Diagnosis and Management of Myelodysplastic Syndromes

A Clinical Guide

Aziz Nazha
Editor

Springer

Diagnosis and Management of Myelodysplastic Syndromes

Aziz Nazha
Editor

Diagnosis and Management of Myelodysplastic Syndromes

A Clinical Guide

Springer

Editor
Aziz Nazha
Department of Hematology
and Medical Oncology
Cleveland Clinic
Cleveland, OH
USA

ISBN 978-3-030-51877-6 ISBN 978-3-030-51878-3 (eBook)
https://doi.org/10.1007/978-3-030-51878-3

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Myelodysplastic syndromes (MDS) are a group of clonal disorders characterized by pancytopenias and the risk of progression to acute myeloid leukemia. The diagnosis of MDS can be challenging as several other diseases could mimic MDS. The outcome of MDS patients is very heterogeneous with some of the patients staying alive decades after their diagnosis while others die within few months of their diagnosis. The only potentially curative option for MDS is allogeneic stem cell transplant, but a significant number of patients will not qualify either due to their age or other comorbidities. The treatment goals for patients with the lower-risk disease is to improve quality of life and decrease transfusion burden while the goal of treating patients with higher risk disease is to prolong their life. There are only three FDA approved drugs for the treatment of MDS patients that include: azacitidine, decitabine, and lenalidomide. Novel therapeutic agents are desperately needed to improve the outcome of these patients.

In this book, 10 groups of international MDS experts have provided a concise but yet comprehensive perspectives on different areas of the disease management from biology, pathophysiology, and clinic presentation of MDS to the disease diagnosis and treatment algorithms for lower and higher-risk diseases. Rena Buckstein reviews the epidemiology and various etiologies of MDS and how the disease presents at diagnosis while Goel and Hasserjian review the pathological diagnosis of MDS and its challenges. Chan et al take a deep dive into the biology and pathophysiology of MDS with del5q while Savona and colleagues review the biology of the MDS at the stem cell level. Haferlach and Schmidts discuss the molecular landscape of MDS while Bejar reviews how to use molecular data to differentiate MDS from its mimics like idiopathic cytopenia of unknown significance or clonal cytopenia of unknown significance. Bewersdorf and Zeidan summarize recent developments in prognostic models. As MDS is mainly characterized as a lower-risk and a higher-risk disease, Hambley and DeZern review the treatment algorithms for lower-risk disease while Fenaux and Ades summarize the treatment algorithms for the higher-risk disease. Finally, Kubasch and Platzbecker review at length the indications and clinical implications of allogeneic stem cell transplants in MDS patients.

I hope the readers will enjoy these highly curated reviews by international MDS experts and find them useful in their clinical practices and research expeditions.

Cleveland, OH, USA Aziz Nazha

Acknowledgment

To my wife Sarah and my kids George and Sophia, without their support and love, this book would never have happened. Thank you for all your sacrifices, I love you and cannot imagine my life without you.

To all the authors and co-authors of this book, thank you for your time and effort.

To all my mentors and colleagues who helped me throughout my career, thank you for your help and guidance.

To all my patients, thank you for making me live a life full of grace and gratitude.

Contents

Contributors

Lionel Adès Service D'hématologie Séniors, Hôpital St Louis, Assistance Publique – Hôpitaux de Paris (APHP) and Université de Paris, Paris, France

Rafael Bejar Moores Cancer Center, University of California San Diego, La Jolla, CA, USA

Jan Philipp Bewersdorf Department of Internal Medicine, Section of Hematology, Yale School of Medicine, Yale University, New Haven, CT, USA

Rena Buckstein Odette Cancer Center, Sunnybrook Health Sciences Center, Toronto, ON, USA

Onyee Chan Department of Medicine, University of South Florida, Tampa, FL, USA

Division of Malignant Hematology, Moffitt Cancer Center, Tampa, FL, USA

Amy E. DeZern Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA

Division of Hematology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Pierre Fenaux Service D'hématologie Séniors, Hôpital St Louis, Assistance Publique – Hôpitaux de Paris (APHP) and Université de Paris, Paris, France

P. Brent Ferrell Department of Medicine and Program in Cancer Biology, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA

Shalini Goel Department of Pathology and Lab Medicine, Medanta – The Medicity Hospital, Gurugram, India

Torsten Haferlach MLL Munich Leukemia Laboratory, Munich, Germany

Bryan C. Hambley Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA

Division of Hematology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Robert P. Hasserjian Department of Pathology-WRN 244, Massachusetts General Hospital, Boston, MA, USA

Anne Sophie Kubasch Medical Clinic and Policlinic, Department of Hematology, Cellular Therapy and Hemostaseology, Leipzig University Hospital, Leipzig, Germany

German MDS Study Group (D-MDS), Leipzig, Germany

The European Myelodysplastic Syndromes Cooperative Group (EMSCO), Leipzig, Germany

Alan List Division of Malignant Hematology, Moffitt Cancer Center, Tampa, FL, USA

Uwe Platzbecker Medical Clinic and Policlinic, Department of Hematology, Cellular Therapy and Hemostaseology, Leipzig University Hospital, Leipzig, Germany

German MDS Study Group (D-MDS), Leipzig, Germany

The European Myelodysplastic Syndromes Cooperative Group (EMSCO), Leipzig, Germany

David Sallman Division of Malignant Hematology, Moffitt Cancer Center, Tampa, FL, USA

Michael R. Savona Department of Medicine and Program in Cancer Biology, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA

Ines Schmidts MLL Munich Leukemia Laboratory, Munich, Germany

Chetasi Talati Division of Malignant Hematology, Moffitt Cancer Center, Tampa, FL, USA

Matthew T. Villaume Department of Medicine and Program in Cancer Biology, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA

Amer M. Zeidan Department of Internal Medicine, Section of Hematology, Yale School of Medicine, Yale University, New Haven, CT, USA

Part I
Biology, Pathophysiology, and Clinical Presentation of MDS

Chapter 1
Epidemiology, Etiology, and Clinical Presentation of Myelodysplastic Syndromes

Rena Buckstein

Introduction

Myelodysplastic syndromes (MDS) encompass a family of clonal myeloid stem cell disorders that increase with age, characterized by dysplastic and ineffective hematopoiesis and high frequency of cytogenetic abnormalities and genetic mutations. The disease frequently presents with peripheral blood cytopenias, macrocytosis, anisocytosis, and poikilocytosis and is diagnosed by bone marrow aspirate + biopsy with cytogenetic testing. The phenotype is ineffective hematopoiesis with a propensity to develop acute myeloid leukemia (AML). It has undergone a number of varied diagnostic criteria and classifications over the years ranging from the French American British (FAB) criteria [1] and the World Health Organization (WHO) classifications in 1999 [2], 2002 [3], 2008 [4], and 2016 [5] (Fig. 1.1). Major differences between the 2008 and 2016 classifications include the replacement of "refractory anemia" with by "MDS" with, the collapsing of "refractory anemia, thrombocytopenia and neutropenia" into "MDS with single lineage dysplasia," and the replacement of "refractory anemia with ring sideroblasts" with MDS with ring sideroblasts (RS) and single lineage dysplasia or multilineage dysplasia (MDS-RS-SLD, MDS-RS-MLD). The international classification of disease (ICD) codes for MDS have evolved over time and they do not encompass all forms of MDS. In the ninth edition of the International Classification of Diseases (ICD-9-CM), MDS was coded as a disease of the blood and blood-forming organs (ICD 238.72–238.75) but was reclassified as a neoplasm in the tenth edition (ICD-10: D46) and the ICD for Oncology Third Edition (ICD-03), the classification system used by population-based cancer registries [6, 7]. When WHO reclassified MDS as a neoplastic disease and ICD-03 was implemented internationally, it became reportable to National Cancer Institute

R. Buckstein (✉)
Odette Cancer Center, Sunnybrook Health Sciences Center, Toronto, ON, USA
e-mail: Rena.buckstein@sunnybrook.ca

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3_1

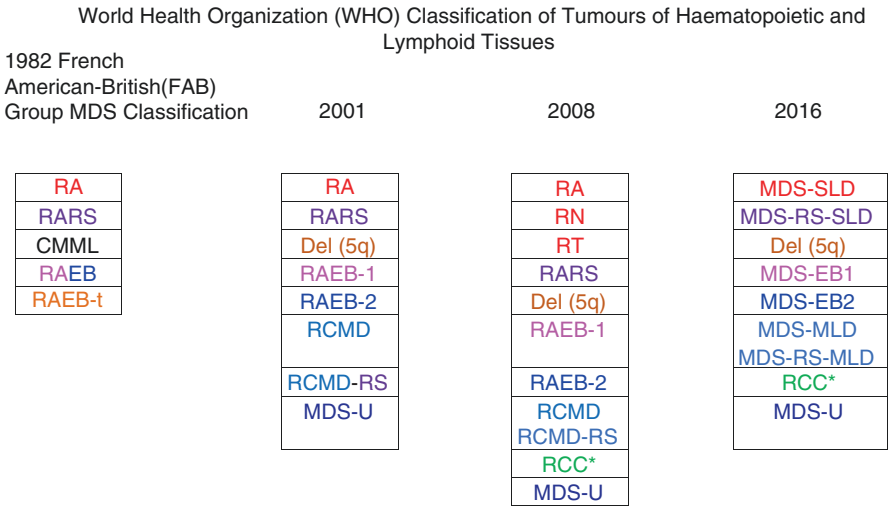

Fig. 1.1 The classification systems for MDS from 1982 to 2016. Entities that encompass the same subtype are color coded. *Legend*: *RA* refractory anemia, *RARS* refractory anemia with ring sideroblasts, *CMML* chronic myelomonocytic leukemia, *RAEB* refractory anemia with excess blasts, RAEB-T refractory anemia with excess blasts in transformation, Del5q MDS with isolated del5q, RAEB-1 refractory anemia with excess blasts type 1, RAEB-2 refractory anemia with excess blasts type 2, RCMD refractory cytopenia with multilineage dysplasia, RCMD refractory cytopenia with multilineage dysplasia and ring sideroblasts, MDS-U MDS unclassifiable, RN refractory neutropenia, RT refractory thrombocytopenia, RCC refractory cytopenia of childhood, MDS-SLD MDS with single lineage dysplasia, MDS-MLD MDS with multilineage dysplasia, MDS-EB1 MDS excess blasts type 1, MDS-EB2 MDS with excess blasts type 2, * provisional. (Adapted from Figure 1 of Zeidan et al. [13])

Surveillance, Epidemiology, and End Results (SEER) Program in 2001 and other cancer registries worldwide. SEER is the authoritative source on cancer incidence and survival in the USA and representing approximately 26.2% of the US population [8]. Using SEER data encompassing the years 2001–2003, US incidence of MDS was first published in 2007 [9] and updated in 2008 with the inclusion of data from North American Association of Cancer Registries (NAACR) which encompasses 82% of the US population [10] and included 24,798 patients with MDS overall. Major findings from both studies included the following: the overall age-adjusted (AA) incidence was 3.3 cases/100,000, this was a disease diagnosed at a median age of 76 with 86% of cases aged ≥60 years, and incidence was increasing dramatically with age (Fig. 1.2); men had a significantly higher incidence rate than women (4.4 versus 2.5 per 100,000/year); (AA) MDS incidence was more common in Caucasian (3.3) than Black (2.4), Asian/Pacific Islander (2.5), and American Indian/Alaska native (1.2) patients; MDS was associated with a 3-year overall and relative survival of only 35% and 42%, respectively. The incident cases (defined by FAB) included 14% refractory anemia (RA), 10% refractory anemia with ring sideroblasts (RARS), 11% refractory anemia with excess blasts (RAEB), 2% refractory anemia with

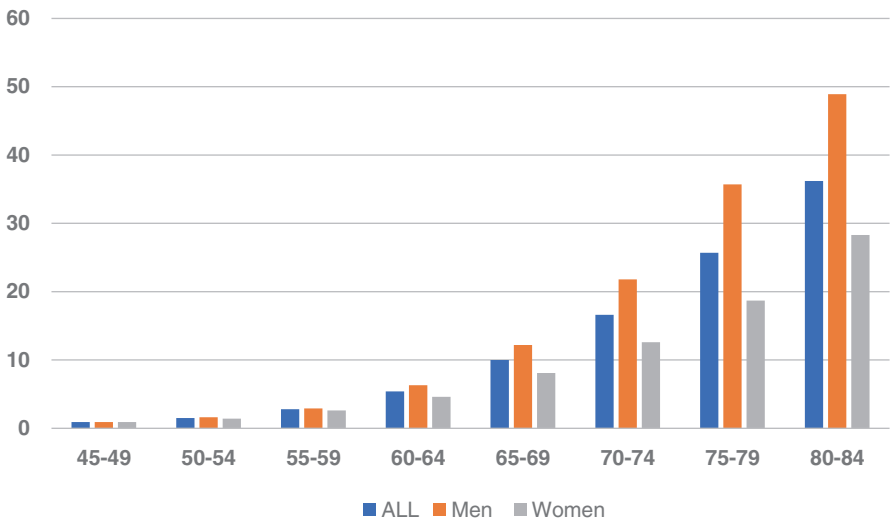

Fig. 1.2 SEER 2001–2003 Incidence/100,000 (*y* axis) according to age categories overall and by sex

excess blasts in transformation (RAEB-T), 2% refractory cytopenia with multi-lineage dysplasia (RCMD), and 2% deletion 5q MDS (del5q). Importantly, 56% incident cases had no MDS subtype specified therefore the observed subtype distribution may not be entirely representative. Incidence increased over the 3 years from 3.3/100,000 in 2001 to 3.8/100,000 in 2004 [10] with an estimated 9700 new cases made in 2004. Increasing incidence after 2001 has been reported by many disease registries [11, 12] and may be a function of increased recognition and reporting in an aging population. Using a SEER November 2017 submission, Zeidan et al. estimated the 2015 incidence to be 4 cases/100,000 with 13,400 new cases of MDS diagnosed annually in the USA [13], suggesting a leveling off in age-adjusted incidence in recent years. The incidence data reported in some other Western countries are summarized in Fig. 1.3 and are very aligned with that of SEER and the North American Association of Central Cancer Registries (NAACR). Differences may relate to sources (registry versus claims-based and chart review), years of case ascertainment, ICD-version codes used, the inclusion or exclusion of entities no longer classified as MDS such as chronic myelomonocytic leukemia (now classified as an overlap MDS/MPN since 2001) or refractory anemia with excess blasts in transformation (now classified as AML in WHO since 2001), or differences in ethnic makeup and population age.

In addition to ICD codes that do not always align with correct or histologically confirmed diagnoses, another significant limitation to relying on cancer registries for disease incidence is their reliance on inpatient reporting. For example, only 4% of the MDS incident cases from NAACR originated from physician's offices [10]. Using a novel, more stringent Medicare claim-based algorithm that looked at blood

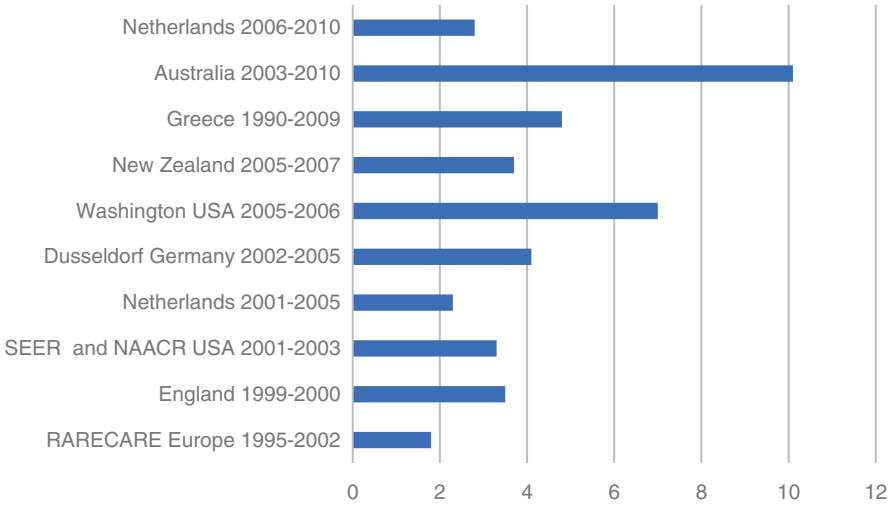

Fig. 1.3 MDS Incidence/100,000 in Western Countries from 1995–2010. *Reference Source Legend:* the Netherlands 2006–2010 [11]; Australia 2003–2010 [27]; Greece 1990–2009 [12]; New Zealand 2005–2007 [75]; Washington, USA, 2005–2006 [76]; Dusseldorf, Germany, 2002–2005 [22]; the Netherlands 2001–2005 [11]; SEER and NAACR, USA, 2001–2003 [10]; England 1999–2000 [77]; RARECARE, Europe, 1995–2002 [78]

work and bone marrow testing applied to beneficiaries residing in a SEER region between 2001–2005, Cogle et al. identified more than 9600 MDS cases not captured by SEER. For 2005, they estimated the MDS incidence to be almost fourfold higher than the SEER incidence in persons 65 years of age or older (75 versus 20 cases/100,000) [6].

