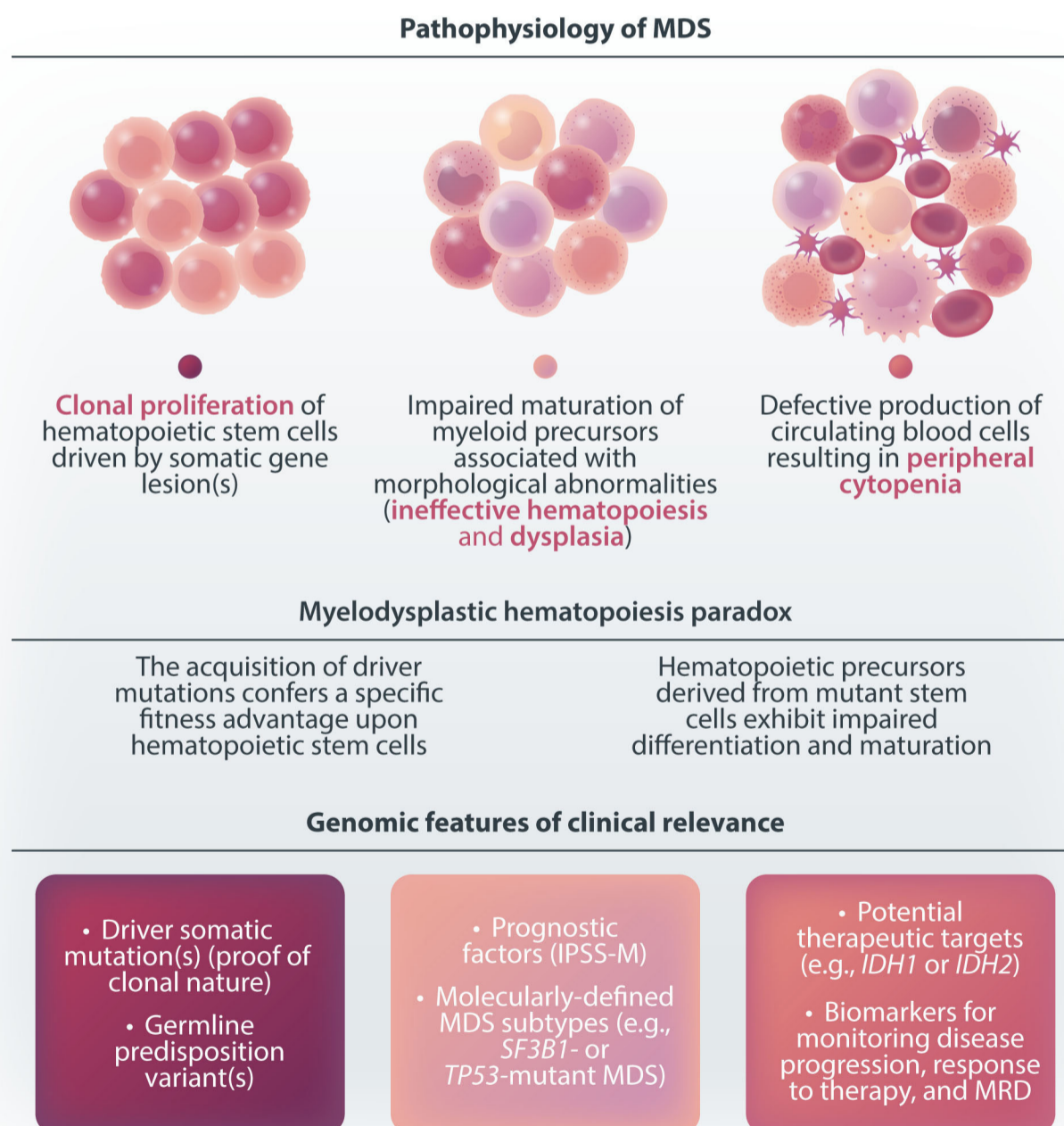


Figure 1. Key steps in the pathophysiology of myelodysplastic syndromes and genomic features that may be relevant to clinical decision-making. MDS: myelodysplastic syndromes; IPSS-M: molecular International Prognostic Scoring System for myelodysplastic syndromes; MRD: minimal residual disease.

bone marrow of the patients showed erythroid hypoplasia but “*the most striking abnormality concerned the megakaryocytes and especially their nuclei, which were generally small, round or oval, and nonlobulated*”.^{15,16} Although a causal relationship between acquired del(5q) and hematologic abnormalities could not be established at that time, these clinical/cytogenetic studies laid the groundwork for later understanding of how haploinsufficiency of multiple genes can lead to a myeloid malignancy.

2009: *TET2* haploinsufficiency, enhanced stem cell function, and clonal proliferation in myeloid malignancies

In 2009, Delhommeau *et al.* reported the occurrence of somatic mutations in *TET2* (including point mutations or gene deletions) in approximately 15% of patients with various myeloid malignancies, including MDS, myeloproliferative neoplasms, and AML.¹⁷ The *TET2* protein is a methylcytosine dioxygenase that plays a key role in regulating DNA methylation status. In both MDS and myeloproliferative neoplasms, mutant *TET2* was detected in primitive CD34⁺CD38⁻ hematopoietic cells, and several observations indicated that the mutant clone was amplified during hematopoietic differentiation. In a parallel study of 102 MDS patients, Langemeijer *et al.* identified acquired deletions as well as missense or nonsense mutations of *TET2* in 26% of subjects and concluded that this was the most frequently mutated gene in MDS.¹⁸

The above observations were consistent with a key role for wild-type *TET2* in the control of normal hematopoiesis. Haploinsufficiency of *TET2*, by deletion or mutation, would confer a fitness advantage to the mutant hematopoietic cells, generating a mutant clone. Two years later, Moran-Crusio *et al.* and Quivoron *et al.* provided an experimental validation of this working hypothesis.^{19,20} Using animal models, these investigators demonstrated that reduction of *TET2* expression or function results in enhanced stem cell function, cell-autonomous competitive advantage of hematopoietic progenitors, and myeloid transformation.

2011: somatic mutations of spliceosome genes in myelodysplastic syndromes as a paradigm shift

In 2010, within the Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium, we used whole exome sequencing to identify somatically acquired point mutations in MDS patients. We initially decided to focus on MDS with ring sideroblasts, reasoning that this distinctive morphological abnormality was likely to be underpinned by a distinctive genetic lesion. Indeed, of the eight MDS patients with ring sideroblasts initially sequenced and reported by Papaemmanuil *et al.* in *The New England Journal of Medicine* in 2011, six had a somatic mutation in *SF3B1*.²¹ Follow-up studies by Malcovati *et al.* showed a strong association between somatically acquired *SF3B1* mutation and ring sideroblasts in myeloid neoplasms.²²

Also in 2011, Yoshida *et al.* reported in *Nature* the results

of whole exome sequencing studies in patients with MDS or related disorders.²³ Unexpectedly, these investigations revealed novel pathway mutations involving multiple components of the splicing machinery, including *SF3B1*, *SRSF2*, *U2FA1*, and *ZRSR2*. The mutations occurred in a mutually exclusive manner, affecting genes involved in the 3' splice site recognition during pre-mRNA processing, and causing abnormal RNA splicing.²³ Subsequent studies have shown that a heterozygous spliceosome mutation is responsible for the mis-splicing of multiple genes, resulting in defective production of their products as illustrated below.