A similar underestimation of true MDS incidence was also reported in a population-based linkage study in Australia with the annual incidence for those aged 65 years and older estimated to be 68/100,000 [14]. Despite these higher incidence rates, most experts acknowledge that this likely *still* represents an underrepresentation of true incidence because of either underreporting of pathologically confirmed cases to cancer registries (if not mandated) or the failure to perform diagnostic bone marrows in the investigation of unexplained anemia, a highly prevalent problem in older adults [15]. Supporting this, an interrogation of electronic pathology reports in Florida during 2006 identified that uncaptured cases of MDS by the Florida cancer registry made up 38% of the total true MDS cases. This led to a calculated incidence of 5.3 cases/100,000 (60% higher than SEER) [7]. Using physician billing claims of the ICD-9 code for MDS (not entirely specific to MDS) in 2003, Goldberg et al. identified the incidence to be 162 cases/100,000 with a median age at diagnosis of 77 and 45,000 newly diagnosed cases. During the 3-year follow-up, 73% of 512 patients suffered cardiac events (62% new) with an age-adjusted odds ratio compared with non-MDS Medicare patients of 2.1 (95% CI 1.7–2.5). MDS patients also had an increased prevalence of diabetes, dyspnea, hepatic diseases, and infectious complications. Of interest was a higher 3-year survival rate of

60% compared with 35% of SEER highlighting the potential referral and reporting biases of cancer registries versus community settings or improved supportive care over time [16].

The epidemiology of MDS reported in Asian countries has been reported to differ from North America and Europe. In one study, Chinese patients with MDS (compared with Western patients) were younger at diagnosis (median 49 vs 65–73 years) and had lower percentages of RARS (2.8 vs 6.6–15.3%) and chronic myelomonocytic leukemia (CMML) (5.2 vs 11.7–31%). Similarly, incidences of single chromosome 5 and 7 abnormalities were lower than those in Western countries (2.2 vs 17.8–42.5%) while complex karyotypes were more common (39% versus 16–25%) [17]. These differences have been observed in other Asian countries such as Korea [18] and Japan [19], and younger age (56–61) at diagnosis has also been reported in Thailand, Turkey, and Central Africa in smaller series (review) [20]. In an analysis of the International Working Group for Prognosis of MDS database (IWG-PM) that encompassed 7012 patients, 300 Japanese (JPN), and 5838 Caucasian (CAUC) patients aged >39 years old were compared. JPN patients were 5.5 years younger at diagnosis (65.5 versus 71), had lower rates of RARS (4 vs 12.6%) and del5q (1.3 versus 4.7%), but higher rates of refractory cytopenia with multilineage dysplasia (41 versus 28%). JPN patients had lower hemoglobin (85 versus 99 g/L), platelet counts (75 vs 130 × 10^9/L), and absolute neutrophil counts (1.3 versus 1.91 × 10^9/L) and were less likely to be red blood cell transfusion dependent at diagnosis (25 versus 33%). While cytogenetic risk categories did not differ, there were some differences in selected karyotypic aberrations. CAUC patients were more likely to fall into very low international prognostic scoring system revised (IPSS-R) (19.5 versus 10%) and Low international prognostic scoring system (IPSS) (38.5 versus 20%) risk categories. Time to AML did not differ between ethnic groups but the OS was significantly longer in JPN even adjusted for age, FAB, and IPSS-R categories. The impact of cytopenias on overall survival (OS) and leukemia-free survival (LFS) was lower in JPN but the impact of BM blasts and cytogenetics risk group was higher [21].

Prevalence

Prevalence is harder to quantify and may be increasing with the aging population and the availability of some disease-modifying agents that extend life. In 2003, it was estimated to be 13/10000 in Dusseldorf, Germany [22], and applying comparable numbers to the USA, the prevalence would be estimated to be 42, 600 cases in 2018 [13]. This is probably still an underestimate. Applying assumptions from the national health and nutrition evaluation survey (NHANES) study on anemia, Sekeres et al. estimated there may have been as many as 170,000 prevalent cases in 2010 [23]. If one applied the estimated prevalence of 155 cases/100,000 (derived from private health insurance claims), the estimated prevalence in the USA could be as high as 500,000 cases [24] although not all insurance claims are histologically proven cases of MDS.

Clinical Presentation

The median age at diagnosis ranges from 71–79 years of age [9, 25–27]. MDS is more common in males than females with a male to female sex ratio of 3–4:2, a ratio that increases with age [9].

The disease usually presents with either symptomatic or asymptomatic cytopenias in one or more cell lines. The most common symptoms may include fatigue (55%), fever and infection (15%), or bleeding (8%) [12] as well as dyspnea on exertion or angina. The blood film indices may show macrocytosis, anisocytosis, tear drops, and a dimorphic population in the red blood cells. The leukocytes may demonstrate a left shift, "pelgeroid" neutrophils, and circulating blasts. Lymphadenopathy and splenomegaly are uncommon but may be seen with the MDS/MPN overlap syndromes. Fifty-two percent present with anemia (hgb < 10 g/L), 18% with neutropenia (ANC < 0.8×10^9/L), and 40% with thrombocytopenia (plt < 100×10^9/L) [26], 35% have bicytopenia and 12% pancytopenia [12] at diagnosis.

In the international working group for myelodysplastic syndromes (IWG-PM) project database ($n = 7012$), 32% of patients were transfusion dependent at diagnosis [26]. In a US physician survey that included 670 newly diagnosed patients, only 22% with lower risk disease were dependent on transfusions compared with 68% of higher risk patients [25]. Similarly, 29% of MDS patients in the European Union MDS registry of lower risk MDS patients (EUMDS) were transfusion dependent at diagnosis [28], however, 41% received transfusions within 1 year of diagnosis with transfusion dose density in the first year correlating with progression-free survival [29]. In the Medicare Standard Analytic File study, the 40% of transfusion-dependent MDS patients suffered a higher rate of clinical complications like infections, dyspnea, hepatic events, diabetes, fungal infections, and cardiac events [16].

Survival, Cause of Death, and Leukemia Rates

The median survival of MDS patients ranges from 0.8–8.8 years overall [26] but varies considerably according to age, comorbidities, transfusion dependence, frailty, karyotype, selected mutations, number and depth of cytopenias, and marrow blast percentage. Prognosis for OS and leukemia-free survival (LFS) according to a number of established risk scores will be discussed in detail in a later chapter. Approximately 25–30% of MDS patients develop AML [30] and the excess mortality in MDS appears to be driven primarily by non-leukemic factors [31]. Three and 5-year overall survival rates are 42% and 29%, respectively [32], and the 3-year relative survival of MDS patients compared with age matched controls is only 45% [10]. Where known, the leading causes of accelerated death in Germany were AML (47%), infection (27%), bleeding (10%), and cardiovascular disease (8%) [30]. Using SEER data from 2001–2011, the most common cause of death in >21,000 patients were MDS/leukemia (50%), cardiovascular disease (19%), infection (5%),

and other (11%) with cardiovascular disease cause of death rates matching that of MDS/leukemia after 5 years [33]. Despite the advent of some disease-modifying agents, the overall survival in MDS has not convincingly improved since 2001 [11, 31, 34].

Association of MDS with Autoimmune Diseases

Autoimmune and inflammatory conditions (AICs) are observed in 7–28% of patients with MDS and may precede, coincide, or follow the diagnosis of MDS [35, 36]. This is not surprising since some of the same immune perturbations that result in AICs (inflammatory cytokines, autoantibodies, increased T regulatory cells, and myeloid-derived suppressor cells) or the treatments to suppress them may contribute to the pathogenesis of MDS. Having an AIC may increase the risk of developing MDS (OR 1.5–2.0) [37, 38] possibly due to chronic immune stimulation although one cannot discount the potentiating or causal effects of anti-inflammatory/immunosuppressive agents used to treat the AIC or a common genetic or environmental susceptibility to both. There may be usually a short latency between AIC and MDS [39] and some but not all studies have found AICs to be more common in younger MDS patients and those with higher risk disease [35]. The AICs associated with MDS span polyarthritis, neutrophilic dermatosis (Sweet's syndrome), connective tissue diseases, vasculitis, hypothyroidism, immune thrombocytopenia purpura (ITP), psoriasis, and autoimmune hemolytic anemia. In a pooled retrospective analysis from Moffit and Kings College Hospital of 1408 patients, 27% had an AIC, the most common being hypothyroidism (44%), ITP (12%), and rheumatoid arthritis (11%). MDS patients with AIC in this series were comprised disproportionately of women (44%), associated more with RCMD, and were less likely to be RBC transfusion dependent. In addition, MDS with AIC had improved overall survival compared with those without (median OS 60 mos. versus 45 mos., $p = 0.011$) even adjusting for IPSS-R and age [40]. However, other smaller studies have either found no effect or inferior OS for MDS patients and AICs [35, 41].

Risk Factors for MDS

Age is one of the biggest risk factors for the development of MDS. One contributing factor may be the acquisition of genetic mutations during aging in hematopoietic stem cells that provide a clonal proliferative advantage but without cytopenias or dysplasia. This phenomenon, deemed age-related clonal hematopoiesis (ARCH) or clonal hematopoiesis of indeterminate potential (CHIP), is observed in 10% of the general population above the age of 60–65 and increases with age. CHIP is associated with an increase in risk of hematologic cancer (HR 11.1–12.9) [42, 43]. Since MDS is a clonal disease whose pathophysiology is linked to chromosomal

abnormalities and somatic mutations in genes that regulate methylation, differentiation, cell signaling, RNA splicing, nuclear transcription, and proliferation, the increased prevalence with age of somatic mutations in genes regulating some of these pathways may explain the higher incidence of MDS with age. This subject is discussed in detail in a later chapter.

In addition, there are hereditary germ-line mutations and syndromes associated with the development of MDS [44] that will be discussed in a later chapter.

A number of occupational, environmental, and lifestyle factors have been associated with MDS.

Pesticide exposure Pesticide exposure appears to be a risk factor for AML in manufacturing workers and pesticide applicators [45], but is this finding applicable to MDS? Because of conflicting case control studies, a large meta-analysis based on 1942 cases and 5359 controls was conducted and included 11 retrospective case-control studies from USA, Italy, UK, France, Serbia, China, and France published between 1990 and 2011. The findings were as follows: A) pesticide exposure was associated with a 95% increased risk of MDS. B) Subgroup analyses showed a stronger effect of pesticide exposure on RA/RARS than on RAEB/RAEB-t with exposed MDS patients having a 63% increased risk of RA/RARS (95% CI 1.06–2.51) and 49% increased risk of RAEB/RAEB-T, respectively (95% CI 0.78–2.84). C) The risk from pesticides was primarily due to exposure to insecticides (OR 1.71, 95% CI 1.22–2.4), not herbicides (OR 1.16, 95% CI 0.55–2.43) and fungicides (OR 0.7, 95% CI 0.2–3.2). D) The adverse effect of pesticide exposure on MDS was observed in Europe (OR 2.13, 95% CI 1.35–3.36) and Asia (OR 2.0, 95% CI 1.17–3.41) but not in the USA (OR 1.52, 95% CI 0.3–7.73) [46].

Obesity and Lifestyle Factors

A prospective cohort study of the national institutes of health (NIH) and the American Association of Retired Persons (AARP) examined the relationship between diet, body mass index (BMI), exercise, and smoking on the development of MDS incident cases identified through state cancer registry databases. Across the USA, 470,000 men and women between the ages of 50–71 were included and 193 incident cases of MDS were identified. Obesity (BMI $\geq$ 30) was associated with a greater than twofold increased risk of MDS and there was a significant positive trend for the relation between BMI and MDS. Physical activity (vigorous physical activity $\geq$3 times/week) had a protective effective effect on MDS development (HR 0.68, 95% CI 0.49–0.95) compared with physical inactivity ($\leq$ 3 x/month). Neither alcohol consumption, fruit and vegetable intake, nor meat intake were associated with MDS [47]. In a meta-analysis of five case-control studies, alcohol consumption was also not significantly associated with MDS [48].

Benzene

Benzene is a volatile organic compound most commonly used for the manufacturing of plastic packaging, insulation, and other products. It is one of the top 20 chemicals produced in the USA, occurs naturally in petroleum products and premium gasoline, and occupational exposure to benzene by inhalation or dermal absorption spans many industries [49]. It is carcinogenic and myelotoxic [50] and its association with acute leukemias is well known for many years [51]. One large hospital-based case control study from China demonstrated a direct exposure-response pattern (threshold >3 parts per million) with refractory cytopenias and multi-lineage dysplasia the most common type of MDS in China [52]. Ambient air exposure to benzene may also be important since it derives from many sources such as automobile emissions, burning wood, cigarette smoke, mining, and many others. Using data from the environmental protection agency (EPA) national air toxics assessment (NATA) program, Teras et al. modeled census tract ambient benzene concentration estimates to examine potential associations with hematologic cancers in a large prospective cohort (n = 115,996) between 1997 and 2013. They found that total ambient benzene was associated with MDS (HR 1.16, 95% CI 1.01–1.33 per $\mu g/m^3$), follicular lymphoma (in men), and T cell lymphomas [49]. In another study, latency (<10 years) from last exposure, total length of occupational exposure (2–10 years), and younger age at first exposure (age < 30) also influenced the associations between benzene and MDS/AML [53]. The conclusive associations between benzene exposure and MDS has been recently expertly reviewed [54].

Smoking

Interestingly, smoking, a significant source of benzene exposure, was not positively associated with all MDS or RCMD in the Chinese study highlighted above [52] but showed associations with RAEB and refractory anemia among men in Japan with a HR for current smokers relative to never smokers of 2.11 (95% CI 0.9–4.9) [55]. The largest meta-analysis of 10 case-control studies evaluating 1800 cases and 2000 controls found an overall risk of 1.45 (95% CI 1.2–1.7) with ever smoking [48]. In the only prospective cohort study of the NIH/AARP, former smokers (HR 1.68, 95% CI 1.17–2.41) and current smokers (HR 3.17, 95% CI 2.02–4.98) had significantly elevated risks of MDS, with the highest risk in those currently smoking more than 1 pack of cigarettes/day (HR 4.70, 95% CI 2.68–8.24) [47]. In one study, patients with chromosomal abnormalities were more likely to be ever smokers (OR 1.92) than patients with normal karyotype [56], and in another study, poor risk karyotypes such as chromosome 7 abnormalities were more associated with smoking as well [57].

Therapy-Related MDS

MDS is deemed therapy related if it follows treatment with cytotoxic chemotherapy or irradiation and is classified as a therapy-related myeloid neoplasm in the WHO classification and is combined with T-AML and T-MDS/myeloproliferative neoplasms (MPN) due to similar prognostic and genetic profiles [4]. T-MDS patients tend to be younger (median age 68), and have a higher proportion of IPSS-R high risk scores compared with primary MDS, have short time to progression to overt AML, and median survivals of 16 months [58].

Therapy-related MDS comprises 10–15% of MDS cases [10, 25] and is associated with karyotypic abnormalities 85–90% of the time (compared with 45–50% in de-novo MDS) [59]. The most frequent primary diseases are non-Hodgkin's lymphoma (28%), breast cancer (16%), myeloma (6%), prostate cancer (96%), Hodgkin's lymphoma (5%), and gastrointestinal tumors with preceding chemotherapy in 75% and radiotherapy in 47%. The most common chemotherapeutic drugs received included alkylating agents (65%), topoisomerase inhibitors (44%), antitubulin agents (26%), and antimetabolites (26%) [58]. In a nation-wide nested case control study from Taiwan of 6300 cancer patients, the adjusted odds ratios for developing MDS after radiotherapy and chemotherapy were 1.53 (95% CI 1.33–1.77) and 1.51 (95% CI 1.25–1.82), respectively, and there was an interaction effect when both chemotherapy and radiotherapy were administered [60]. Radiation has also been linked with increased risk of MDS in a number of tumors including breast cancer [61, 62], prostate cancer [63], lymphoma [64, 65], and thyroid cancer [66], although the absolute increased risks are often small . After involved field radiotherapy, the risk appears to peak at 2 years and normalize after 10–15 years [67].

MDS that develops after exposure to alkylating agents (cyclophosphamide, melphalan, chlorambucil, etc.) often has a latency of 5–10 years and is associated with deletions and unbalanced translocations affecting chromosome 5 and 7 or complex karyotypes, often with associated *TP53* mutations. MDS that develops after exposure to topo-isomerase-2 inhibitors (adriamycin, topotecan, etoposide) is less common, occurs earlier (2–3 years), and is associated with an mixed lineage leukemia (MLL) translocation at 11q23 or RUNX1/AML1 at 21q22 [68]. T-MDS is also linked with exposures to nucleoside analogs (e.g., fludarabine) [69] and anti-metabolites (Imuran) [68, 70]. ARCH or CHIP may also be linked to T-MDS possibly due to the clonal selection advantage upon bone marrow reconstitution post chemotherapy. This is relevant for both lymphoma [71] and solid tumor patients [72, 73] and has been linked with pretreatment *TP53* and PPM1D mutations [72]. The risk of T-MDS/AML post autologous stem cell transplant (ASCT) ranges from 1% to 20% and has been associated with cumulative doses of alkylating agents, total body irradiation, graft source, and preparative regimens [64], so it is notable that clonal mutations were found in the stem cell product in 67% of the 12/401 patients with non-Hodgkin's lymphoma who underwent an ASCT and went on to develop a therapy-related myeloid neoplasm (TMN) [74]. The 10-year cumulative incidence for T-MN was 14.1% vs 4.3% for those with and without clonal mutations, respectively; $P = 0.002$.