2014: clonal hematopoiesis

In 2014, two papers in *The New England Journal of Medicine* reported the results of whole-exome sequencing studies in peripheral blood cells from populations of subjects unselected for hematologic phenotypes.^{24,25} Both studies identified somatic mutations in genes that are recurrently mutated in myeloid cancers, predominantly *DNMT3A*, *TET2*, and *ASXL1*. Their prevalence was negligible below the age of 40–50 years but increased significantly after the age of 50 years. As the median variant allele frequency (VAF) of somatic mutations was around 0.10, the median number of clonal cells was about 20%. This indicates that in the individuals studied, hematopoiesis was largely (approximately 80%) polyclonal, with a smaller (20%) clonal component. Thus, *strictu sensu*, “clonal hematopoiesis” is not the best definition of this condition: “oligoclonal hematopoiesis” would be a better definition (Lucio Luzzatto, personal communication, 2023). Clonal hematopoiesis is associated with an increased risk of hematologic cancers, coronary artery disease, and other common conditions.^{24–28} For a complete understanding of the consequences of clonal hematopoiesis, the reader is referred to a recent, very comprehensive review article by Weeks and Ebert.²⁹

The somatic genomic landscape of myelodysplastic syndromes

Between 2011 and 2014, several studies showed that multiple mutation-driver genes, belonging to different biological pathways, may be responsible for MDS.^{21,23,30–33} More recently, the International Working Group for Prognosis in MDS (IWG-PM) has analyzed the genomic landscape of 2,957 clinically well-annotated patients with MDS.³⁴ We found 3,186 cytogenetic alterations in 41% of patients and 9,254 oncogenic mutations across 121 genes in 90% of patients; these genetic lesions are summarized in Table 1. Overall, 94% of patients had at least one genetic lesion; 53% had gene mutations only, 4% had cytogenetic alterations only, and 37% had both gene mutations and cytogenetic alterations. The median number of genetic lesions per patient was four and this number correlated with disease severity.³⁴

As shown in Table 1, recurrently mutated genes include

epigenetic regulators (DNA methylation and histone modification), as well as genes of RNA splicing machinery, transcription regulation, DNA repair control, cohesin complex, signaling, and other biological pathways. Only three genes (*TET2*, *ASXL1*, and *SF3B1*) are mutated in at least 20% of MDS patients, while an additional four genes (*DNMT3A*, *SRSF2*, *RUNX1*, and *TP53*) are mutated in 10% to 20% of cases. The remaining genes are mutated less frequently, while most patients have combinations of pathway mutations.

Somatic mutations in the epigenetic regulators *DNMT3A*, *TET2*, and *ASXL1*, collectively termed DTA, drive clonal outgrowth of mutant stem cells because the inactivation of one allele enhances their fitness without significantly impairing their differentiation and maturation capacity. These epigenetic regulators are typically mutated also in clonal hematopoiesis of indeterminate potential (CHIP) and clonal cytopenia of undetermined significance (CCUS).^{24,35-40}

Somatically acquired mutations in spliceosome genes, namely *SF3B1*, *SRSF2*, *U2AF1*, and *ZRZS2* result in haploinsufficiency of multiple downstream genes expressed in hematopoietic cells. Spliceosome mutations are heterozygous, meaning that a single cell produces approximately equal amounts of normal and mutant splicing factors. As a result, half of the 100,000 spliceosomes operating in each cell are wild-type, while the other half are neomorphic due to the mutant splicing factor. Neomorphic spliceosomes can misplace downstream genes, with *GPATCH8*, a factor involved in quality control of branch point selection, being required for mutant *SF3B1*-induced splicing alterations.⁴¹ Mis-splicing, in turn, results in aberrant transcripts that can be rapidly degraded by nonsense-mediated decay or translated into a variant protein.¹ Haploinsufficiency of specific genes has various consequences, including a fitness advantage of mutant stem cells (clonal expansion) and abnormal maturation of hematopoietic progenitors and precursors. For example, in MDS with ring sideroblasts, neomorphic spliceosomes with mutant *SF3B1* factor cause coordinated mis-splicing of the mitochondrial transporters *TMEM14C* and *ABCB7*, leading to iron sequestration in mitochondria and resulting in ring sideroblast formation.⁴²⁻⁴⁴ More generally, *SF3B1* mutations have been found to induce specific proteome remodeling and cellular metabolic reprogramming.⁴⁵ For functional implications of other genes and pathways mutated in MDS, the reader is referred to the comprehensive review article by Ogawa.⁴⁶

Germline genetic predisposition to myeloid malignancies

The cancer predisposition revolution began in 1969 when Li and Fraumeni described four families characterized by the occurrence of sarcomas in children and cancers in their parents and relatives.⁴⁷ Subsequently, the germline transmission of a mutated *TP53* gene was identified in families with Li-Fraumeni syndrome.^{48,49} In recent decades, several

Table 1. Mutated genes and cytogenetic alterations detected in the 2,957 patients with myelodysplastic syndromes studied by the International Working Group for Prognosis in Myelodysplastic Syndromes.³⁴

Mutated genes*
Epigenetic regulators (DNA methylation and histone modification) <i>TET2</i> (>20% of pts) <i>ASXL1</i> (>20% of pts) <i>DNMT3A</i> (10-20% of pts) <i>EZH2</i> , <i>BCOR</i> , <i>IDH2</i> , <i>IDH1</i> , <i>PHF6</i> , <i>BCORL1</i> , <i>ZBTB33</i> , <i>EP300</i> , <i>KMT2D</i>
RNA splicing <i>SF3B1</i> (>20% of pts) <i>SRSF2</i> (10-20% of pts) <i>U2AF1</i> , <i>ZRSR2</i> , <i>PRPF8</i> , <i>U2AF2</i>
Transcription regulation <i>RUNX1</i> (10-20% of pts) <i>CUX1</i> , <i>MLL</i> (<i>KMT2A</i>), <i>ETV6</i> , <i>CEBPA</i> , <i>CTCF</i> , <i>WT1</i> , <i>ZBTB33</i> , <i>GATA2</i> , <i>NFE2</i>
DNA repair control <i>TP53</i> (10-20% of pts) <i>PPM1D</i> , <i>BRCC3</i>
Cohesin complex <i>STAG2</i> , <i>SMC1A</i> , <i>RAD21</i>
Signaling <i>CBL</i> , <i>NRAS</i> , <i>KRAS</i> , <i>JAK2</i> , <i>MPL</i> , <i>SH2B3</i> , <i>PTPN11</i> , <i>GNB1</i> , <i>FLT3</i>
Miscellanea <i>SETBP1</i> , <i>DDX41</i> , <i>ETNK1</i> , <i>KMT2C</i> , <i>CSNK1A1</i> , <i>NPM1</i> , <i>GNAS</i> , <i>ARID2</i>
Cytogenetic alterations*
<i>del(5q)</i> (10-20% of pts) complex karyotype +8, -Y, -7, <i>del(7q)</i> , <i>del(11q)</i> , -13, +21, <i>del(4q)</i> , <i>del(1p)</i>

Only somatic genetic lesions found in at least 1% of patients are shown. The mutated genes and cytogenetic alterations utilized for calculation of the molecular International Prognostic Scoring System for myelodysplastic syndromes (IPSS-M) are underlined. *Listed in order of decreasing frequency within each biological pathway. Pts: patients.

conditions with germline genetic predisposition to hematologic malignancies, including MDS, have been identified.^{50,51} The use of massively parallel DNA sequencing has advanced the field tremendously: Table 2 provides a list of conditions that are associated with an increased risk of developing MDS.⁵²⁻⁷³ These predisposition disorders are now included in the current classifications of tumors of hematopoietic and lymphoid tissues.^{10,11}

The age of onset for MDS or other myeloid malignancies varies considerably among individuals with a genetic predisposition, serving as a surrogate for the underlying biological pathway.⁵² It is important to note that while individual predisposition disorders are rare, they may collectively contribute to up to 15% of MDS cases.⁷⁴ A recent study found that 7% of MDS patients who received allogeneic stem cell transplantation from a family donor had pathogenic germline variants.⁷⁵ This percentage was based on variants that were detectable in both the patient and donor, so the actual frequency may be

Table 2. Conditions with germline genetic predisposition to myeloid malignancies, including myelodysplastic syndromes. Provisional estimates suggest that up to 15% of all patients with myelodysplastic syndromes, regardless of age, may carry deleterious germline variants of the genes associated with these conditions.

Conditions	References
Conditions without clinical phenotype before the development of MDS <i>DDX41</i> -associated predisposition to myeloid malignancies <i>CHECK2</i> -associated predisposition to myeloid malignancies	Polprasert <i>et al.</i> , ⁵³ Sebert <i>et al.</i> , ⁵⁴ Li <i>et al.</i> , ⁵⁵ Makishima <i>et al.</i> , ⁵⁶ Makishima <i>et al.</i> , ⁵⁷ Cheloor Kovilakam <i>et al.</i> , ⁵⁸ Freiman <i>et al.</i> ⁵⁹
Familial platelet disorders, characterized by thrombocytopenia and/or platelet dysfunction Hereditary platelet disorder due to germline variants in <i>RUNX1</i> Hereditary platelet disorder due to germline variants in <i>ETV6</i> Hereditary platelet disorder due to germline variants in <i>ANKRD26</i>	Homan <i>et al.</i> , ⁶⁰ Homan <i>et al.</i> , ⁶¹ Homan <i>et al.</i> , ⁶² Cunningham <i>et al.</i> ⁶³
Telomere biology disorders	Kam <i>et al.</i> , ⁶⁴ Niewisch <i>et al.</i> , ⁶⁵ Revy <i>et al.</i> ⁶⁶
Bone marrow failure syndromes Fanconi anemia Shwachman-Diamond syndrome Diamond-Blackfan anemia <i>ERCC6L2</i> -associated bone marrow failure syndrome	Olson ⁶⁷ Myers <i>et al.</i> , ⁶⁸ Reilly & Shimamura ⁶⁹ Da Costa <i>et al.</i> ⁷⁰ Hakkarainen <i>et al.</i> ⁷¹
GATA2-spectrum disorders (GATA2 deficiency syndrome)	Calvo & Hickstein ⁷²
<i>SAMD9</i> and <i>SAMD9L</i> syndromes	Sahoo <i>et al.</i> ⁷³

higher. Despite inherent limitations, a retrospective analysis revealed that 25% (115 out of 464) of patients with myeloid malignancies who underwent germline testing had a deleterious germline variant.⁷⁶

The Chronic Malignancies Working Party of the European Society for Blood and Marrow Transplantation (EBMT) recently published a position paper on how to consider germline predisposition traits in allogeneic hematopoietic stem cell transplantation for MDS.⁷⁷ The paper recommends that germline testing should be clinical practice for all MDS patients undergoing allogeneic transplantation, regardless of their age at diagnosis or other features. Given the current limitations regarding the availability of germline testing, the authors recommend prioritizing children and younger adults (aged <50 years). Their recommendations for germline variant testing include guidelines for germline DNA source, sequencing platforms, and variant interpretation. Additionally, they suggest the implementation of genetic counseling and surveillance of donors and families with inherited traits.⁷⁷

Genomic profiling in the diagnosis and risk stratification of myelodysplastic syndromes

Diagnosis of myelodysplastic syndromes and related disorders

The diagnostic approach to MDS typically begins with a patient presenting persistent cytopenia after common causes of anemia, neutropenia, or thrombocytopenia have been ruled out. Assessment of morphological dysplasia in the

bone marrow and peripheral blood, along with conventional cytogenetics for identifying recurrent chromosomal abnormalities, has been the cornerstone of the diagnostic approach so far.¹ While still recognizing the fundamental importance of these features, the Bone Marrow Pathology Group now underlines the need for genetic testing.⁷⁸ Previous studies have shown that the assessment of morphological dysplasia can be subject to significant inter-observer variability; furthermore, the threshold of 10% dysplastic cells is arbitrary.⁷⁹ In addition, the aforementioned IWG-PM study of 2,957 MDS patients detected cytogenetic alterations in only 41% of cases, indicating that chromosome band analysis fails to provide any information on clonality in approximately 60% of subjects.³⁴ In contrast, somatic gene mutations were present and documented the clonal nature of the disease in 90% of patients.

Figure 2 illustrates the benefits of genomic profiling in diagnosing MDS. The utility of targeted gene sequencing to differentiate myeloid malignancies from other cytopenic conditions was first demonstrated in an unselected cohort of 683 consecutive patients evaluated for unexplained cytopenia.³⁸ This finding has subsequently been prospectively validated in the US National MDS Natural History Study, which enrolls and banks samples from patients with cytopenia and suspected MDS. A recent report from this collaborative group has demonstrated how genomic profiling enhances the diagnostic accuracy of MDS when combined with histopathology.⁸⁰ At most academic institutions in the USA and Western Europe, gene panel sequencing is now a routine procedure complementing chromosome banding analysis. It should be noted, however, that gene panel sequencing does not routinely include the assessment of copy-number

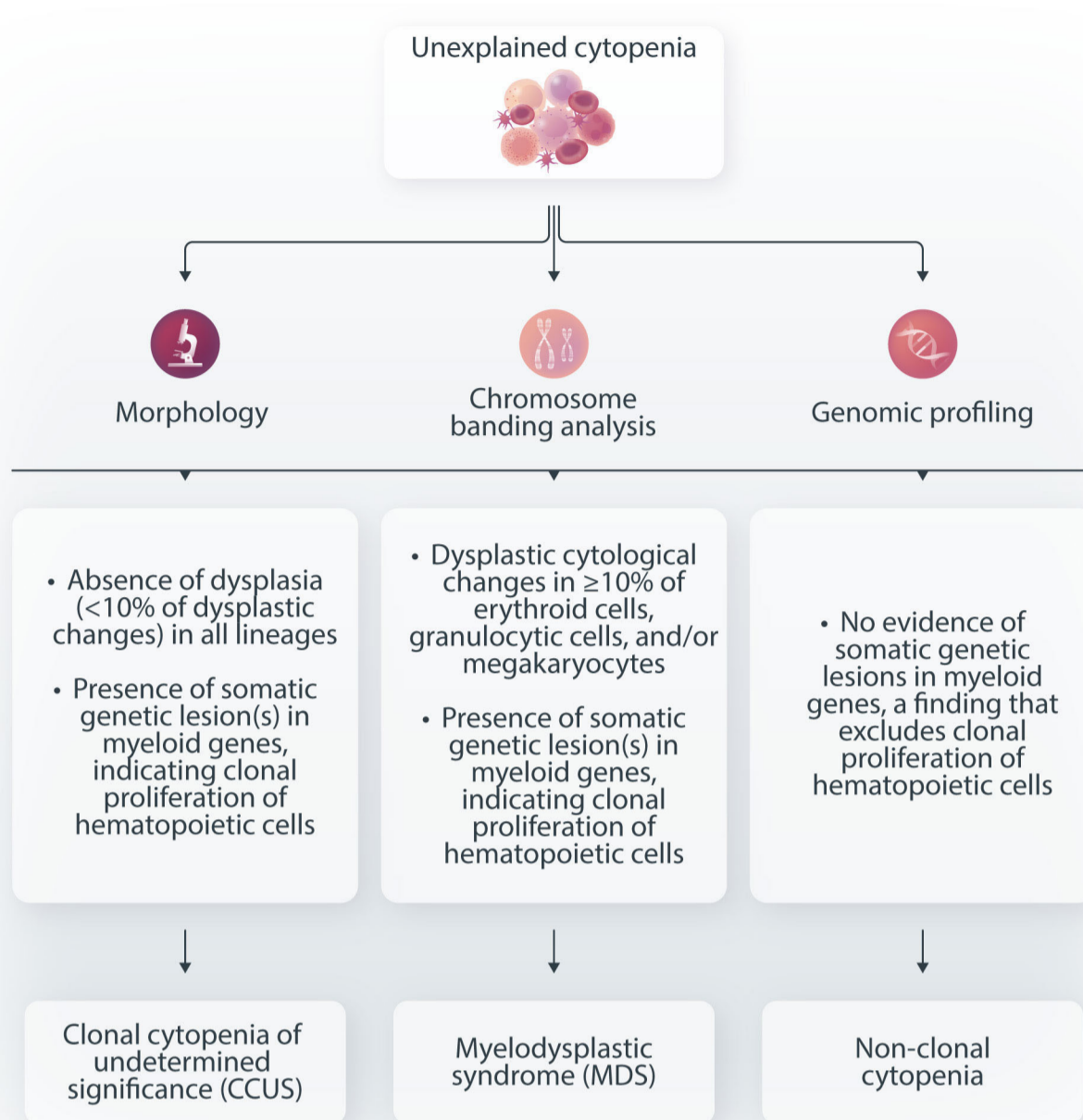


Figure 2. Benefits of genomic profiling in the diagnostic approach to myelodysplastic syndromes and related disorders. Genomic profiling is a fundamental tool for the diagnosis of clonal cytopenia of undetermined significance, as well as for the identification of non-clonal disorders.

alterations and copy-neutral loss of heterozygosity, which are essential for identifying an *TP53* multi-hit state.⁸¹

Whole-genome sequencing has the potential to replace both conventional cytogenetic and sequencing approaches in the near future, providing rapid and accurate comprehensive genomic profiling.^{82,83} To make this a reality, specialized infrastructures need to be established within public health systems. One illustrative example is the Genomic Medicine Sweden initiative, a national infrastructure with the objective of implementing precision medicine in clinical settings and of strengthening collaboration between Swedish healthcare institutions, universities, and the life-sciences industry.⁸⁴

The molecular International Prognostic Scoring System for myelodysplastic syndromes

The revised International Prognostic Scoring System (IPSS-R) has been extensively utilized for risk stratification of MDS, therapeutic decision-making in clinical practice, and clin-

ical trial design.⁶ However, the IPSS-R does not consider somatic gene mutations, which have been demonstrated to have independent prognostic significance.^{1,30}

The IWG-PM recently developed the molecular International Prognostic Scoring System for MDS (IPSS-M) with the goal of achieving molecular precision. We studied a well-characterized cohort of 2,957 MDS patients who were profiled for mutations in 152 genes.³⁴ The IPSS-M risk score is a continuous index that is defined as the weighted sum of the following prognostic variables: hemoglobin concentration, platelet count, bone marrow blast percentage, IPSS-R cytogenetic category, 17 binary features derived from the presence of mutations in 16 prognostic genes, and a feature representing the number of mutated genes (Nres) from a residual set of 15 genes.³⁴ The IPSS-M risk score was standardized such that a score of 0 represents an average patient, meaning a hypothetical patient with average scores on all variables. Negative scores indicate

lower risk, while positive scores indicate higher risk. To facilitate the use of IPSS-M in clinical settings, a schema of six risk categories was established based on score cutoffs. Table 3 summarizes the different risk groups and their clinical outcomes. In clinical practice, the cutoff of 0 enables clinicians to distinguish between patients with lower-risk MDS (score ≤ 0) and those with higher-risk MDS (score > 0). These two groups differ significantly in terms of the risk of leukemic transformation and overall survival (Table 3).