Summary

MDS is a heterogeneous clonal bone marrow malignancy diagnosed primarily in older patients aged 71–76 with an age-adjusted incidence derived from cancer registries of 4–5 cases/100,000 that increases tenfold above the age of 80. Incidence and prevalence have increased since its initial definition as a disease in 1982 primarily due to better recognition, investigation of anemia, the availability of therapies, and the aging population. These data are likely significant underestimates since incidence data derived from chart reviews and reimbursement claim databases are significantly higher. MDS is more common in men and Caucasians. The expected survival of an MDS patient is curtailed by >50% due to disease-related complications that include acute myeloid leukemia, infections, bleeding, and cardiovascular disease and is dominated by non-leukemic causes. The WHO classification of MDS has undergone three revisions over a 15-year period, is continuously evolving, and is currently based on the degrees of bone marrow dysplasia, blast %, the presence of ring sideroblasts, and karyotype. While age is the biggest risk factor, environmental exposures to radiation, pesticides, benzene, and lifestyle factors that include smoking, obesity, and physical inactivity have been associated with higher rates of MDS. Exposure to mutagenic chemotherapy and radiotherapy is associated with therapy-related MDS, a devastating condition that accounts for 10–15% of all MDS and is expected to rise in prevalence as the population ages and the number of cancer survivors increase. Finally, age-related clonal hematopoiesis and selected germ line mutations are also risk factors for the development of de-novo and T-MDS.

References

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol. 1982;51(2):189–99.
2. Harris NL, Jaffe ES, Diebold J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol. 1999;17(12):3835–49.
3. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100(7):2292–302.
4. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937–51.
5. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
6. Cogle CR, Craig BM, Rollison DE, List AF. Incidence of the myelodysplastic syndromes using a novel claims-based algorithm: high number of uncaptured cases by cancer registries. Blood. 2011;117(26):7121–5.
7. Cogle CR, Iannacone MR, Yu D, et al. High rate of uncaptured myelodysplastic syndrome cases and an improved method of case ascertainment. Leuk Res. 2014;38(1):71–5.
8. Park HS, Lloyd S, Decker RH, Wilson LD, Yu JB. Overview of the surveillance, epidemiology, and end results database: evolution, data variables, and quality assurance. Curr Probl Cancer. 2012;36(4):183–90.

9. Ma X, Does M, Raza A, Mayne ST. Myelodysplastic syndromes: incidence and survival in the United States. Cancer. 2007;109(8):1536–42.
10. Rollison DE, Howlader N, Smith MT, et al. Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs. Blood. 2008;112(1):45–52.
11. Dinmohamed AG, Visser O, van Norden Y, et al. Trends in incidence, initial treatment and survival of myelodysplastic syndromes: a population-based study of 5144 patients diagnosed in the Netherlands from 2001 to 2010. Eur J Cancer. 2014;50(5):1004–12.
12. Avgerinou C, Alamanos Y, Zikos P, et al. The incidence of myelodysplastic syndromes in Western Greece is increasing. Ann Hematol. 2013;92(7):877–87.
13. Zeidan AM, Shallis RM, Wang R, Davidoff A, Ma X. Epidemiology of myelodysplastic syndromes: why characterizing the beast is a prerequisite to taming it. Blood Rev. 2019;34:1–15.
14. McQuilten ZK, Wood EM, Polizzotto MN, et al. Underestimation of myelodysplastic syndrome incidence by cancer registries: results from a population-based data linkage study. Cancer. 2014;120(11):1686–94.
15. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood. 2004;104(8):2263–8.
16. Goldberg SL, Chen E, Corral M, et al. Incidence and clinical complications of myelodysplastic syndromes among United States Medicare beneficiaries. J Clin Oncol. 2010;28(17):2847–52.
17. Chen B, Zhao WL, Jin J, et al. Clinical and cytogenetic features of 508 Chinese patients with myelodysplastic syndrome and comparison with those in Western countries. Leukemia. 2005;19(5):767–75.
18. Lee JH, Lee JH, Shin YR, et al. Application of different prognostic scoring systems and comparison of the FAB and WHO classifications in Korean patients with myelodysplastic syndrome. Leukemia. 2003;17(2):305–13.
19. Oguma S, Yoshida Y, Uchino H, Maekawa T, Nomura T, Mizoguchi H. Clinical characteristics of Japanese patients with primary myelodysplastic syndromes: a co-operative study based on 838 cases. Anemia Study Group of the Ministry of Health and Welfare. Leuk Res. 1995;19(3):219–25.
20. Germing U, Neukirchen J, Haas R. The epidemiology of myelodysplastic syndromes. Clin Leuk. 2008;2(1):34–8.
21. Miyazaki Y, Tuechler H, Sanz G, et al. Differing clinical features between Japanese and Caucasian patients with myelodysplastic syndromes: analysis from the International Working Group for Prognosis of MDS. Leuk Res. 2018;73:51–7.
22. Neukirchen J, Schoonen WM, Strupp C, et al. Incidence and prevalence of myelodysplastic syndromes: data from the Dusseldorf MDS-registry. Leuk Res. 2011;35(12):1591–6.
23. Sekeres MA. The epidemiology of myelodysplastic syndromes. Hematol Oncol Clin North Am. 2010;24(2):287–94.
24. Cogle CR, Kurtin SE, Bentley TG, et al. The incidence and health care resource burden of the myelodysplastic syndromes in patients in whom first-line hypomethylating agents fail. Oncologist. 2017;22(4):379–85.
25. Sekeres MA, Schoonen WM, Kantarjian H, et al. Characteristics of US patients with myelodysplastic syndromes: results of six cross-sectional physician surveys. J Natl Cancer Inst. 2008;100(21):1542–51.
26. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454–65.
27. McQuilten ZK, Polizzotto MN, Wood EM, Sundararajan V. Myelodysplastic syndrome incidence, transfusion dependence, health care use, and complications: an Australian population-based study 1998 to 2008. Transfusion. 2013;53(8):1714–21.
28. de Swart L, Smith A, Fenaux P, et al. Early mortality in 1000 newly diagnosed MDS patients with low- and intermediate-1 risk MDS in the European Leukemianet MDS (EUMDS) registry. Blood. 2012;120(21):3830.

29. de Swart L, Crouch S, Hoeks M, et al. Impact of red blood cell transfusion dose density on progression-free survival in lower-risk myelodysplastic syndromes patients. Haematologica. 2020;105(3):632–9.
30. Nachtkamp K, Stark R, Strupp C, et al. Causes of death in 2877 patients with myelodysplastic syndromes. Ann Hematol. 2016;95(6):937–44.
31. Nomdedeu M, Pereira A, Ramos F, et al. Excess mortality in the myelodysplastic syndromes. Am J Hematol. 2017;92(2):149–54.
32. Ma X. Epidemiology of myelodysplastic syndromes. Am J Med. 2012;125(7 Suppl):S2–5.
33. Brunner AM, Blonquist TM, Hobbs GS, et al. Risk and timing of cardiovascular death among patients with myelodysplastic syndromes. Blood Adv. 2017;1(23):2032–40.
34. Gangat N, Patnaik MM, Begna K, et al. Survival trends in primary myelodysplastic syndromes: a comparative analysis of 1000 patients by year of diagnosis and treatment. Blood Cancer J. 2016;6(4):e414.
35. Wolach O, Stone R. Autoimmunity and inflammation in myelodysplastic syndromes. Acta Haematol. 2016;136(2):108–17.
36. Grignano E, Jachiet V, Fenaux P, Ades L, Fain O, Mekinian A. Autoimmune manifestations associated with myelodysplastic syndromes. Ann Hematol. 2018;97(11):2015–23.
37. Anderson LA, Pfeiffer RM, Landgren O, Gadalla S, Berndt SI, Engels EA. Risks of myeloid malignancies in patients with autoimmune conditions. Br J Cancer. 2009;100(5):822–8.
38. Kristinsson SY, Bjorkholm M, Hultcrantz M, Derolf AR, Landgren O, Goldin LR. Chronic immune stimulation might act as a trigger for the development of acute myeloid leukemia or myelodysplastic syndromes. J Clin Oncol. 2011;29(21):2897–903.
39. Mekinian A, Grignano E, Braun T, et al. Systemic inflammatory and autoimmune manifestations associated with myelodysplastic syndromes and chronic myelomonocytic leukaemia: a French multicentre retrospective study. Rheumatology (Oxford). 2016;55(2):291–300.
40. Komrokji RS, Kulasekararaj A, Al Ali NH, et al. Autoimmune diseases and myelodysplastic syndromes. Am J Hematol. 2016;91(5):E280–3.
41. Montoro J, Gallur L, Merchan B, et al. Autoimmune disorders are common in myelodysplastic syndrome patients and confer an adverse impact on outcomes. Ann Hematol. 2018;97(8):1349–56.
42. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488–98.
43. Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371(26):2477–87.
44. West AH, Churpek JE. Old and new tools in the clinical diagnosis of inherited bone marrow failure syndromes. Hematology Am Soc Hematol Educ Program. 2017;2017(1):79–87.
45. Van Maele-Fabry G, Duhayon S, Lison D. A systematic review of myeloid leukemias and occupational pesticide exposure. Cancer Causes Control. 2007;18(5):457–78.
46. Jin J, Yu M, Hu C, et al. Pesticide exposure as a risk factor for myelodysplastic syndromes: a meta-analysis based on 1,942 cases and 5,359 controls. PLoS One. 2014;9(10):e110850.
47. Ma X, Lim U, Park Y, et al. Obesity, lifestyle factors, and risk of myelodysplastic syndromes in a large US cohort. Am J Epidemiol. 2009;169(12):1492–9.
48. Du Y, Fryzek J, Sekeres MA, Taioli E. Smoking and alcohol intake as risk factors for myelodysplastic syndromes (MDS). Leuk Res. 2010;34(1):1–5.
49. Teras LR, Diver WR, Deubler EL, et al. Residential ambient benzene exposure in the United States and subsequent risk of hematologic malignancies. Int J Cancer. 2019;145(10):2647–60.
50. Robert Schnatter A, Kerzic PJ, Zhou Y, et al. Peripheral blood effects in benzene-exposed workers. Chem Biol Interact. 2010;184(1–2):174–81.
51. Vigliani EC, Saita G. Benzene and Leukemia. N Engl J Med. 1964;271(17):872–6.
52. Copley GB, Schnatter AR, Armstrong TW, et al. Hospital-based case-control study of MDS subtypes and benzene exposure in Shanghai. J Occup Environ Med. 2017;59(4):349–55.
53. Linet MS, Gilbert ES, Vermeulen R, et al. Benzene exposure response and risk of myeloid neoplasms in Chinese workers: a multicenter case-cohort study. J Natl Cancer Inst. 2019;111(5):465–74.

54. Li W, Schnatter AR. Benzene risk assessment: does new evidence on myelodysplastic syndrome justify a new approach? Crit Rev Toxicol. 2018;48(6):417–32.
55. Ugai T, Matsuo K, Sawada N, et al. Smoking and alcohol and subsequent risk of myelodysplastic syndromes in Japan: the Japan Public Health Centre-based Prospective Study. Br J Haematol. 2017;178(5):747–55.
56. Strom SS, Gu Y, Gruschkus SK, Pierce SA, Estey EH. Risk factors of myelodysplastic syndromes: a case-control study. Leukemia. 2005;19(11):1912–8.
57. Bjork J, Albin M, Mauritzson N, Stromberg U, Johansson B, Hagmar L. Smoking and myelodysplastic syndromes. Epidemiology. 2000;11(3):285–91.
58. Kuendgen A, Tuechler H, Nomdedeu M, et al. An analysis of prognostic markers and the performance of scoring systems in 1837 patients with therapy-related myelodysplastic syndrome – a study of the International Working Group (IWG-PM) for Myelodysplastic Syndromes (MDS). Blood. 2015;126(23):609.
59. Mauritzson N, Albin M, Rylander L, et al. Pooled analysis of clinical and cytogenetic features in treatment-related and de novo adult acute myeloid leukemia and myelodysplastic syndromes based on a consecutive series of 761 patients analyzed 1976-1993 and on 5098 unselected cases reported in the literature 1974-2001. Leukemia. 2002;16(12):2366–78.
60. Sun LM, Lin CL, Lin MC, Liang JA, Kao CH. Radiotherapy- and chemotherapy-induced myelodysplasia syndrome: a nationwide population-based nested case-control study. Medicine (Baltimore). 2015;94(17):e737.
61. Kaplan H, Malmgren J, De Roos AJ. Risk of myelodysplastic syndrome and acute myeloid leukemia post radiation treatment for breast cancer: a population-based study. Breast Cancer Res Treat. 2013;137(3):863–7.
62. Zeidan AM, Long JB, Wang R, et al. Risk of myeloid neoplasms after radiotherapy among older women with localized breast cancer: a population-based study. PLoS One. 2017;12(9):e0184747.
63. Wang R, Zeidan AM, Yu JB, et al. Myelodysplastic syndromes and acute myeloid leukemia after radiotherapy for prostate cancer: a population-based study. Prostate. 2017;77(5):437–45.
64. Pedersen-Bjergaard J, Andersen MK, Christiansen DH. Therapy-related acute myeloid leukemia and myelodysplasia after high-dose chemotherapy and autologous stem cell transplantation. Blood. 2000;95(11):3273–9.
65. Yamasaki S, Suzuki R, Hatano K, et al. Therapy-related acute myeloid leukemia and myelodysplastic syndrome after hematopoietic cell transplantation for lymphoma. Bone Marrow Transplant. 2017;52(7):969–76.
66. Sawka AM, Thabane L, Parlea L, et al. Second primary malignancy risk after radioactive iodine treatment for thyroid cancer: a systematic review and meta-analysis. Thyroid. 2009;19(5):451–7.
67. Radivoyevitch T, Sachs RK, Gale RP, et al. Defining AML and MDS second cancer risk dynamics after diagnoses of first cancers treated or not with radiation. Leukemia. 2016;30(2):285–94.
68. Smith SM, Le Beau MM, Huo D, et al. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. Blood. 2003;102(1):43–52.
69. Leleu X, Soumerai J, Roccaro A, et al. Increased incidence of transformation and myelodysplasia/acute leukemia in patients with Waldenstrom macroglobulinemia treated with nucleoside analogs. J Clin Oncol. 2009;27(2):250–5.
70. Godley LA, Larson RA. Therapy-related myeloid leukemia. Semin Oncol. 2008;35(4):418–29.
71. Takahashi K, Wang F, Kantarjian H, et al. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. Lancet Oncol. 2017;18(1):100–11.
72. Coombs CC, Zehir A, Devlin SM, et al. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. Cell Stem Cell. 2017;21(3):374–82.. e374
73. Gillis NK, Ball M, Zhang Q, et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. Lancet Oncol. 2017;18(1):112–21.

74. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. J Clin Oncol. 2017;35(14):1598–605.
75. Rodger EJ, Morison IM. Myelodysplastic syndrome in New Zealand and Australia. Intern Med J. 2012;42(11):1235–42.
76. De Roos AJ, Deeg HJ, Onstad L, et al. Incidence of myelodysplastic syndromes within a nonprofit healthcare system in western Washington state, 2005-2006. Am J Hematol. 2010;85(10):765–70.
77. Phekoo KJ, Richards MA, Moller H, Schey SA, South Thames Haematology Specialist Committee. The incidence and outcome of myeloid malignancies in 2,112 adult patients in southeast England. Haematologica. 2006;91(10):1400–4.
78. Visser O, Trama A, Maynadie M, et al. Incidence, survival and prevalence of myeloid malignancies in Europe. Eur J Cancer. 2012;48(17):3257–66.

Chapter 2
Morphological, Flow Cytometry, and Cytogenetic Diagnosis of MDS

Shalini Goel and Robert P. Hasserjian

Introduction

Myelodysplastic syndromes (MDS) are a group of clonal hematological proliferations presenting with unremitting cytopenias and morphologic dyspoiesis in one or more hematopoietic cell lineages. The hallmark dyspoiesis can be appreciated morphologically in the peripheral blood and bone marrow, and is associated with aberrant patterns of antigen expression on hematopoietic cells detected by flow cytometry (FCM). FCM can be used as a part of the diagnostic algorithm in suspected cases of MDS; however, in the revised 4th edition WHO Classification of Myeloid Neoplasms published in 2017, the presence of FCM abnormalities alone in the absence of conclusive morphologic features is not considered sufficient to establish a diagnosis of MDS [1].