The IWG-PM has created the IPSS-M web-based calculator (IPSS-M Risk Calculator: <https://mds-risk-model.com>), which provides not only an individual patient's score, but also the expected leukemia-free survival, overall survival, and risk of AML transformation. It accounts for missing values, in which case the IPSS-M is calculated under the best, average, and worst scenarios.

In a real-world study of 2,876 patients, the IPSS-M has been validated and found to improve prognostic discrimination for all clinical endpoints compared to the IPSS-R.⁸⁵ In particular, the IPSS-M may result in a more effective selection of candidates for allogeneic transplantation.

Genomic profiling and molecular taxonomy of myelodysplastic syndromes

The existing classifications of MDS include only two molecularly defined entities, namely *SF3B1*-mutant and *TP53*-mutant MDS, in addition to the already established MDS with isolated del(5q), or MDS-del(5q).^{10,11} These defining genetic lesions, namely *SF3B1* mutation, *TP53* mutation, and del(5q), share key features: they are all founding or early genetic lesions in the clonal ontogeny, major determinants of disease phenotype, and relevant prognostic factors.⁸⁶ Overall, the current classifications are still largely based on morphological criteria, and there is a need to

utilize the wealth of molecular data available to develop a mechanistic classification.⁸⁷

Recent reports have shown that clinical and molecular data can be combined to define additional distinct MDS entities.⁸⁸ For instance, mutations in spliceosome genes *SRSF2* and *U2AF1* are typically associated with MDS subtypes that are characterized by high genetic complexity and poor clinical outcomes. Conversely, DTA genes define MDS subtypes with relatively good clinical outcomes when present as single genetic lesions. These considerations led the IWG to develop a molecular taxonomy of MDS.⁸⁹ In a study of 3,233 MDS patients, the IWG-PM investigators derived gene mutations, copy-number alterations, and copy-neutral loss of heterozygosity events from targeted sequencing of a 152-gene panel. The molecular features were then used for unsupervised clustering analysis through Bayesian Dirichlet processes.⁸⁹ This approach has enabled the characterization of 18 distinct MDS molecular subgroups, including the already established MDS with isolated del(5q), *SF3B1*-mutant, and *TP53*-mutant MDS. Interestingly, the subgroup labeled as “no-event” (6% of all MDS patients) was characterized by the absence of any recurrent driver and was associated with a very benign clinical course and a low risk of leukemic transformation. These patients were diagnosed with MDS based solely on morphological criteria, but likely most of them did not have a clonal (malignant) disorder.

The molecular taxonomy of MDS developed by the IWG-PM investigators now needs to be translated into a mechanistic classification of these disorders, following the previously described methodology.⁸⁷ MDS entities that may be considered in future classifications are summarized in Figure 3.

Table 3. Molecular International Prognostic Scoring System for myelodysplastic syndromes risk score, risk categories, and clinical outcomes.*

Risk category (% of patients)	IPSS-M score	Median leukemia-free survival in years	Median overall survival in years	AML transformation by 1 year, %
Six-category risk schema				
Very low (14)	≤ -1.5	9.7	10.6	0
Low (33)	> -1.5 to -0.5	5.9	6.0	1.7
Moderate low (11)	> -0.5 to 0	4.5	4.6	4.9
Moderate high (11)	> 0 to 0.5	2.3	2.8	9.5
High (14)	> 0.5 to 1.5	1.5	1.7	14.3
Very high (17)	> 1.5	0.7	1.0	28.2
Lower-risk vs. higher-risk MDS				
Lower-risk MDS (58)	≤ 0 (negative value)	6.0 (95% CI: 5.7-6.7)	6.3 (95% CI: 5.8-7.2)	2.0
Higher-risk MDS (42)	> 0 (positive value)	1.2 (95% CI: 1.1-1.3)	1.5 (95% CI: 1.4-1.6)	18.9

*Information from Bernard *et al.*³⁴ IPSS-M: molecular International Prognostic Scoring System for myelodysplastic syndromes; AML: acute myeloid leukemia; pts: patients; MDS: myelodysplastic syndromes; 95% CI: 95% confidence interval.

Genomically defined nosological entities in the field of myelodysplastic syndromes and related disorders

Based on the diagnostic approach illustrated in Figure 2 and the schematic representation of Figure 3, we will now focus on a few representative disorders.

Clonal cytopenia of undetermined significance

Patients with CCUS lack the morphological criteria for the diagnosis of MDS (they have less than 10% of dysplastic cells in each myeloid lineage), but carry somatic mutations with a VAF $\geq 2\%$ in genes that are recurrently mutated in MDS.^{35-37,90,91} In the US National MDS Natural History Study on patients with cytopenia and suspected MDS, about one-fourth of the patients enrolled were eventually diagnosed

with CCUS, indicating that this condition is more common than thought.⁸⁰

We have previously shown that patients with CCUS have a significantly higher risk of developing a myeloid neoplasm than those with cytopenia and no evidence of clonality, indicating that CCUS represents a precursor condition for myeloid neoplasms with myelodysplasia with a variable risk of disease progression.³⁸ We also found that clone metrics (type of mutation driver genes, clone size, and comutation patterns) enable the estimation of disease progression risk and may inform clinical decision making in these patients.⁹² Subsequently, Weeks *et al.* developed a clonal hematopoiesis risk score (CHRS) that can predict the risk of progression to a myeloid neoplasm in individuals with clonal hematopoiesis, including patients with CCUS.⁹³ Furthermore, Gu *et al.* analyzed data from 454,340 UK Biobank participants, focusing on subjects who developed a myeloid neoplasm up to 15 years after recruitment.⁹⁴ These

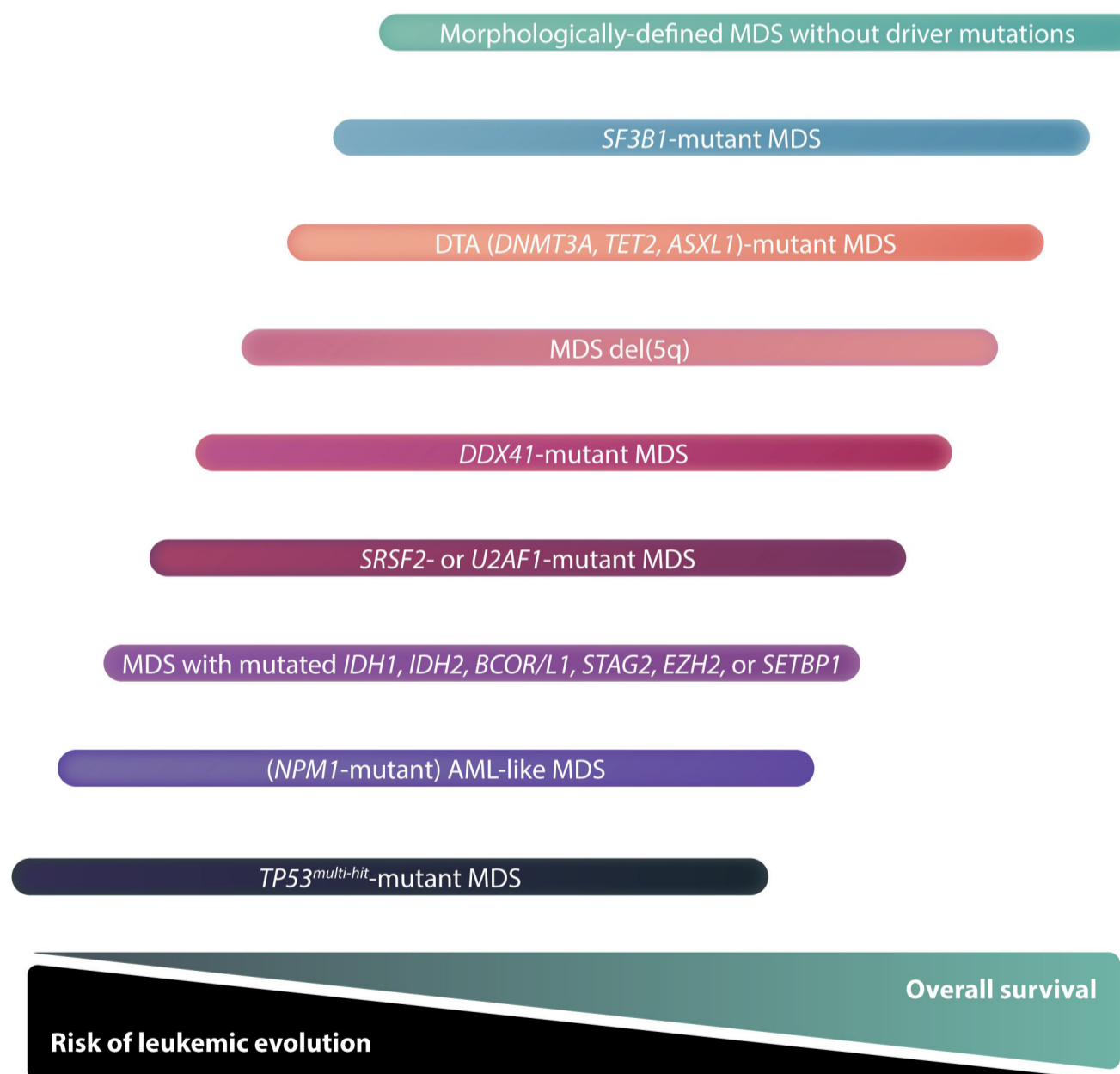


Figure 3. Schematic representation of molecularly defined subtypes of myelodysplastic syndromes in relation to clinical outcomes. Please note that the scheme depicted here, based on the molecular taxonomy of myelodysplastic syndromes (MDS) and related disorders recently developed by Bernard *et al.*,⁸⁹ is not a classification, but rather a representation of the genetic heterogeneity of MDS and its potential clinical relevance. The development of a genomic classification of MDS will require *ad hoc* procedures.⁸⁷

investigators were able to construct “MN-predict”, a web application that generates time-dependent predictions of myeloid neoplasm with the input of basic blood tests and genetic data.

CCUS has only recently been defined and no specific treatment has been approved yet. Several clinical trials testing therapeutic interventions have been registered at ClinicalTrials.gov. These interventions include drugs aimed at reducing inflammation, such as statins, canakinumab, and curcumin, as well as ascorbic acid and metformin. Additionally, enasidenib is being tested in patients with *IDH2* mutation and ivosidenib in those with *IDH1* mutation.

VEXAS syndrome and myelodysplastic syndromes

VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome is a monogenic disease of adulthood caused by somatic mutations in *UBA1* in hematopoietic progenitor cells and is characterized by inflammatory and hematologic symptoms.^{95,96}

Patients with VEXAS syndrome are predisposed to hematologic malignancies, such as MDS and plasma cell dyscrasias.⁹⁵ Specifically, MDS has been diagnosed in about one-third of patients with VEXAS syndromes and most cases are classified as relatively low-risk MDS.^{96,97} Gutierrez-Rodriguez *et al.* recently showed that while patients with VEXAS syndrome have an enrichment of typical clonal hematopoiesis mutations, particularly in *DNMT3A* and *TET2*, *UBA1* mutations are primarily responsible for myeloid clonal expansion, and both the inflammatory and hematologic phenotype.⁹⁸

In a recent study, *UBA1* mutations were identified in 1% of all MDS patients, and inflammatory manifestations were observed in 83% of patients with pathogenic *UBA1* mutations.⁹⁹ Therefore, in the case of a patient with MDS and inflammatory symptoms, sequencing of the *UBA1* gene is recommended to diagnose a potential VEXAS syndrome. Although these patients respond poorly to a variety of therapeutic strategies, they may benefit from treatment with JAK inhibitors, most notably ruxolitinib.¹⁰⁰ Additionally, allogeneic stem cell transplantation may be a viable option in selected patients with VEXAS syndrome.¹⁰¹

Somatic *SF3B1* mutation, ring sideroblasts, and myelodysplasia

MDS with ring sideroblasts was morphologically defined as a distinct MDS subtype in both the FAB and the 2001 WHO classifications.^{2,3} When we evaluated the prognostic value of the WHO classification, the median survival of MDS patients with ring sideroblasts was approximately 9 years, while the mortality of those 70 years of age or older was not significantly different from that of the general population.¹⁰² Therefore, we concluded that MDS patients with ring sideroblasts had an overall indolent clinical course. In 2011, we found that most – but not all – MDS patients with ring sideroblasts had a somatic *SF3B1* mutation,^{21,22}

and subsequent studies established a causal mechanistic relationship between *SF3B1* mutation and ring sideroblast formation.^{42,43} MDS patients with ring sideroblasts and somatic *SF3B1* mutation represented a homogeneous subgroup with isolated erythroid dysplasia, ineffective erythropoiesis, and a favorable prognosis.^{103,104} In contrast, MDS with ring sideroblasts and wild-type *SF3B1* was found to be frequently associated with *SRSF2* or *TP53* mutations and characterized by an unfavorable prognosis.^{103,105}

The association between *SF3B1* mutation and clinical outcomes has recently been found to be modulated by comutation patterns.³⁴ Patients with no gene mutations other than *SF3B1*, or simple comutation patterns involving only *DNMT3A*, *TET2*, and/or *ASXL1*, had favorable outcomes across all clinical endpoints. In contrast, patients with concomitant del(5q) or comutation patterns in other genes such as *BCOR*, *BCORL1*, *NRAS*, *RUNX1*, or *STAG2* had worse clinical outcomes. These comutation patterns are incorporated into the IPSS-M, which is therefore able to capture their prognostic differences when applied to individual patients.¹⁰⁶ Todisco *et al.* have recently performed a comprehensive evaluation of the genomic and transcriptomic profiles of a prospective cohort of 129 patients with MDS and ring sideroblasts.¹⁰⁷ The study found that 64% of patients had a somatic mutation in *SF3B1*, 11% in *SRSF2*, and 10% in *TP53* (multi-hit state), with the remaining showing miscellaneous lesions. Each mutation category was associated with a distinct clinical outcome; of note, *SF3B1*-mutant MDS had a benign clinical course and *TP53*-mutant MDS had a poor outcome.

Figure 4 summarizes the molecular subtypes that may be associated with ring sideroblasts in MDS. The ICC diagnosis of *SF3B1*-mutant MDS requires exclusion of comutation in *RUNX1* and concomitant del(5q), and basically defines the MDS with isolated mutation in *SF3B1* or simple comutation with DTA mutations.¹⁰ Patients with *SF3B1*-mutant MDS have a benign disease with low risk of leukemic transformation and are very likely to respond to luspatercept with amelioration of anemia.¹⁰⁸

Myelodysplastic syndrome with 5q deletion

The MDS subtype identified by van den Berghe *et al.* in 1974 is unique in several respects.¹² The driver mutation is the deletion on the long arm of chromosome 5 that leads to haploinsufficiency of multiple genes mapping to 5q32-q33. This explains the clonal proliferation of mutant hematopoietic stem cells, the clinical manifestations (macrocytic anemia with mild thrombocytosis), and the efficacy of lenalidomide treatment.¹ Haploinsufficiency production of casein kinase 1A1, encoded by the *CSNK1A1* gene, confers a fitness advantage to del(5q)-heterozygous hematopoietic stem cells, causing their clonal expansion.¹⁰⁹ Haploinsufficiency production of *RPS14* results in defective ribosome biogenesis with activation of the innate immune system and the p53 pathway in mutant erythroblasts, which in