The term clonal signifies that the abnormal hematopoiesis is due to recurrent genetic abnormalities affecting the MDS stem cells, the proliferation of which overtakes normal hematopoiesis, leading to ineffective hematopoiesis and peripheral blood cytopenias. For nearly 50 years, these genetic abnormalities were detected by bone marrow karyotype, which provides a global view of the full chromosome complement. By conventional karyotyping, about 50% of MDS cases have cytogenetic abnormalities, which usually result in unbalanced losses or gains of genetic material. Specific chromosomal aberrations, along with the degree of cytopenias and percentage of blasts in bone marrow, represent a cornerstone of MDS risk stratification. The International Prognostic Scoring System (IPSS) was defined in 1997 for predicting the prognosis and overall survival in MDS cases and was subsequently

S. Goel
Department of Pathology and Lab Medicine, Medanta – The Medicity Hospital, Gurugram, India

R. P. Hasserjian (✉)
Department of Pathology-WRN 244, Massachusetts General Hospital, Boston, MA, USA
e-mail: rhasserjian@partners.org

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3_2

revised in 2012 [2]. Due to rapid advances in the field of molecular genetics, subkaryotypic genetic abnormalities detected by single nucleotide polymorphism (SNP) arrays and next-generation sequencing (NGS) techniques have increased the percentage of MDS cases with detectable genetic aberrations to over 90%. This chapter addresses the use of morphology (in peripheral blood and bone marrow), FCM, and cytogenetics to diagnose and classify MDS.

Diagnosis and Classification of MDS

In the initial approach to a putative MDS case, the diagnostician must first determine if the basic underlying criteria of MDS are fulfilled and exclude possible non-neoplastic reactive mimics of MDS; the latter are discussed in another chapter. The basic prerequisites to establish a diagnosis of MDS are as follows: (1) the presence of at least one unexplained cytopenia, which is most often anemia (with or without thrombocytopenia and/or neutropenia) and is less commonly isolated neutropenia or thrombocytopenia; and (2) morphologic dysplasia (with or without an increase in blast cells) in at least one of the three hematopoietic lineages manifesting in the bone marrow and/or blood smear. MDS is known to be a clonal disease and this clonality can often be documented by abnormal bone marrow karyotype and/or specific mutations detected by NGS. Additionally, FCM often demonstrates phenotypic abnormalities in myeloblasts and maturing hematopoietic elements. However, while genetic evidence of clonality and abnormal FCM immunophenotype can be supportive of a diagnosis of MDS (and conversely, normal FCM and lack of detectable mutations on a large MDS-directed NGS panel tend to argue against MDS), these findings are insufficient to establish a diagnosis of MDS in the absence of the two prerequisites mentioned above. The only exception is in cases bearing certain MDS-defining cytogenetic abnormalities (discussed later), which can establish a diagnosis of MDS in a cytopenic patient even in the absence of sufficient morphologic dysplasia.

Once a primary diagnosis of MDS is established, the disease must be classified in order to help guide patient management according to the expected disease behavior. The classification of MDS has changed over the years. These hematological conditions were first described in the early 1900s and were labelled as “refractory anemia/preleukemia.” The French-American-British (FAB) co-operative group in 1976 gave them the name “dysmyelopoietic syndromes,” which comprised refractory anemia with excess blasts (RAEB) and chronic myelomonocytic leukemia (CMML). The term was modified to “myelodysplastic syndromes” in 1982, in order to acknowledge the wide range of morphologic findings in the peripheral blood and bone marrow seen in the disease. MDS according to the FAB comprised 5 entities: refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), RAEB, refractory anemia with excess blasts in transformation to AML (RAEB-t), and CMML. This classification was widely used for the next 20 years by clinicians and pathologists and had prognostic value; however, there was broad variation in patient outcome in each category. The World Health Organization (WHO) classification in

2001 built upon the FAB system and added new entities to this category, including the distinction between single and multilineage dysplasia in refractory cytopenia with multilineage dysplasia (RCMD), blast count stratification splitting RAEB into two sub-categories, and the first use of a cytogenetic aberration, an isolated del(5q), to define a new MDS subtype [3]. Additionally, CMML was removed and placed in the MDS/myeloproliferative neoplasm (MDS/MPN) category and RAEB-t was reclassified as AML by reducing the AML-defining blast count from 30% to 20%. In 2008, the WHO also added a provisional category of MDS in children and an unclassifiable MDS (MDS-U) category and also allowed for patients with cytopenias other than anemia (diagnostic names changed to "refractory cytopenia" rather than "refractory anemia") [3].

The latest classification of MDS was published in 2017 by the WHO and is currently the most widely used classification system [1]. This classification removed the terms cytopenia, anemia, neutropenia, and thrombocytopenia from the MDS names and instead used the terms "single lineage dysplasia" or "multilineage dysplasia." Another major change was that cases with erythroid predominance (>50% bone marrow erythroids) and non-erythroid blast count of ≥20%, previously considered to be the erythroid/myeloid erythroleukemia subtype of AML, were re-classified as MDS based on the blast percentage of total bone marrow cells. The following are the MDS disease categories according to the 2017 WHO Classification [1]:

I. Myelodysplastic syndrome with single lineage dysplasia (MDS-SLD)
II. Myelodysplastic syndrome with multilineage dysplasia (MDS-MLD)
III. Myelodysplastic syndrome with ring sideroblasts (MDS-RS)
    (a) MDS-RS and single lineage dysplasia (MDS-RS-SLD)
    (b) MDS-RS and multilineage dysplasia (MDS-RS-MLD)
IV. Myelodysplastic syndrome with isolated del(5q)
V. Myelodysplastic syndrome with excess blasts (MDS-EB)
    (a) MDS-EB-1
    (b) MDS-EB-2
VI. Myelodysplastic syndrome, unclassifiable (MDS-U)
VII. Refractory cytopenia of childhood (provisional)
VIII. Therapy-related MDS (t-MDS)

Morphology in Peripheral Blood and Bone Marrow

Peripheral Blood

Examination of the peripheral blood smear is the first step in the diagnosis of MDS. By definition, all MDS patients have at least one peripheral cytopenia at presentation. The peripheral smear may show the presence of blasts as well as with

dyspoietic granulocytes, red cells, and abnormal platelets. In a five-part cell differential analyzer, certain parameters have been shown to be associated with an underlying MDS. These include NEUT-X and NEUT-Y, which are parameters for neutrophil structure and maturation. NEUT-X is the direct measurement of side scatter diffraction, corresponding to channel number, and is representative of the internal structure of the neutrophils. It correlates with hypogranularity of neutrophils and when taken into consideration with anemia, abnormalities of NEUT-X can be suggestive of an underlying MDS. NEUT-Y is the direct measurement of the fluorescence intensity. These parameters can allow a more detailed workup of cases with higher likelihood of MDS in places with limited resources and also aid in differentiating MDS from secondary causes of cytopenia(s), such as megaloblastic anemia [4]. In the latter condition, patients often present with pancytopenia and dyserythropoiesis, potentially mimicking MDS. However, unlike MDS, NEUT-X and NEUT-Y are noted to be high in these cases, helping differentiate them from MDS and facilitating early diagnosis and cost effectiveness [5].

The dyspoiesis seen in MDS peripheral blood granulocytes is illustrated in Fig. 2.1. It includes pseudo-Pelger–Huët abnormality, hypogranularity, and abnormal nuclear lobation (typically hypolobation or non-lobated nuclei, but also less commonly hypersegmentation). There can be a mild left shift noted in the granulocytic series, with the presence of a variable number of blasts, which are usually myeloblasts (Fig. 2.1 G, H), but can also show features of monocytic differentiation. The percentage of circulating blasts in MDS is variable, but it is always <20%. Auer rods can be seen in the blasts or in immature circulating granulocytes, and if present in MDS, they upgrade the disease to MDS with excess blasts-2 (MDS-EB2). Dyspoietic features seen in the red cells (Fig. 2.2) include basophilic stippling, Howell–Jolly bodies, and poikilocytosis, as well as circulating nucleated RBCs. The platelets can show dyspoiesis in the form of giant platelets, vacuolated and hypogranulated platelets, and megakaryocytic fragments (Fig. 2.2).

Bone Marrow

The bone marrow in MDS usually shows increased cellularity relative to the patient's age; this finding, in the required setting of peripheral cytopenias, exemplifies the ineffective hematopoiesis inherent to MDS (Figs. 2.3, 2.4 and 2.5). However, in 10–20% of cases the marrow is normocellular or hypocellular; the latter is sometimes termed "hypoplastic MDS," although not a formal MDS subtype in the WHO Classification.

As mentioned above, morphologic dysplasia is a defining feature of MDS and there must be significant dyspoiesis in one or more hematopoietic lineages. The WHO suggests that at least 10% of a given lineage should be dyspoietic to consider it significant. However, due to inter-observer variations, dysplasia can be missed or overcalled. Moreover, the presence of dyspoiesis is not pathognomonic of MDS and can be seen in patients with non-MDS conditions. Since the

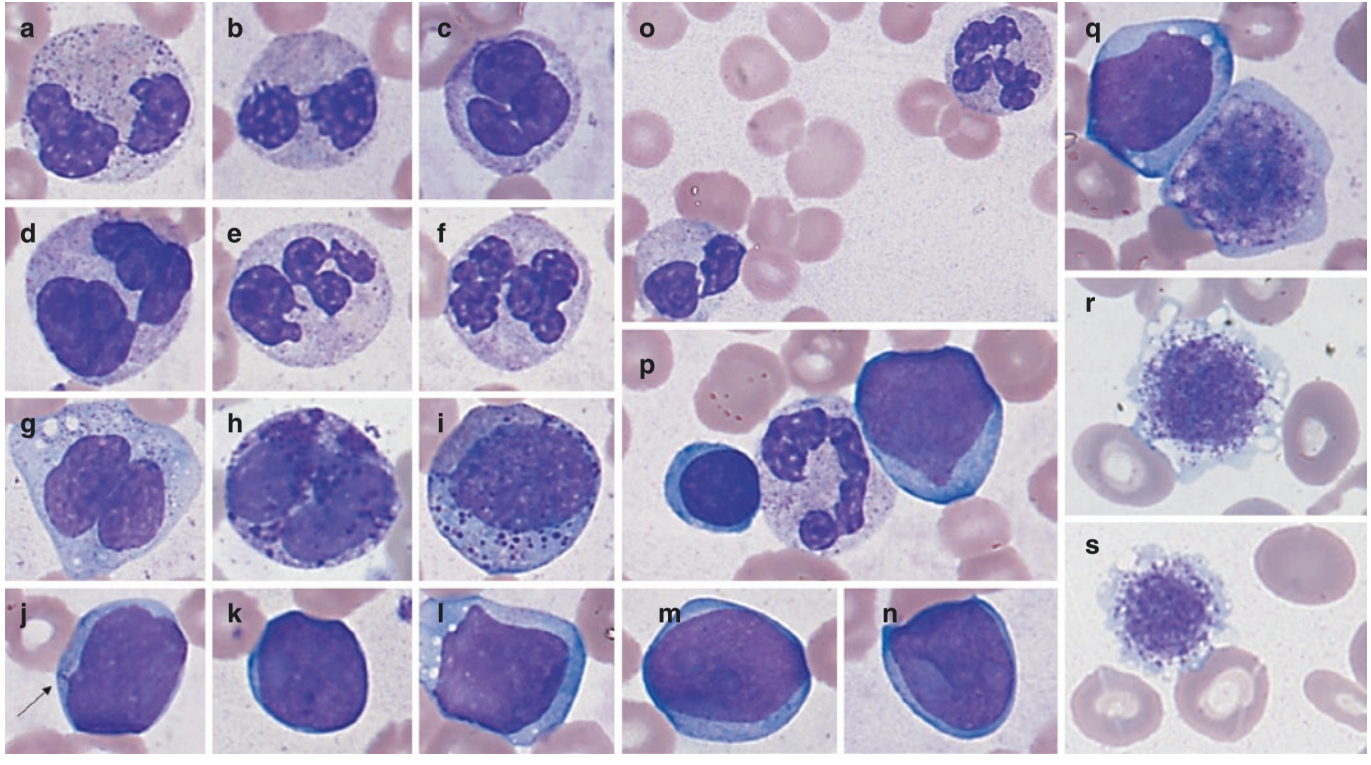

Fig. 2.1 Peripheral smears stained with Leishman and Giemsa (100×). (**a**–**i**) Dyspoietic changes in myeloid cells in MDS. Pseudo–Pelger–Huët cells (**a**, **b**); abnormal lobation of nuclei, including hypersegmentation, and hypogranulation of neutrophils (**c**–**f**); abnormal monocyte (**g**); hypogranular basophil (**h**); abnormally granulated eosinophilic myelocyte (**i**). (**j**–**n**) Circulating blasts in MDS, including small forms (**j**, **k**; arrow in **j** indicating an Auer rod); monoblast (**l**); large forms with prominent nucleoli (**m**, **n**). (**o**, **p**) Combined findings on peripheral smear in MDS. RBC anisocytosis with Pseudo–Pelger–Huët cell and hypersegmented neutrophil (**o**). Blast with a giant dyspoietic neutrophil and nucleated RBC (**p**). (**q**–**s**) Platelet abnormalities in MDS, including platelet giant forms with cytoplasmic vacuolation

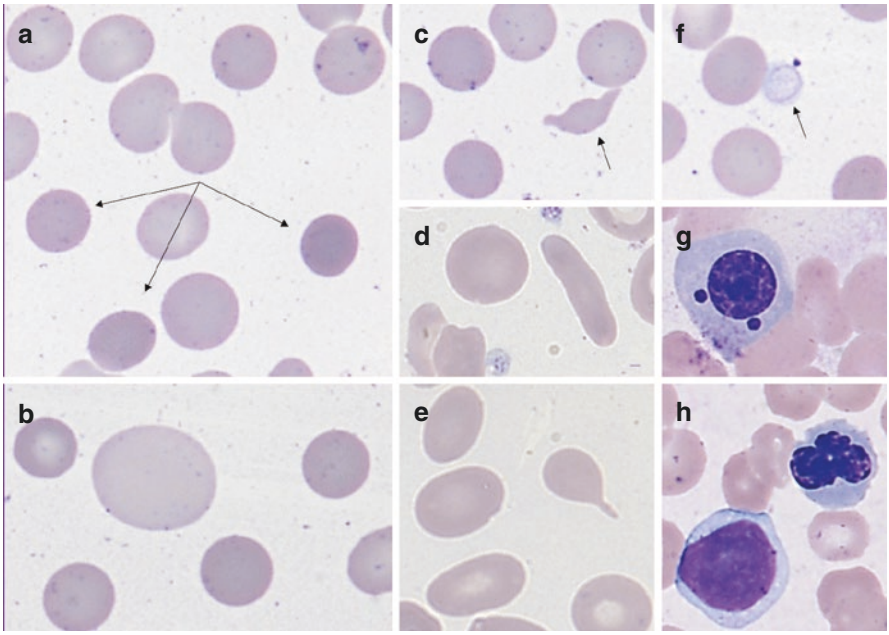

Fig. 2.2 Peripheral smears stained with Leishman and Giemsa (100×) showing abnormalities on red cells and erythroids in MDS, including anisopoikilocytosis with spherocytes (**a**); macrocytes (**b**); fragmented RBCs (**c**); elliptocytes (**d**); teardrop cells (**e**); cabot rings (**f**); basophilic stippling (**g**); and dyspoietic nRBCs (**g**, **h**)

mainstay of diagnosis of MDS is morphology, the various hematological disorders that may show dyspoiesis associated with cytopenias are close differentials. These disorders need to be ruled out before rendering a diagnosis of MDS. One of the most common differential in developing countries is megaloblastic anemia, which presents with macrocytic anemia (with or without other cytopenias) and often with significant dyserythropoiesis and megaloblastoid change, potentially mimicking MDS [4]. Marrow recovery from chemotherapy and infections such as parvovirus B19 can elicit variable dyspoiesis in myeloid and erythroid lineages. Another differential, which is seen in some parts of the world, is the intake of different forms of medicinal therapies that may include heavy metals such a lead, arsenic, or zinc as a constituent. These agents can induce significant trilineage dyspoiesis and sometimes an increase in blast percentage in the peripheral blood and bone marrow. Certain hereditary conditions such as autosomal dominant Pelger–Huët abnormality as well as paroxysmal nocturnal hemoglobinuria, autoimmune disorders, and lymphomas involving the marrow can also cause dyspoiesis [6]. A careful, complete history and laboratory evaluation, including drug history and ancillary microbiological studies, can help in most of the differentials.