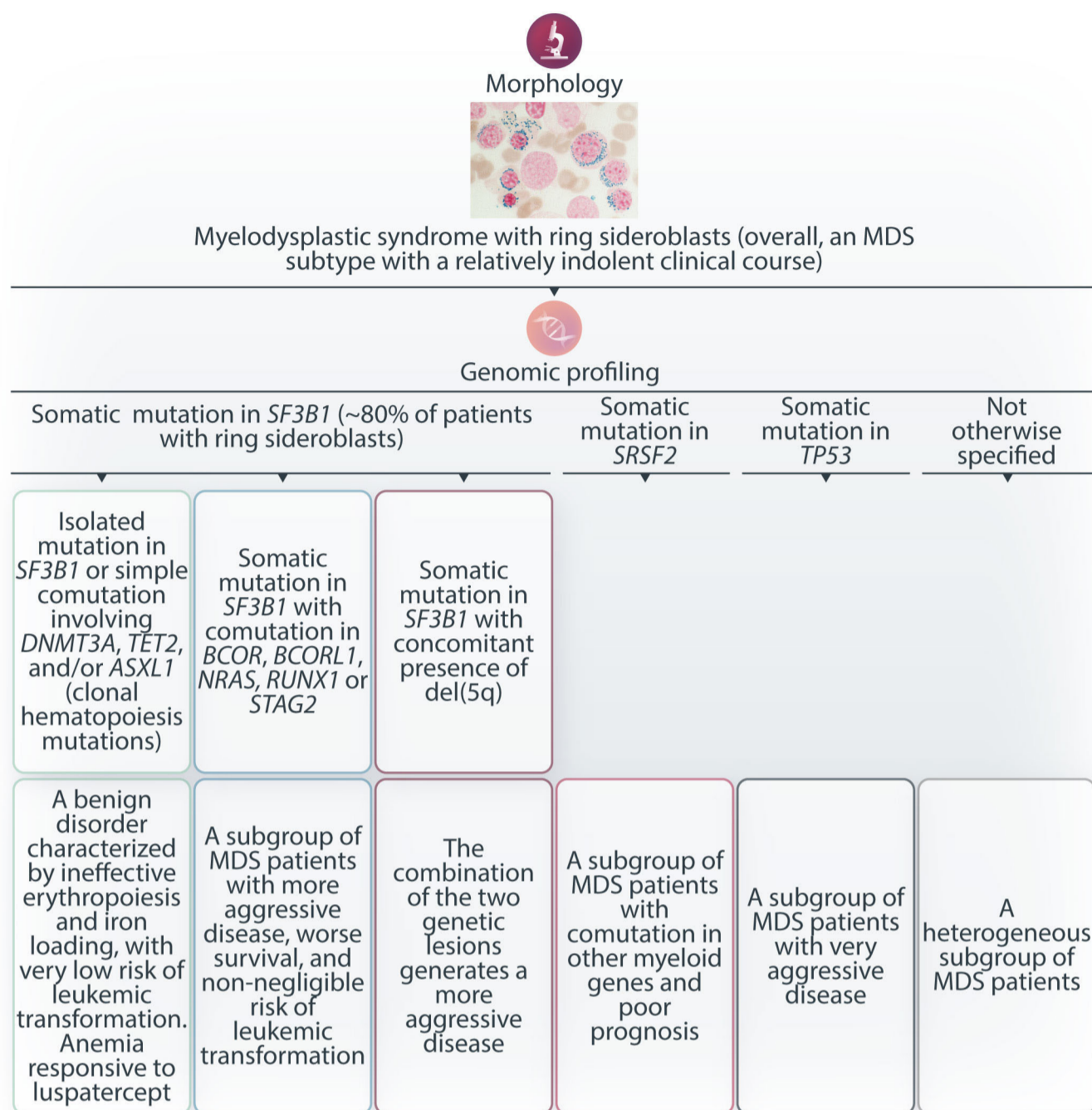


Figure 4. The diagnosis of myelodysplastic syndromes with ring sideroblasts is made through the use of Perls staining on a bone marrow aspirate. Within this morphologically defined condition, genomic profiling enables the identification of distinct molecular subtypes with different clinical outcomes. The image of ring sideroblasts is reproduced from the paper by Malcovati & Cazzola,¹⁰⁵ with permission from John Wiley and Sons. MDS: myelodysplastic syndromes.

turn causes excessive apoptosis of erythroid precursors, defective red blood cell production, and macrocytic anemia.¹¹⁰⁻¹¹² Haploinsufficient production of the physiological repressors of megakaryocytopoiesis *MIR145* and *MIR146A* in megakaryocytes is responsible for the abnormal maturation of these hematopoietic precursors, characterized by dysplastic features (hypolobated nucleus) with an increase in platelet production.^{113,114}

Lenalidomide treatment can lead to transfusion independence in approximately two-thirds of MDS-del(5q) patients, with many achieving cytogenetic remission.¹¹⁵ This immunomodulatory agent induces hematopoietic stem cell failure by promoting the ubiquitination and proteasomal degradation of casein kinase 1A1.^{109,116} In MDS-del(5q), mutant hematopoietic cells have only one copy of the *CSNK1A1* gene, rendering them more sensitive to lenalidomide than normal cells with two intact alleles.¹¹⁶

This explains why lenalidomide treatment can selectively suppress the del(5q) clone and restore normal hematopoiesis in a subset of patients, thus representing a truly targeted therapy. Nevertheless, the efficacy of lenalidomide may be diminished by additional genetic lesions present in patients. For instance, patients with MDS-del(5q) and concomitant *TP53* mutation are less likely to respond with amelioration of anemia and restoration of polyclonal hematopoiesis.¹¹⁷

The median duration of response to lenalidomide is a couple of years, after which most patients show re-emergence of the del(5q) clone and recurrence of anemia, with a high rate of leukemic transformation.^{1,118} It is now established that lenalidomide treatment may be associated with disease progression due to the selection of adverse subclones. This is particularly true for patients harboring recurrent variants of *TP53* or *RUNX1*, in whom a

selective pressure mechanism leads to the expansion of lenalidomide-resistant *TP53*- or *RUNX1*-mutant cells.^{117,119} It is noteworthy that a recent study has demonstrated that lenalidomide treatment confers a selective advantage to *TP53*-mutant hematopoietic stem and progenitor cells, which may lead to the development of therapy-related myeloid neoplasms.¹²⁰

The above observations, summarized in Figure 5, suggest that the distinction between MDS-del(5q) with or without concomitant *TP53* mutations may be clinically relevant for therapeutic decision-making. In a study of 682 patients with MDS-del(5q), approximately one-fifth of patients were found to carry a somatic mutation in *TP53*.¹²¹ A biallelic *TP53* inactivation or monoallelic mutation with a VAF >20% was associated with inferior survival and a higher risk of leukemic transformation.¹²¹ While *ad hoc* studies are needed, we believe that genomic profiling should be used to guide and monitor lenalidomide treatment in patients with MDS-del(5q).

***TP53*-mutant myelodysplastic syndrome**

TP53 is the most frequently mutated gene in human cancer, as evidenced by the 42% of samples carrying this genetic lesion in the Pan-Cancer cohort.¹²² Somatic mutations in this gene are found in 10% to 20% of patients with MDS and are common in all myeloid neoplasms, defining a group of disorders with an overall poor prognosis.¹²³ Since *TP53* encodes a tumor suppressor protein, somatic mutations can be either monoallelic or biallelic: the latter condition is better defined as a multi-hit *TP53* state, which can be the result of different mechanisms (Figure 6).

The IWG-PM conducted a study in a group of 3,324 peri-diagnostic and treatment-naïve patients with MDS or closely related myeloid neoplasms. The purpose of the study was to evaluate the effect of *TP53* allelic state on genomic stability, clinical presentation, outcome, and response to therapy.⁸¹ Of the total number of patients, 378 patients had

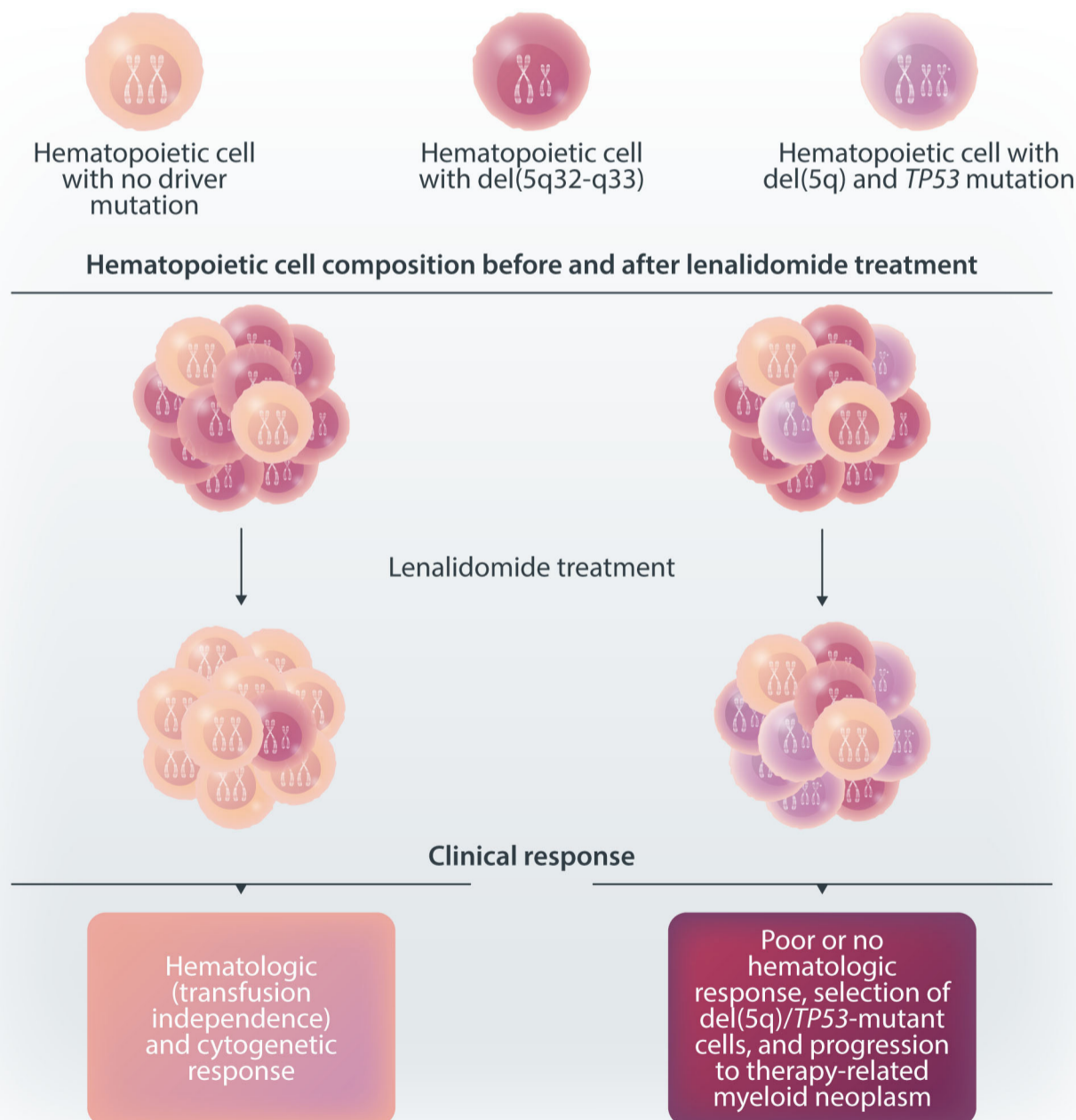


Figure 5. A schematic representation of the bone marrow hematopoietic cell composition in patients with myelodysplastic syndrome-del(5q) and its relation to potential outcomes of lenalidomide treatment. The efficacy of lenalidomide treatment is negatively affected by the presence of subclones with a *TP53* mutation, which may also be responsible for the progression to therapy-related myeloid neoplasms^{117,120} (see also Figure 6).

oncogenic mutations in *TP53*: one-third had monoallelic mutations, while two-thirds had multiple hits (multi-hit) consistent with biallelic targeting. The allelic state of *TP53* was found to have relevant clinical implications.⁸¹ MDS patients with monoallelic mutations did not differ from *TP53* wild-type patients with respect to clinical outcomes and response to therapy. By contrast, *TP53* multi-hit patients had a median overall survival of approximately 9 months with a very high risk of leukemic transformation. These patients responded poorly to medical treatment, namely hypomethylating agents and lenalidomide. In addition, they had high relapse rates after allogeneic transplantation. A recent analysis by the Blood and Marrow Transplant Clinical Trials Network, however, has provided somewhat different results regarding allogeneic transplantation.¹²⁴ This study showed that reduced-intensity transplantation improves

survival in patients with *TP53*-mutant MDS, regardless of *TP53* allelic state, although the absolute survival benefit was modest.

The prognosis of MDS patients with *TP53* mutation may be negatively affected by other factors such as clone size and blast count. For instance, in patients with therapy-related MDS, a *TP53* mutation VAF $\geq 10\%$ and a blast count $>10\%$ were associated with a poor prognosis.¹²⁵ Collectively, the available evidence suggests that assessment of *TP53* allelic state and *TP53* mutation VAF is critical for diagnostic and prognostic accuracy in MDS. Patients with *TP53*-mutant MDS with biallelic inactivation and/or adverse-risk cytogenetics have exceptionally poor outcomes.¹²⁶ Dedicated prospective randomized trials are needed to determine who may benefit from medical treatment or allogeneic transplantation.

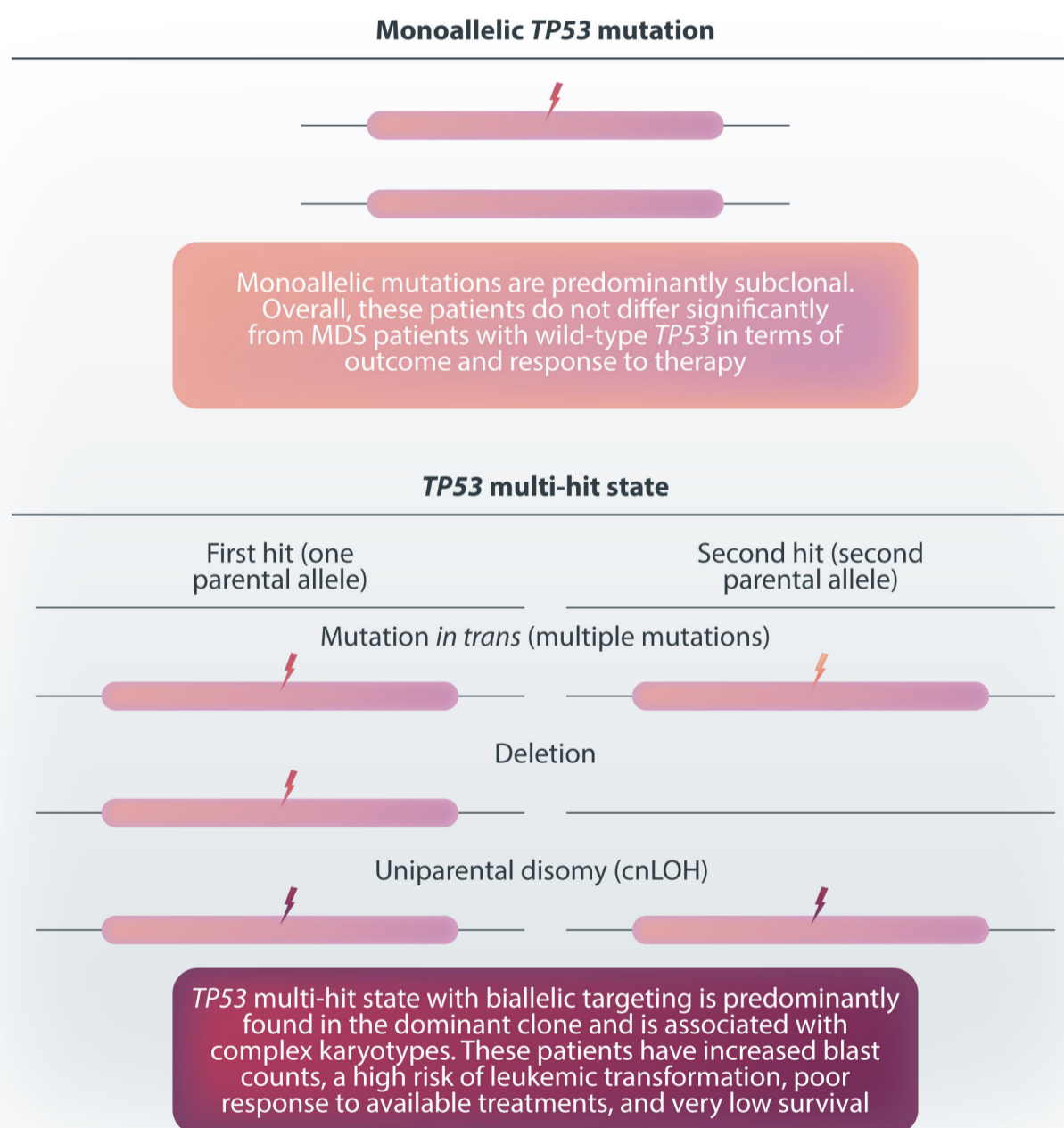


Figure 6. Schematic representation of the allelic state of myelodysplastic syndromes with mutated *TP53*, illustrating the mechanisms that can be responsible for the inactivation of the second allele. In the study by Bernard *et al.*,⁸¹ 33% of *TP53*-mutated patients had a monoallelic mutation, while 67% had a multi-hit state. It is important to underline that a precise methodology for discerning a *TP53* multi-hit state necessitates the implementation of *ad hoc* copy number and loss of heterozygosity analysis.⁸¹ Without this complete approach, it is not possible to make any reliable claims concerning the allelic state. MDS: myelodysplastic syndromes; cnLOH: copy neutral loss of heterozygosity.