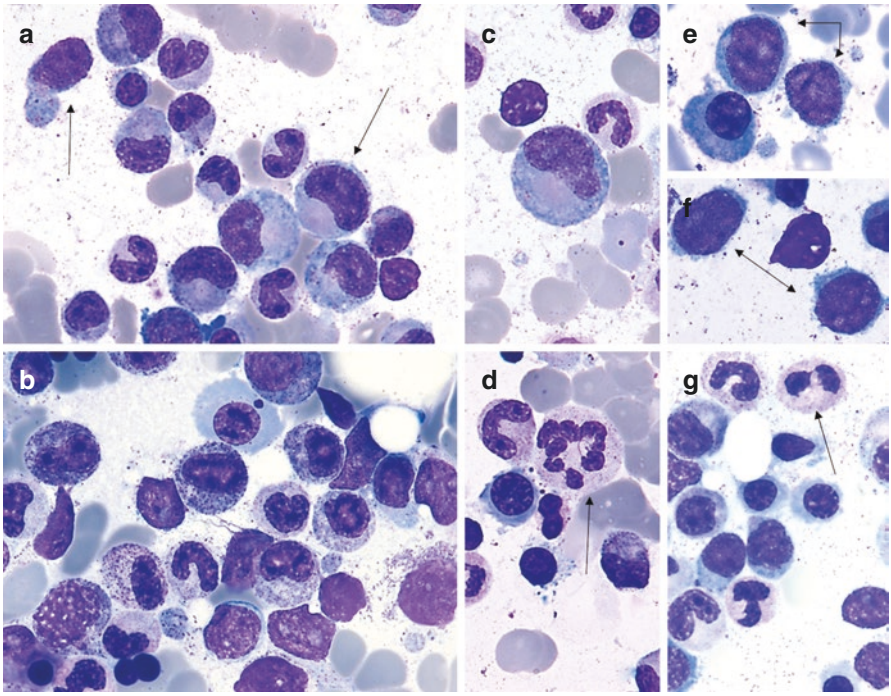

Fig. 2.3 Bone marrow aspirate in MDS showing dyspoiesis in the myeloid lineage, including hypogranulated, variably sized myelocytes (**a**); uneven granulation (**b**); abnormal nuclear lobation in large hypogranular myelocyte (**c**); abnormal nuclear hypersegmentation in neutrophil (**d**); blasts (**e** and **f**); and pseudo-Pelger–Huët cell (**g**). (Leishman and Giemsa, 100×)

Interobserver variations can result from differences in specimen quality, preparation, and staining between different institutions, and hence a high quality of staining is of utmost importance in the overall diagnosis of MDS. Bone marrow aspirate smears are stained by Wright–Giemsa or May–Grunwald–Giemsa and they optimally should contain spicules. Interpretation should be done in areas in which the cells are well spread (Figs. 2.3 and 2.4) rather than crowded. Staining by the Leishman–Giemsa stain enhances nuclear and cytoplasmic details [7]. An iron stain should also be performed on the bone marrow aspirate in any putative MDS case in order to allow for the identification of ring sideroblasts. The bone marrow biopsy should be of sufficient length to include several intertrabecular areas of active hematopoiesis and should be decalcified for as short a period of time as possible to permit sectioning while avoiding deleterious effects that many decalcifying agents have on the morphology and immunostaining results. Thin sectioning (2 to 3 microns) enhances evaluation of the cytology. In addition to Hematoxylin & Eosin staining, a reticulin stain is recommended, as a subset of MDS cases manifests increased reticulin fibrosis. Giemsa staining can be helpful to facilitate the identification of early erythroid elements and distinguish them from myeloblasts. A Perls iron stain

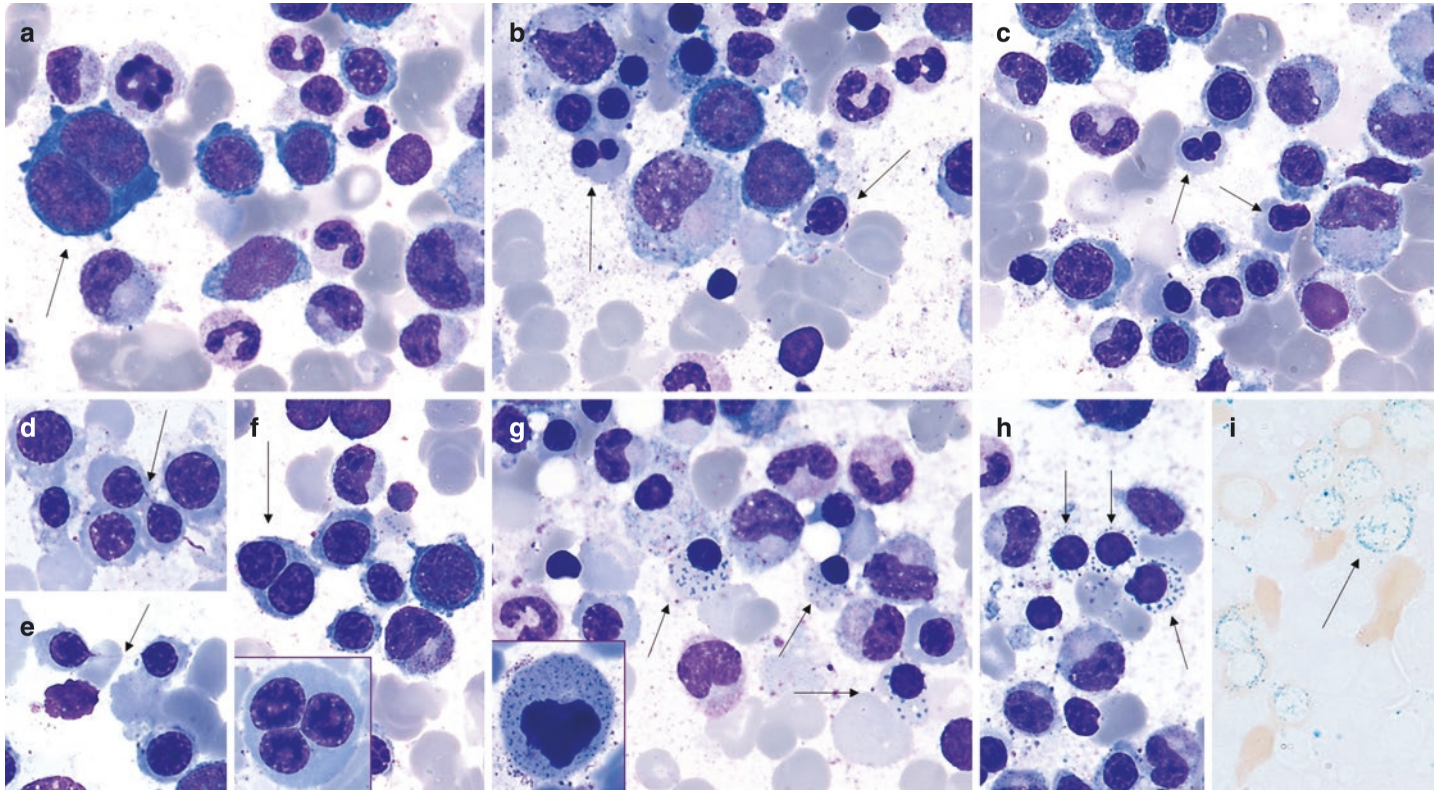

Fig. 2.4 Bone marrow aspirate in MDS showing dyspoiesis in the erythroid lineage, including megaloblasts (**a**, arrow shows a binucleate megaloblast); nuclear budding (**b** and **c**); nuclear bridging (**d** and **e**); binucleate and trinucleate erythroid precursors (**f**, with inset); basophilic stippling (**g**, with inset, and **h**). (Leishman and Giemsa, 100×) Perls Prussian blue stain highlights the presence of ring sideroblasts in a case of MDS-RS (**i; 100x**)

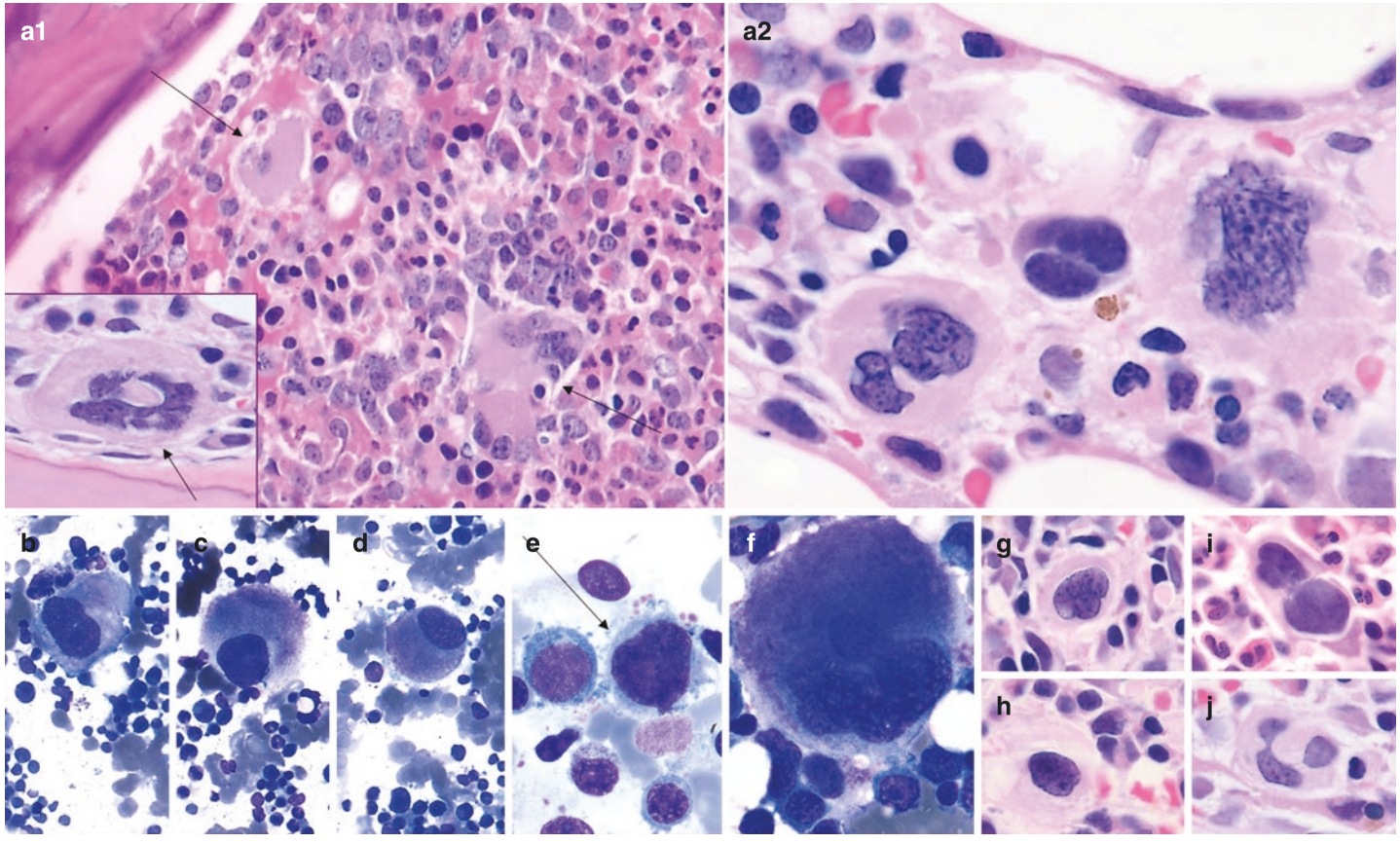

Fig. 2.5 Abnormal megakaryocyte findings in MDS. Bone marrow biopsy (Hematoxylin and Eosin) shows increased cellularity with paratrabecular location of megakaryocytes [**a**1; 40× and 100× (inset)] and loose megakaryocyte clustering (**a**1; 40× and **a**2; 100×). The bone marrow aspirate (Leishman and Giemsa) shows megkaryocytes with hypolobated and non-lobated nuclei (**b–d**; 40×); a megakaryoblast (**e**; 100×); and an abnormally lobated megkaryocyte nucleus (**f**; 100×). In the bone marrow biopsy there are variably sized megakaryocytes with non-lobated, hypolobated, and abnormally lobated nuclei (**g–j**; 100×)

on the core biopsy is not recommended, as the ring sideroblasts are usually not visible in decalcified, paraffin-embedded material.

Granulocytic dyspoiesis in the bone marrow (Fig. 2.3) includes all the features described above in the peripheral blood, as well as hypogranularity, uneven granulation, or abnormally shaped granules in early granulocytic forms. There may be abnormally prominent nucleoli in myelocytes and abnormal nuclear lobation in precursor cells, such as ring-shaped nuclei. The blasts can be normal or increased (≥5% of all cells) in number, and in the biopsy may show abnormal clustering away from the bone trabecular surface where blasts and early myeloid elements normally reside (see Fig. 2.6a1, a3–a4). Blasts can be abnormally large or small in size and show abnormal nuclear features (Fig. 2.3). Erythroid dyspoiesis includes both cytologic dyspoiesis and abnormal disruption of the topographic erythroid islands normally found in the biopsy (see Fig. 2.6b2). Erythroid elements are often left-shifted in MDS. Cytologic erythroid dysplasia is illustrated in Fig. 2.4 and includes megaloblastic changes; nuclear abnormalities such as budding, fragmentation, inter-nuclear bridging, bi-nucleation, and multi-nucleation; and cytoplasmic abnormalities such as blebbing and basophilic stippling. Perls stain for iron should be performed on all bone marrow aspirate smears of possible MDS cases in order to investigate for the presence of ring sideroblasts (Fig. 2.4i). Ring sideroblasts can help establish a diagnosis of MDS (since they are by definition dysplastic erythroids) and may also suggest the specific subcategory of MDS-RS-SLD or MDS-RS-MLD. Megakaryocytic dyspoiesis, illustrated in Fig. 2.5, includes abnormal clustering and paratrabecular localization of megakaryocytes in the bone marrow biopsy. Cytologically, the megakaryocytes usually show pleomorphism, with non-lobated or hypolobated nuclei, abnormal nuclear lobation, widely separated, rounded nuclear lobes, and abnormally small size, including so-called "micromegakaryocytes" (Fig. 2.6c2).

As an adjunct to the morphology on routine stains of the bone marrow aspirate and biopsy, an immunohistochemical profile on the biopsy can also help in the final diagnosis of these conditions. CD34 aids in identifying blasts and their abnormal clustering and may help achieve a more accurate enumeration of blasts in cases in which the aspirate is compromised due to hemodilution or preparation artifacts (Fig. 2.6a1–a4). CD71, E-cadherin, glycophorin, and other erythroid markers can highlight abnormal disruption or localization of erythroid islands (Fig. 2.6b1, b2). CD61, CD42b, or Factor VIII highlight abnormal megakaryocyte topography and cytology, particularly very small forms (so-called micromegakaryocytes) that are often missed on routine stains (Fig. 2.6c1, c2). P53 can also be a diagnostic aid, since if strongly expressed in many hematopoietic cells, it supports a diagnosis of MDS and usually correlates with a *TP53* mutation and an adverse prognosis (particularly in the therapy-related setting).

Flow Cytometry

The clonal hematopoietic stem cells in MDS usually exhibit aberrant antigenic expression and thus show an abnormal pattern when interrogated by FCM, as highlighted below. In the WHO revised fourth edition, FCM is not a required diagnostic

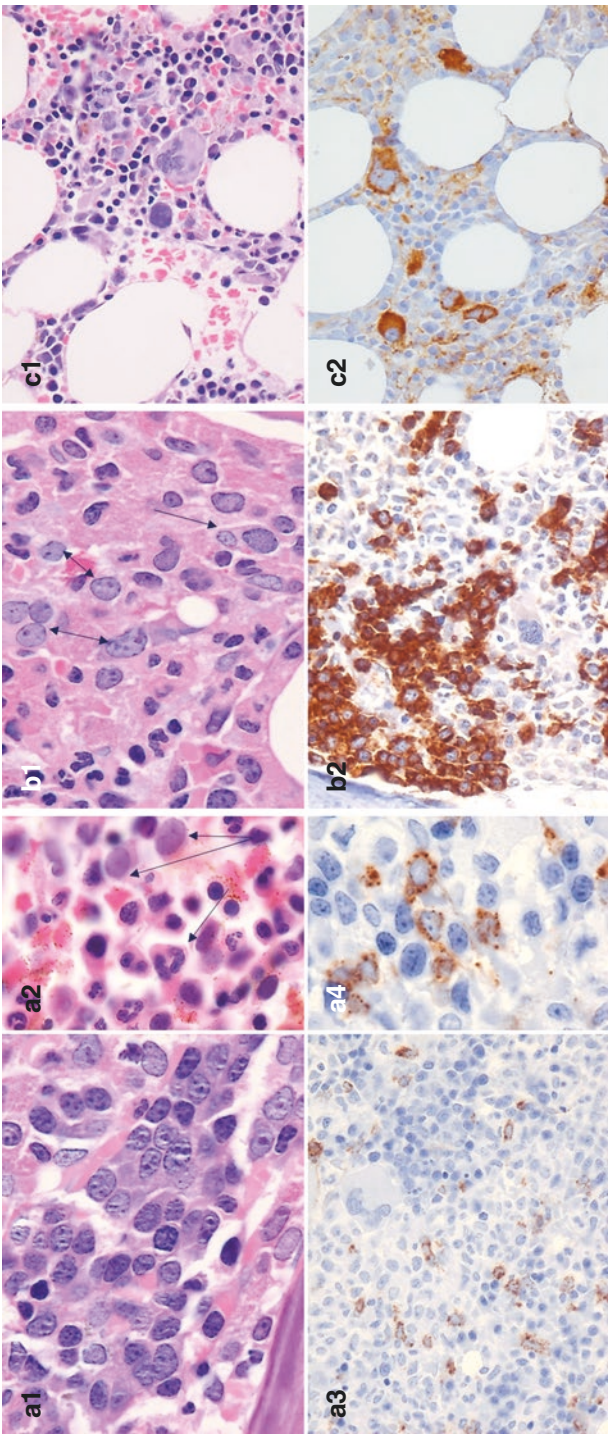

Fig. 2.6 Bone marrow biopsy (Hematoxylin and Eosin) findings in MDS. Increased blasts can be paratrabecular (**a1**; 100×) or interstitial (**a2** double arrow; 100×) in location; abnormal lobation in a neutrophil is also apparent in **a2** (single arrow). CD34 immunostain shows mildly increased interstitial blasts, scattered singly (**a3**; 40×) and in small clusters (abnormal localization of immature precursors, ALIP) (**a4**; 100×). The erythroid lineage shows singly scattered and small aggregates of pronormoblasts (**b1**; 100×). CD71 immunostain highlights an erythroid island in an abnormal paratrabecular location (**b2**; 40×). There are loose clusters of megakaryocytes with dysmegakaryopoiesis (**c1**; 40×). Factor VIII immunostain highlights the micromegakaryocytes, some of which are difficult to appreciate on routine histologic stains (**c2**; 40×)

procedure for MDS. However, multiple immunophenotypic abnormalities involving one or more myeloid linages can be considered as suggestive or supportive of MDS [8]. With the advancement to multi-color cytometers, the development of monoclonal antibodies to an increased number of antigens, and new fluorochromes, there has been significant improvement in use of FCM to support the diagnosis of MDS, particularly in early and lower-grade MDS subtypes, where the morphologic abnormalities may be subtle and the karyotype is often normal. Recent ELN (International/European LeukemiaNet Working Group) guidelines included FCM as a recommended diagnostic procedure for MDS, if performed according to the published guidelines. The ELN has recommended methods of cell sampling, handling, and processing in order to standardize the results obtained by FCM across laboratories, which remains a challenge [9].