Myelodysplastic syndrome/acute myeloid leukemia

The ICC of myeloid neoplasms and acute leukemia has introduced the new category of MDS/AML.¹⁰ Patients with this entity have 10% to 19% bone marrow blasts, any cytogenetic aberration except those that define AML, and any gene mutation except mutant *NPM1*, *CEBPA*, or *TP53*. Patients with MDS/AML should be eligible for both MDS and AML trials.¹⁰

A distinct condition is MDS/AML with mutated *TP53*, which is characterized by 10% to 19% bone blasts and *TP53* mutation VAF >10%. Grob *et al.* performed a molecular and clinical analysis of patients with *TP53*-mutant AML (bone marrow blasts \geq 20%) compared with patients with *TP53*-mutant MDS with excess blasts (bone marrow blasts 10-19%).¹²⁷ These studies demonstrated that the two conditions do not differ in molecular characteristics and survival and should, therefore, be considered a single molecular entity.

DDX41-mutant myelodysplastic syndrome

The concept of a genetic predisposition to MDS is best illustrated by *DDX41*-associated susceptibility to myeloid neoplasms.⁵³ This condition is associated with heterozygous germline mutations in the *DDX41* gene and, in the vast majority of subjects, has no clinical phenotype prior to progression.⁵⁶ Nevertheless, in a subset of patients, hypocellular bone marrow and mild cytopenia may be observed.⁵¹ In approximately half of cases, progression to MDS is associated with the acquisition of a somatic mutation in the second *DDX41* allele, which typically occurs in the sixth or seventh decade of life. The lifetime risk of developing a myeloid malignancy is approximately 50% in mutant *DDX41* carriers, with a higher prevalence observed in males than in females.⁵⁶

Patients with *DDX41*-mutant MDS typically present with excess blasts and have a high risk of leukemic transformation. This risk is essentially limited to individuals carrying truncating variants, a molecular feature that is much more predictive of leukemic transformation than both the IPSS-R and IPSS-M.⁵⁶ Additionally, since almost all truncating variants are germline, the identification of a truncating *DDX41* allele on genomic profiling to detect somatic mutations should prompt germline genetic testing.⁵⁷

Patients with *DDX41*-mutant MDS are potential candidates for allogeneic transplantation and germline genetic testing is mandatory for donor selection to ensure that a related donor does not carry the deleterious germline variant.⁵⁰ Additionally, patients with deleterious germline *DDX41* variants frequently develop severe acute graft-versus-host disease after allogeneic stem cell transplantation, a complication that may be caused by a pro-inflammatory milieu that stimulates donor-derived T cells.⁵⁷ The use of post-transplant cyclophosphamide may reduce the risk of severe acute graft-versus-host disease in these patients.⁷⁶

Residual polyclonal hematopoiesis, assessment of treatment response, and minimal residual disease in myelodysplastic syndromes

Whole-genome sequencing studies of hematopoietic stem cells and their progeny indicate that hematopoiesis in normal individuals is massively polyclonal until approximately the age of 65 years.¹²⁸ Using passenger mutations as barcodes, Mitchell *et al.* estimated that a stable population of 20,000 to 200,000 hematopoietic cells contributes equally to blood cell production in adults less than 65 years of age.¹²⁸ This estimate is consistent with previous calculations based on sequencing data from nearly 50,000 healthy individuals.¹²⁹ In contrast, in individuals aged over 70 years, hematopoiesis was oligoclonal, with a significant proportion of circulating blood cells being derived from only 12 to 18 hematopoietic clones.¹²⁸ This dramatic decrease in clonal diversity may suggest that more than 99% of hematopoietic stem cells are lost by the seventh to eighth decade of life.¹³⁰ While most surviving hematopoietic clones do not carry known driver mutations, this period of life is also typically characterized by the emergence of hematopoietic clones carrying mutations in genes involved in epigenetic regulation, splicing, and apoptosis.¹³¹

In MDS patients, hematopoiesis is by definition clonal, with the vast majority of bone marrow and circulating blood cells being derived from the dominant clone.^{87,132} In most cases, this clone is heterogeneous, with one or more subclones associated with additional driver mutations. The extent and presence of residual polyclonal hematopoiesis in patients with MDS are uncertain. The majority of patients diagnosed with these disorders are between the ages of 65 and 70 years, and hematopoiesis is oligoclonal in nature at this age. Observations on residual normal hematopoiesis are mainly available in MDS-del(5q). In a randomized clinical trial, one-third of patients receiving lenalidomide (10 mg daily) achieved complete cytogenetic remission, suggesting restoration of normal hematopoiesis.¹¹⁵ A study was conducted to specifically investigate the del(5q) lesion in subsets of hematopoietic cells during lenalidomide treatment.¹¹⁸ The study demonstrated that rare del(5q)-mutant stem cells persist even after complete clinical and cytogenetic remission, suggesting resistance to lenalidomide that was later associated with the recurrence of the del(5q) clone and clinical relapse.¹¹⁸

MDS-del(5q) has a relatively simple molecular pathophysiology, resulting from a single genetic lesion that causes clonal proliferation and all associated clinical manifestations.¹ In other MDS subtypes, especially higher-risk MDS, the pathogenesis of disease is typically multistep and more complex. Uy *et al.* used genomic profiling of serial bone marrow samples to monitor MDS tumor burden during treatment with decitabine.¹³³ They found that all patients,

even those in complete remission, showed a persistent measurable tumor burden. More recently, Nannya *et al.* studied higher-risk MDS patients who were treated with azacitidine.¹³⁴ Targeted-capture sequencing was used to analyze somatic gene mutations in bone marrow samples before and after treatment. In most cases, pre-treatment mutations persisted even after treatment, but the clone size decreased significantly in subjects who achieved complete remission. Overall, these observations suggest that hypomethylating agents are unlikely to alter the clonal architecture of MDS significantly, even in patients who achieve a complete hematologic response. They also raise the question of whether elderly patients with higher-risk MDS have a significant number of normal (unmutated) residual stem cells.

Genomic profiling can be a valuable tool for monitoring the response to allogeneic transplantation in patients with MDS. Duncavage *et al.* used enhanced exome sequencing to achieve this objective and identified at least one validated somatic mutation in almost all patients.¹³⁵ Patients who had persistent mutations with a VAF of at least 0.5% in their bone marrow 30 days after transplantation had a higher risk of disease progression. Tobiasson *et al.* recently conducted a study on the association between measurable residual disease and relapse after allogeneic transplantation.¹³⁶ Patient-specific mutations were identified using a targeted sequencing panel and then followed up with droplet digital polymerase chain reaction analysis, a rapid and clinically feasible technique with a sensitivity of 0.1%. In 42 out of 44 cases, disease relapse was preceded by positive measurable residual disease in the bone marrow. This approach could be employed in clinical trials on pre-emptive therapeutic strategies with the objective of improving transplantation outcomes.

Conclusions

The application of genome sequencing has the potential to markedly improve the management of MDS and related

disorders, enabling the implementation of precision medicine approaches. This potential should be fully exploited at all levels, from the design of clinical trials to the management of patients.

The vast majority of clinical trials conducted thus far in MDS have not incorporated genome sequencing into their trial design. This must be rectified, primarily for the benefit of the patients who generously donate cells, time, and energy to allow clinicians to perform clinical studies. Genomic profiling is of paramount importance for the evaluation of the effect of investigational treatments on hematopoietic cell populations. This approach can indeed document the suppression of mutant clones with the restoration of normal hematopoiesis, as well as the emergence of new abnormal clones under selection pressure.

The implementation of genome sequencing in clinical settings is now a realistic prospect, but it will require the establishment of specialized infrastructures within public health systems, involving close collaboration between healthcare institutions, academia, and the life-sciences industry. It thus becomes a matter of allocating public funds properly, with the objective of facilitating the realization of this prospect.

Disclosures

No conflicts of interest to disclose.

Contributions

MC and LM conceived and wrote this article.

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