There are different FCM scoring systems which have been developed in the past decade, based on the interpretation of the surface marker abnormalities and quantitative differences in immature progenitors versus normal counterparts. In the proposed guidelines of the ELN group, the "Ogata score" can be used as a screening test. It includes the percentage of CD34+ myeloid progenitor cells, the frequency of B-cell precursors within the CD34+ compartment, CD45 expression on myeloid progenitors compared to lymphocytes, and evaluation of neutrophil granularity by comparison to the light scatter pattern of lymphocytes. A score of ≥ 2 has been considered to be reasonably specific for MDS after various validation cohort studies [9]. However, some cases of reactive conditions can have high scores as well [9]. A more comprehensive immunophenotypic panel has been suggested by the ELN group, in which an aberrant finding in at least three tested features affecting at least two cell lineages has been associated with an MDS or MDS/MPN diagnosis in several studies [8]. Examples of flow cytometry aberrations in MDS in both the blast compartment and in maturing myeloid cells are shown in Fig. 2.7.

Abnormalities in Progenitor Myeloid Cells Progenitor myeloid cells in MDS may have an increased side scatter (SSC); decreased expression of CD45 and/or CD117; and increased expression of HLA-DR, CD11b, and CD13/33. The CD34+ blast compartment contains fewer CD19+ and CD38−/dim cells and CD34+/CD117+ cells may show abnormal expression of CD5, CD56, and/or CD7 [9]. However, while aberrant expression of CD5 and CD7 in blasts is relatively specific for MDS, the percentage of MDS cases showing these abnormalities is small (<2% and <10%, respectively). The antigens collectively showing abnormal expression patterns in more than 50% of MDS cases are HLA-DR, CD13, CD33, CD38, and CD117 [10].

Abnormalities in Mature Myeloid Cells The morphometric parameter of hypogranularity in neutrophils in the "Ogata score" has a good specificity for MDS (nearly 90%) but can be discordant in hemodiluted aspirates. Abnormal expression patterns of CD13/CD16 and CD11b/CD16, aberrant expression of CD56, and lack of CD33 and CD64 expression can also be seen. The ELN Working Group has suggested a strong association of MDS with an asynchronous expression of CD34; aberrant pattern of CD11b/CD16; and abnormal expression of CD5, CD56, and

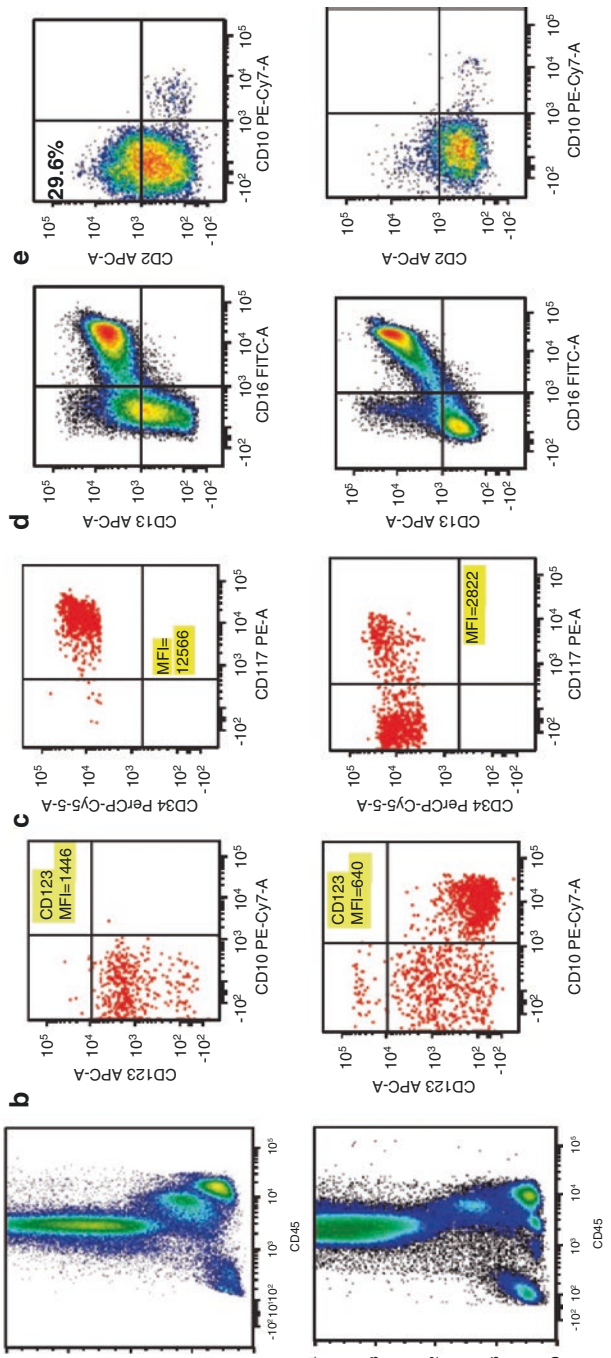

Fig. 2.7 Examples of flow cytometry abnormalities in MDS, with MDS cases shown in the upper panels and normal marrow in the lower panels. (**a**) In CD45 versus side-scatter (SSC), granulocytes show decreased SSC in MDS compared to the normal control. (**b**, **c**) The CD34+ blasts (red) in MDS show abnormally increased CD123 (**b**) and CD117 (**c**) expression compared to the normal control; also note the numerous CD10+ B-lymphoid precursors (hematogones) in Panel B in the normal control. (**d**) Maturing granulocytes in the MDS case show an abnormal pattern of CD16 versus CD13 expression in MDS, deviating from the normal "check-mark" pattern seen in the normal control. (**e**) Monocytes in this MDS case show abnormal expression of CD2, which is normally negative on monocytes. Figure reprinted (with permission) from *Diagnosis of Blood and Bone Marrow Disorders* (Wang SA and Hasserjian RP, eds, Springer Nature 2018.)

CD7 in maturing granulocytes. Additionally, they documented an increased expression of CD117, HLA-DR, CD36, and aberrant patterns of CD15/CD10 and HLA-DR/CD11b [9].

Abnormalities in Monocytes Maturing monocytes in MDS may have decreased SSC and decreased expression of CD45, HLA-DR, and CD11b; abnormal expression of CD36; and an aberrant pattern of CD36/CD14 and HLA-DR/CD11b. There is a strong association of MDS with asynchronous CD34 expression; abnormal CD16 expression in CD11b+ monocytes; and abnormal CD5, CD56, and CD7 expression.

Abnormalities in Erythroid Lineage The ELN group describes four major FCM abnormalities in erythroids in MDS: an increased percentage of CD117 positive erythroid precursors, abnormally heterogeneous and low expression of CD36 and CD71, and an aberrant pattern of CD71/CD235 expression [9]. Recently, increased expression of CD105 in immature erythroid precursors has also been suggested [10].

Cytogenetic Studies

MDS is characterized by recurrent genetic abnormalities which can manifest as gross chromosomal alterations, smaller chromosomal deletions or gains, or mutations in specific genes. The first recurrent genetic abnormality associated with MDS was reported in 1974 by geneticist Herman van den Berghe and his colleagues as deletion in the long arm of chromosome 5 (del5q), which was associated with anemia and thrombocytosis. The first point mutation reported in MDS was in the *NRAS* gene in 1987, followed by *KRAS* mutations [3]. Since then there has been rapid growth in this field of molecular biology from the traditional techniques such as karyotyping, to the use of FISH and SNP arrays, and finally sequencing, including next-generation sequencing (NGS), which has increased the rate of detection of the genetic abnormalities in MDS cases from 50% detected by conventional karyotyping to approximately 90% detected by conventional karyotype plus NGS [11, 12]. Cytogenetic abnormalities play a significant role in the diagnosis, including specific abnormalities defining particular disease subtypes within MDS and also strongly influence the prognosis. Thus, a conventional karyotype should be performed on bone marrow aspirate material in all putative MDS cases. FISH panels which interrogate for the most common MDS-associated cytogenetic abnormalities, do not substitute for a conventional karyotype, but can be used if the karyotype fails or is insufficient [13]. Previously, in the 2008 4th edition WHO Classification, the only genetic marker used in MDS classification was del(5q). In the recent 2017 revised 4th edition WHO classification, the presence of an *SF3B1* mutation (detected by molecular genetic methods rather than karyotype) can define MDS-RS even when the RS count is <15%, provided other diagnostic features are fulfilled and there are at least 5% RS [1]. The role of NGS in the diagnosis of MDS and its mutational landscape are discussed in a separate chapter.

The large majority of identified cytogenetic abnormalities in MDS consists of loss or gain of large segments of chromosomes, the most frequent being −7, del(5q), and +8. Deletions or losses of chromosomal material may also result from unbalanced translocations. These cytogenetic aberrations can be dynamic, increasing in complexity or with some abnormalities disappearing over time with the progression of the disease, with or without superimposed treatment [14–16]. The various cytogenetic abnormalities found in MDS strongly correlate with prognosis [17–20]. Thus, karyotypes have been used in various MDS prognostic systems over the years, as discussed in another chapter. Aside from their influence on prognosis, cytogenetic abnormalities can also be diagnostically useful: some abnormalities are considered to be pathognomonic for MDS in a cytopenic patient in the WHO classification (Table 2.1). Of note, the presence of a del(5q) cytogenetic abnormality, present

Table 2.1 MDS categories with peripheral blood counts, morphology, and cytogenetic abnormalities

MDS categories	Lineages with significant dysplastic changes	Lineages with Cytopenias	Blast (%)	Cytogenetic abnormalities
MDS-SLD	1	1–2	<5% BM; <1% PB	Up to 50%; usually simple karyotype
MDS-MLD	2–3	1–3	<5% BM; <1% PB	~50%; more frequent than in MDS-SLD and MDS-RS-SLD
MDS-RS	≥5% RS with *SF3B1;* ≥15% RS without *SF3B1*		<5% BM; <1% PB	
SLD	1	1–2		
MLD	2–3	1–3		
MDS with isolated del(5q)	1–2	1–2	<5% BM; <1% PB	Del(5q) only or any 1 additional abnormality except del(7q)
MDS-EB	Any	Any	<20% on BM/PB	Clonal abnormalities are more frequent in MDS-EB than in MDS-SLD/MLD; often complex/high-risk karyotype
EB-1	1–3	1–3	2–4% PB; 5–9% BM	
EB-2	1–3	1–3	5–19% PB; 10–19% BM; Auer rods	
MDS-U				
SLD with pancytopenia	1	3	<1% PB; <5% BM	Any
Any MDS category with 1% blasts	1–3	1–3	1 PB; <5% BM	Any
Defining cytogenetic abnormality	0	1–3	<1% PB; <5% BM	MDS-defining abnormality

Abbreviations: *BM* bone marrow; *PB* peripheral blood; Other abbreviations defined in text.

alone or with a single additional abnormality (that does not involve loss of chromosome 7) in a cytopenic patient is associated with a specific MDS subtype, MDS with isolated del(5q), discussed below and in a separate chapter. Illustrations of some of the common karyotype findings in MDS are shown in Fig. 2.8a.

MDS Subtypes

The peripheral blood and bone marrow features described above are common to different subtypes of MDS. The key diagnostic points of each subtype, including the peripheral blood cytopenia(s), morphology, and defining genetic abnormalities, are described below along with a brief discussion of the overall clinical behavior (Table 2.2).

Myelodysplastic Syndrome with Single Lineage Dysplasia (MDS-SLD)

This entity strictly is defined by significant unilineage dysplasia with cytopenia in one or two lineages and no increase in bone marrow or blood blasts. The most common scenario is isolated anemia with isolated erythroid lineage dysplasia. However, of note the dysplastic lineage often does not coincide with the cytopenic lineage(s) (e.g., isolated anemia with megakaryocytic dysplasia but without significant erythroid lineage dysplasia still qualifies as MDS-SLD).

Cytogenetic abnormalities are present at diagnosis in up to 50% of patients and tend to be relatively simple. These characteristics are consistent with the relatively indolent behavior of MDS-SLD [21]. However, progression to AML can occur, particularly in cases with high-risk or complex karyotypes.

Myelodysplastic Syndrome with Multilineage Dysplasia (MDS-MLD)

MDS-MLD is one of the most common MDS subtypes. It is characterized by significant bilineage or trilineage dysplasia with variable cytopenias and no increase in blasts in bone marrow or blood. There is some degree of interobserver discordance in distinguishing MDS-MLD from MDS-SLD, as distinguishing single lineage from multilineage dysplasia is subjective [22].

Cytogenetic abnormalities are present in approximately 50% of patients and tend to be more frequent than in MDS-SLD or MDS-RS, but there are no specific or

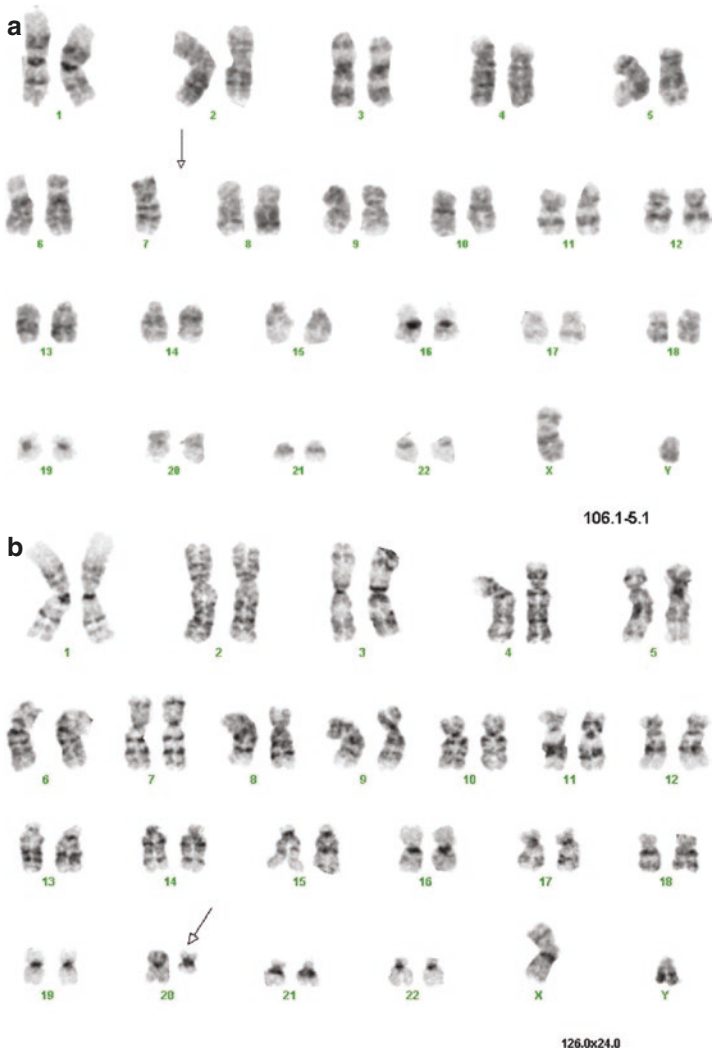

Fig. 2.8 Examples of common cytogenetic abnormalities in MDS. (**a**) Loss of the entire chromosome 7 (or just the long arm) is a relatively common finding in MDS and is considered to be a high-risk finding. (**b**) Deletion 20q is a common finding in MDS, but unlike the −7 abnormality illustrated in Panel **a**, it is not considered to be MDS-defining in isolation. (**c**) A complex karyotype in a case of MDS with excess blasts, illustrating numerical and structural abnormalities of multiple chromosomes and "marker chromosomes" (designated by "A" at the bottom left), which cannot be assigned to a specific chromosome number. Highly complex karyotypes in MDS (at least 4 independent cytogenetic aberrations, as in this case) are associated with very high risk. (**d**) If the bone marrow karyotype fails or is insufficient, interphase FISH studies can help confirm MDS-type cytogenetic abnormalities. In this case of MDS with excess blasts and marked bone marrow fibrosis, the presence of loss of 5q and 7q (red) signals indicated the presence of del(5q) and del(7q) abnormalities, supporting the diagnosis of MDS

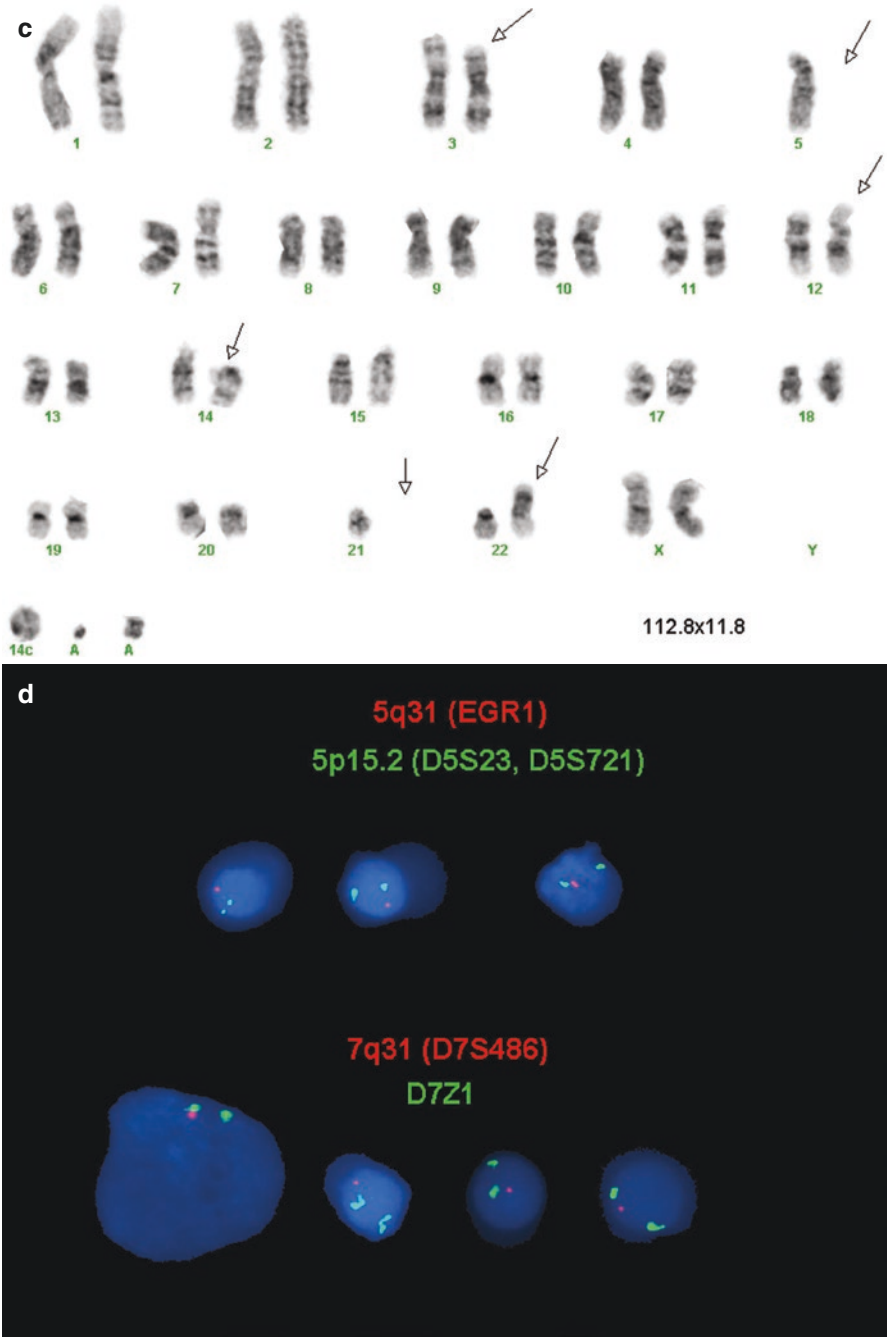

Fig. 2.8 (continued)

Table 2.2 Recurrent cytogenetic abnormalities in myelodysplastic syndrome (MDS)

I. Gain or loss of chromosomal material (relatively common)
–7/del(7q)
del(5q)
+8[a]
+21, −21
–17 and unbalanced translocations at 17p
–20/del(20q)[a]
del(11q)
–Y[a]
del(9q)
+6
del(12p) and unbalanced translocations at 12p
-13/del(13q)
II. Other translocations and inversions (relatively uncommon)
t(3;3)(q21;q26), inv3(q21q26), t(3;21)(q26;q22), and other 3q21 and 3q26 translocations
t(1;7)(p11;p11)
t(2;11)(p21;q23)
t(11;16)(q23;p13)
t(6;9)(p23;q34)
t(2;11)(p21;q23)
i(17q)

[a]Del (20q), +8, and –Y abnormalities, although common findings in MDS, are not considered MDS defining and cannot in isolation be used to make a diagnosis of MDS

defining cytogenetic abnormalities [23, 24]. The prognosis is inferior to MDS-SLD [21, 23, 25–29].

Disease morbidity is usually due to evolving peripheral cytopenias and not to the development of AML.

Myelodysplastic Syndrome with Ring Sideroblasts (MDS-RS)

MDS-RS is a category of MDS which shows ring sideroblasts (RS) on Perls stain for iron. It is subcategorized into MDS-RS with single lineage dysplasia (MDS-RS-SLD) and MDS-RS with multilineage dysplasia (MDS-RS-MLD), with similar dysplasia criteria as MDS-SLD and MDS-MLD, respectively.

In the latest WHO classification, the presence of *SF3B1* mutation is considered to be supportive of this diagnosis and is associated with favorable prognosis. Morphologically, on iron stain, the presence of ≥15% RS with or without *SF3B1* mutation and ≥5% RS accompanied by an *SF3B1* mutation is diagnostic of this entity [1]. However, if there are excess blasts in bone marrow or blood, then the case is classified under MDS-EB.

It is noted that patient survival of MDS-RS-SLD is similar to MDS-SLD with a low rate of progression to AML [23, 25, 26, 30]. The prognosis of MDS-RS-SLD is better than MDS-RS-MLD, which may have *TP53* and *ASXL1* mutations and more aggressive clinical behavior [31].

Myelodysplastic Syndrome with Isolated del(5q) (MDS-del5q)

This entity is defined by the presence of macrocytic anemia and variable dyspoiesis in the erythroid lineage and prominent megakaryocytic dysplasia. The myeloid series is usually relatively unaffected, with <10% dyspoiesis and no neutropenia. The platelet count may be normal or increased and there is usually an increase in megakaryocytes in the bone marrow with predominantly non-lobated forms. There are no increased blasts in bone marrow or blood.

Patients presenting with these classic features usually show an isolated del(5q) abnormality, which as mentioned previously was among the first cytogenetic abnormalities to be detected in MDS [3]; in the most recent WHO classification, a single additional cytogenetic abnormality is allowed, except for those involving deletion of chromosome 7 [1].

These patients generally have a favorable prognosis (although worsened if there is a concomitant *TP53* mutation) and are more likely to respond to the drug lenalidomide than MDS patients lacking del(5q) [23, 25, 26].

Myelodysplastic Syndrome with Excess Blasts (MDS-EB)

This category is defined by the presence of increased blasts in the bone marrow and/or blood in a background of variable degree of dyspoiesis and any number of cytopenias. Based on the blast count of all nucleated cells and the presence of Auer rods, MDS-EB is further classified as EB-1 and EB-2. Due to variable distribution of blasts, CD34 estimation on the bone marrow biopsy can be done to corroborate the aspirate smear blasts count.

The presence of any Auer rods in blasts classifies the disease as MDS-EB-2 irrespective of the blast count, superseding all other MDS categories mentioned above.

Previously in the 2008 4th edition WHO classification, the entity acute erythroid leukemia, erythroid/myeloid subtype encompassed cases with >50% bone marrow erythroid cells in which the blasts comprised ≥20% of the non-erythroid cells, even if they were <20% of all nucleated cells. These cases are now classified as MDS-EB in the 2017 revised 4th edition WHO classification, with the blast count being taken from all nucleated cells [1]. This change has been made on the basis that such cases of erythroid leukemias did not always have an aggressive clinical course and the cytogenetic and mutation profile was more akin to MDS than to de novo AML [32–35].

Clonal cytogenetic abnormalities are more frequent in MDS-EB than in MDS-SLD or MDS-RS and more often show complex or high-risk karyotype abnormalities. The median survival is shorter and disease progression to AML is higher in EB-2 when compared to EB-1.

Myelodysplastic Syndrome, Unclassifiable (MDS-U)

This entity encompasses three specific scenarios which do not fit into the above categories. This is based on the prognostic differences with the above entities.

1. Cases with features of MDS-SLD or MDS-RS-SLD with pancytopenia. Prognostically, these cases have a more aggressive behavior, akin to MDS-MLD, and are placed in the MDS-U category [36, 37].

2. MDS-SLD, MDS-MLD, MDS-RS, or MDS-del(5q) with exactly 1% blasts in the blood, confirmed independently on two separate occasions. These cases appear prognostically similar to MDS-EB and are placed in the MDS-U category [38].

3. The presence of MDS-defining cytogenetic abnormalities on karyotype in the absence of significant dysplasia in any lineage in a patient with persistent unexplained cytopenia. These cases are placed in the MDS-U category because their clinical behavior is uncertain.

Refractory Cytopenia of Childhood (RCC)

This is a provisional entity in the most recent WHO classification, encompassing cases of MDS in the pediatric population that lack excess bone marrow or blood blasts and typically show a hypocellular marrow [1].

The main differential diagnosis is with aplastic anemia.

Therapy-Related MDS (t-MDS)

Any of the above MDS subtypes occurring in patients with prior exposure to cytotoxic chemotherapy (for a neoplastic or non-neoplastic condition) and/or significant bone marrow radiation exposure is considered to be therapy related.

Compared to non-therapy-related cases, t-MDS has a poorer prognosis, mainly due to a much higher incidence of *TP53* mutations and complex karyotypes.

References

1. Hasserjian RP, Orazi A, Brunning RD, Germing U, Le Beau MM, Porwit A, et al. Myelodysplastic syndromes. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer; 2017. p. 98–120.
2. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12): 2454–65. Epub 2012 Jun 27. PubMed PMID: 22740453; PubMed Central PMCID: PMC4425443.

3. Steensma DP. Historical perspectives on myelodysplastic syndromes. Leuk Res. 2012;36(12):1441–52. https://doi.org/10.1016/j.leukres.2012.08.007. Epub 2012 Aug 23. PubMed PMID: 22921019.
4. Goel S, Sachdev R, Gajendra S, Jha B, Sahni T, Dorwal P, et al. Picking up myelodysplastic syndromes and megaloblastic anemias on peripheral blood: use of NEUT-X and NEUT-Y in guiding smear reviews. Int J Lab Hematol. 2015;37(2):e48–51. https://doi.org/10.1111/ijlh.12285. Epub 2014 Aug 6. PubMed PMID: 25132616.
5. Goel S, Gajendra S, Sahni T, Jha B, Dang M, Rawat HS, et al. Role of NEUT-X & NEUT-Y in picking up megaloblastic anaemia on peripheral blood & in differentiating from other macrocytic anaemia. Indian J Med Res. 2017;145(3):395–8. https://doi.org/10.4103/ijmr.IJMR_201_15. PubMed PMID: 28749404; PubMed Central PMCID: PMC5555070.
6. Steensma DP. Dysplasia has A differential diagnosis: distinguishing genuine myelodysplastic syndromes (MDS) from mimics, imitators, copycats and impostors. Curr Hematol Malig Rep. 2012;7(4):310–20. https://doi.org/10.1007/s11899-012-0140-3. Review. PubMed PMID: 23015360.
7. Gajendra S, Jha B, Goel S, Sahni T, Sharma R, Shariq M, et al. Leishman and Giemsa stain: a new reliable staining technique for blood/bone marrow smears. Int J Lab Hematol. 2015;37(6):774–82. https://doi.org/10.1111/ijlh.12408. Epub 2015 Jul 30. PubMed PMID: 26224117.
8. Bento LC, Correia RP, Pitangueiras Mangueira CL, De Souza BR, Rocha FA, Bacal NS, et al. The use of flow cytometry in myelodysplastic syndromes: a review. Front Oncol. 2017;7:270. https://doi.org/10.3389/fonc.2017.00270. eCollection 2017. Review. PubMed PMID: 29188193; PubMed Central PMCID: PMC5694750.
9. Porwit A, van de Loosdrecht AA, Bettelheim P, Brodersen LE, Burbury K, Cremers E, et al. Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes-proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. Leukemia. 2014;28(9):1793–8. https://doi.org/10.1038/leu.2014.191. Epub 2014 Jun 12. PubMed PMID: 24919805.
10. Aanei CM, Picot T, Tavernier E, Guyotat D, Campos CL. Diagnostic utility of flow cytometry in myelodysplastic syndromes. Front Oncol. 2016;6:161. https://doi.org/10.3389/fonc.2016.00161. eCollection 2016. Review. PubMed PMID: 27446807; PubMed Central PMCID: PMC4921489.
11. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241–7. https://doi.org/10.1038/leu.2013.336. Epub 2013 Nov 13. PubMed PMID: 24220272; PubMed Central PMCID: PMC3918868.
12. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122(22):3616–27; quiz 3699. https://doi.org/10.1182/blood-2013-08-518886. Epub 2013 Sep 12. PubMed PMID: 24030381; PubMed Central PMCID: PMC3837510.
13. He R, Wiktor AE, Durnick DK, Kurtin PJ, Van Dyke DL, Tefferi A, et al. Bone marrow conventional karyotyping and fluorescence in situ hybridization: defining an effective utilization strategy for evaluation of myelodysplastic syndromes. Am J Clin Pathol. 2016;146(1):86–94. https://doi.org/10.1093/ajcp/aqw077. Epub 2016 Jun 27. PubMed PMID: 27353768.
14. Pedersen-Bjergaard J, Philip P. Cytogenetic characteristics of therapy-related acute nonlymphocytic leukaemia, preleukaemia and acute myeloproliferative syndrome: correlation with clinical data for 61 consecutive cases. Br J Haematol. 1987;66(2):199–207. PubMed PMID: 3606956.
15. Raskind WH, Tirumali N, Jacobson R, Singer J, Fialkow PJ. Evidence for a multistep pathogenesis of a myelodysplastic syndrome. Blood. 1984;63(6):1318–23. PubMed PMID: 6326894.
16. Tomonaga M, Tomonaga Y, Kusano M, Ichimaru M. Sequential karyotypic evolutions and bone marrow aplasia preceding acute myelomonocytic transformation from myelodysplastic syndrome. Br J Haematol. 1984;58(1):53–60. PubMed PMID: 6590091.

17. Vallespí T, Imbert M, Mecucci C, Preudhomme C, Fenaux P. Diagnosis, classification, and cytogenetics of myelodysplastic syndromes. Haematologica. 1998;83(3):258–75. Review. PubMed PMID: 9573680.
18. Olney HJ, Le Beau MM. The cytogenetics and molecular biology of myelodysplastic syndromes. In: Bennett JM, editor. The myelodysplastic syndromes, pathobiology and clinical management. New York: Marcel Dekker, Inc; 2002. p. 89–119.
19. Raimondi SC. Cytogenetics in MDS. In: Lopes LFHH, editor. Myelodysplastic and myeloproliferative disorders in children. Sao Paulo: Le Mar; 2003. p. 119–61.
20. Brunning RD, Hasserjian RP, Porwit A, et al. Refractory cytopenia with unilineage dysplasia. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer; 2008. p. 94–5.
21. Cermák J, Michalová K, Brezinová J, Zemanová Z. A prognostic impact of separation of refractory cytopenia with multilineage dysplasia and 5q- syndrome from refractory anemia in primary myelodysplastic syndrome. Leuk Res. 2003;27(3):221–9. PubMed PMID: 12537974.
22. Font P, Loscertales J, Soto C, Ricard P, Novas CM, Martín-Clavero E, et al. Interobserver variance in myelodysplastic syndromes with less than 5% bone marrow blasts: unilineage vs. multilineage dysplasia and reproducibility of the threshold of 2% blasts. Ann Hematol. 2015;94(4):565–73. https://doi.org/10.1007/s00277-014-2252-4. Epub 2014 Nov 13. PubMed PMID: 25387664.
23. Germing U, Gattermann N, Strupp C, Aivado M, Aul C. Validation of the WHO proposals for a new classification of primary myelodysplastic syndromes: a retrospective analysis of 1600 patients. Leuk Res. 2000;24(12):983–92. PubMed PMID: 11077111.
24. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89(6):2079–88. Erratum in: Blood 1998;91(3):1100. PubMed PMID: 9058730.
25. Germing U, Strupp C, Kuendgen A, Isa S, Knipp S, Hildebrandt B, et al. Prospective validation of the WHO proposals for the classification of myelodysplastic syndromes. Haematologica. 2006;91(12):1596–604. PubMed PMID: 17145595.
26. Malcovati L, Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, et al. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol. 2005;23(30):7594–603. Epub 2005 Sep 26. PubMed PMID: 16186598.
27. Germing U, Gattermann N, Aivado M, Hildebrandt B, Aul C. Two types of acquired idiopathic sideroblastic anaemia (AISA): a time-tested distinction. Br J Haematol. 2000;108(4):724–8. PubMed PMID: 10792275.
28. Howe RB, Porwit-MacDonald A, Wanat R, Tehranchi R, Hellström-Lindberg E. The WHO classification of MDS does make a difference. Blood. 2004;103(9):3265–70. Epub 2003 Dec 18. PubMed PMID: 14684416.
29. Lee JH, Lee JH, Shin YR, Lee JS, Kim WK, Chi HS, et al. Application of different prognostic scoring systems and comparison of the FAB and WHO classifications in Korean patients with myelodysplastic syndrome. Leukemia. 2003;17(2):305–13. PubMed PMID: 12592327.
30. Brunning RD, Matutes E, Harris NL, et al. Acute myeloid leukemias. In: Jaffe EJHN, Stein H, Vardiman JW, editors. World Health Organization classification of tumours: pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2001. p. 75–105.
31. Malcovati L, Karimi M, Papaemmanuil E, Ambaglio I, Jädersten M, Jansson M, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015;126(2):233–41. https://doi.org/10.1182/blood-2015-03-633537. Epub 2015 May 8. PubMed PMID: 25957392; PubMed Central PMCID: PMC4528082.
32. Bacher U, Haferlach C, Alpermann T, Kern W, Schnittger S, Haferlach T. Comparison of genetic and clinical aspects in patients with acute myeloid leukemia and myelodysplastic syndromes all with more than 50% of bone marrow erythropoietic cells. Haematologica.

2011;96(9):1284–92. https://doi.org/10.3324/haematol.2011.043687. Epub 2011 May 23. PubMed PMID: 21606170; PubMed Central PMCID: PMC3166098.
33. Hasserjian RP, Zuo Z, Garcia C, Tang G, Kasyan A, Luthra R, et al. Acute erythroid leukemia: a reassessment using criteria refined in the 2008 WHO classification. Blood. 2010;115(10):1985–92. https://doi.org/10.1182/blood-2009-09-243964. Epub 2009 Dec 29. PubMed PMID: 20040759; PubMed Central PMCID: PMC2942006.
34. Grossmann V, Bacher U, Haferlach C, Schnittger S, Pötzinger F, Weissmann S, et al. Acute erythroid leukemia (AEL) can be separated into distinct prognostic subsets based on cytogenetic and molecular genetic characteristics. Leukemia. 2013;27(9):1940–3. https://doi.org/10.1038/leu.2013.144. Epub 2013 May 7. PubMed PMID: 23648669.
35. Zuo Z, Medeiros LJ, Chen Z, Liu D, Bueso-Ramos CE, Luthra R, et al. Acute myeloid leukemia (AML) with erythroid predominance exhibits clinical and molecular characteristics that differ from other types of AML. PLoS One. 2012;7(7):e41485. https://doi.org/10.1371/journal.pone.0041485. Epub 2012 Jul 23. PubMed PMID: 22844482; PubMed Central PMCID: PMC3402404.
36. Germing U, Strupp C, Giagounidis A, Haas R, Gattermann N, Starke C, Aul C. Evaluation of dysplasia through detailed cytomorphology in 3156 patients from the Düsseldorf Registry on myelodysplastic syndromes. Leuk Res. 2012;36(6):727–34. https://doi.org/10.1016/j.leukres.2012.02.014. Epub 2012 Mar 13. PubMed PMID: 22421409.
37. Verburgh E, Achten R, Louw VJ, Brusselmans C, Delforge M, Boogaerts M, et al. A new disease categorization of low-grade myelodysplastic syndromes based on the expression of cytopenia and dysplasia in one versus more than one lineage improves on the WHO classification. Leukemia. 2007;21(4):668–77. Epub 2007 Feb 15. PubMed PMID: 17301818.
38. Knipp S, Strupp C, Gattermann N, Hildebrandt B, Schapira M, Giagounidis A, Aul C, Haas R, Germing U. Presence of peripheral blasts in refractory anemia and refractory cytopenia with multilineage dysplasia predicts an unfavourable outcome. Leuk Res. 2008;32(1):33–7. Epub 2007 Apr 6. PubMed PMID: 17412418.

Chapter 3
Biology and Pathophysiology of MDS with del(5q)

Onyee Chan, Chetasi Talati, David Sallman, and Alan List

Introduction

Myelodysplastic syndromes (MDS) are a group of heterogeneous clonal hematopoietic stem cell malignancies characterized by morphological dysplasia and ineffective hematopoiesis. Clinical manifestations depend upon the lineage(s) affected, with anemia the most common accompanied by red blood cell transfusion dependence in approximately one-third of patients at diagnosis [1]. Understanding of the clinical outcomes associated with specific chromosomal aberrations in MDS has led to the development of prognostic models, including the International Prognostic Scoring System (IPSS) and later revised-IPSS (R-IPSS) [2, 3]. Cytogenetic aberrations are frequently observed in de novo MDS patients with some studies reporting them in >50% of patients [4, 5]. The most common aberration is an interstitial deletion of chromosome 5q (del(5q)) occurring in approximately 15% of patients with most having an isolated del(5q) [4]. As molecular testing such as next-generation sequencing (NGS) became more widely available, several recurring somatic gene mutations were identified in MDS that carry prognostic significance [6]. In particular, *TP53* gene mutations confer the worst overall survival, associated with a significantly greater risk of acute myeloid leukemia (AML) transformation [6, 7]. *TP53* mutations occur in 20% of patients with isolated del(5q) and in 70–100% of patients with complex karyotype including del(5q) supporting a strong correlation with *TP53* mutations and del(5q) [8].

O. Chan
Department of Medicine, University of South Florida, Tampa, FL, USA

Division of Malignant Hematology, Moffitt Cancer Center, Tampa, FL, USA

C. Talati (✉) · D. Sallman · A. List
Division of Malignant Hematology, Moffitt Cancer Center, Tampa, FL, USA
e-mail: Chetasi.talati@moffitt.org

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3_3

MDS with isolated del(5q) represents a distinct pathologic subtype defined by the 2016 World Health Organization (WHO) as the constellation of dysplasia in 1 to 3 lineages, bone marrow blasts <5%, circulating peripheral blasts <1%, and the presence of del(5q) alone or with 1 additional chromosomal abnormality with the exception of chromosome 7 deletion or del(7q) [1]. Some of the unique features associated with del(5q) were first recognized in 1974 by Van den Berghe et al. [9]. It was initially referred to as the "5q- syndrome" and was described in a series of patients with macrocytic anemia, dyserythropoiesis with erythroid hypoplasia, a normal or elevated platelet count, and hypolobulated megakaryocytes with an indolent natural history. Our understanding of the biology and pathophysiology of del(5q) MDS has been transformed in recent years with the sequencing of the human genome (Fig. 3.1). Herein, we will describe each major milestone and the remarkable story of discovering how haploinsufficiency accounts for the hematological phenotype and selective sensitivity to the drug lenalidomide in del(5q) MDS.

Pathobiology of del(5q) MDS

In the mid-1990s, genetic mapping techniques were utilized for molecular delineation of the commonly deleted region (CDR) of chromosome 5q in myeloid malignancies, encoded within a 1–1.5 Mb segment [10]. Further investigations in patients with 5q- syndrome localized the distal CDR to 5q32-33 containing 40 genes, of which 33 were transcriptionally haplodeficient in CD34+ cells, representing candidate genes possibly contributing to the disease [11, 12]. A second, more proximal CDR was identified at 5q31 and thought to contain tumor suppressor gene(s) in patients predominantly with therapy-related MDS (excluding cases of 5q- syndrome) and AML [13]. However, subsequent studies indicate most patients have large interstitial deletions that span both CDRs [14]. Notably, only rare somatic point mutations were identified in the remaining alleles, suggesting that the hematological phenotype is dictated simply through genetic haploinsufficiency [15–17]. A number of genes in both the distal and proximal CDRs have since been implicated in the pathogenesis of del(5q) MDS through haploinsufficiency, and they are summarized in Fig. 3.2 [18].

Haploinsufficiency Underlies Hematologic Phenotype

In seminal investigations by Ebert and colleagues using short hairpin RNAs (shRNA) to knockdown each of the distal CDR candidate genes in normal CD34+ human hematopoietic progenitor cells identified the ribosomal processing S14 (*RPS14*) gene as the key determinant of the hypoplastic anemia in del(5q) MDS [19]. The *RPS14* gene encodes a key component of the 40S ribosomal subunit, which when underexpressed results in a severe decrease in production and survival

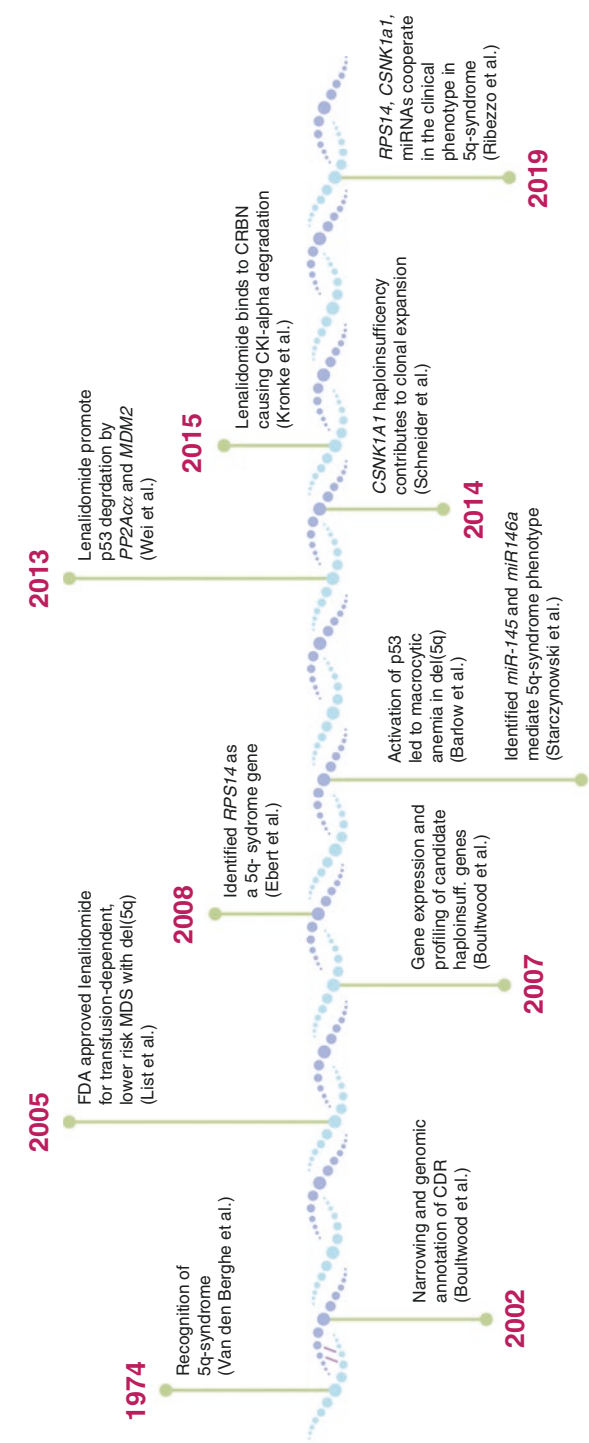

Fig. 3.1 Major milestones in the study and molecular pathogenesis of MDS with del(5q) overtime

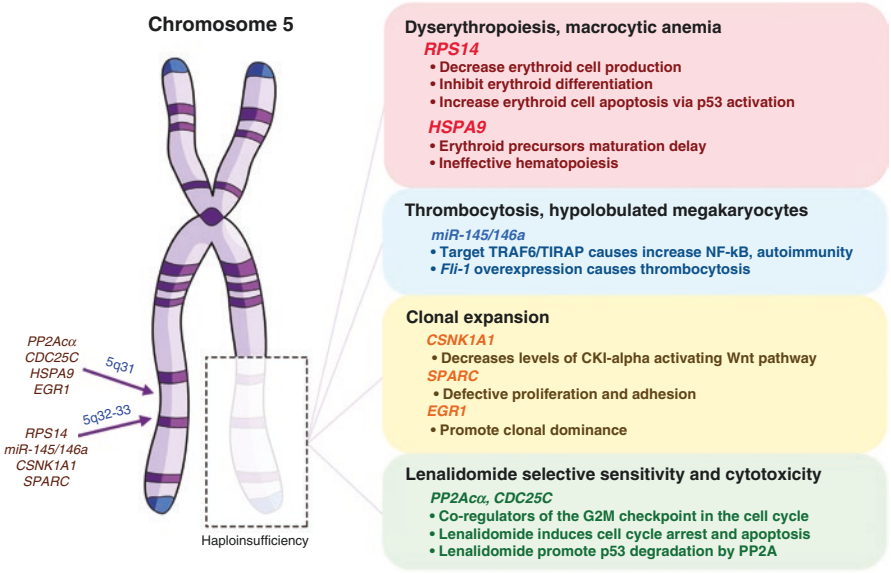

Fig. 3.2 Haploinsufficiency underlies hematologic phenotype, clonal expansion, and lenalidomide selective sensitivity

of differentiating erythroid cells. The level of RPS14 protein expression after knockdown was approximately half that in control cells supporting the hypothesis of haploinsufficiency [19]. In addition, investigators were able to restore erythroid differentiation in bone marrow cells of patients with 5q- syndrome through *RPS14* overexpression, but not in patients lacking del(5q). This established RPS14 deficiency as the principal genetic driver of the dyserythropoiesis in MDS with isolated del(5q) [19]. Interestingly, Diamond–Blackfan anemia, known for its profound erythroid hypoplasia, is a heritable disorder of aberrant ribosome biogenesis caused by haploinsufficiency of a different ribosomal processing gene, *RPS19* [20].

In the last decade, animal studies provided evidence for p53-dependent mechanism in the pathophysiology of the 5q- syndrome [21, 22]. Using large-scale chromosomal engineering, Barlow and colleagues created a mouse model with allelic deletion of the syntenic genes of the human CDR that phenocopied the morphological and hematologic features of the 5q- syndrome [21]. Mechanistically, accumulation of the p53 protein was indispensable for the phenotype, which was validated by crossing the CDR haplodeficient mice with p53-deficient mice, demonstrating complete rescue of the pathologic features. Mouse double minute 2 protein (MDM2) is a key negative regulator of p53 [23]. Free ribosomal proteins (RP) such as RPL11 are liberated as a consequence of *RPS14* haploinsufficiency, binds to MDM2, thereby inhibiting p53 ubiquitination [22]. Activation of p53 induces the programmed death of erythroid precursors ultimately manifest clinically as hypoplastic anemia [24]. In a recent study, Youn and colleagues created a zebrafish model with *RPS14* deficiency that mirrors the anemia phenotype of del(5q) MDS and also demonstrated the induction of matrix metallopeptidase 9 (*MMP9*) expression, a

collagenase known to augment solid tumor growth and invasion, which has been implicated in the initiation and progression of hematological malignancies [25, 26]. Treatment with MMP9 inhibitors partially rescued the erythroid defect. Using a double knockdown technique in human bone marrow progenitor cells, the negative regulatory effect of enhanced *MMP9* expression on erythroid development in *RPS14* knockdown cells was confirmed, supporting its contribution to anemia [26].

Heat shock protein family A (Hsp70) member 9 (*HSPA9*), a gene located in the proximal CDR (5q31.1), was also found to contribute to ineffective erythropoiesis. HSPA9, also known as mortalin, is the only HSP70 homolog localized in the mitochondria matrix that serves as chaperone for the client proteins p53 and S100A9 [27] and has a key role in iron-sulfur (Fe-S) biogenesis [28]. Knockdown of *HSPA9* in the mouse model and in human cells results in erythroid precursor maturation delay, growth arrest, and excess cell death [29]. Liu and colleagues demonstrated that *HSPA9* haploinsufficiency induces overexpression of *TP53*, increased apoptosis, and inhibition of cell growth [30]. However, simultaneous knockdown of *HSPA9* and *TP53* mitigates this effect, suggesting the erythroid defects related to *HSPA9* deficiency is p53-dependent, analogous to the pathogenesis of anemia in del(5q) MDS.

MicroRNA (miRNA) are small, noncoding RNA molecules that post-translationally silence genes by binding to complementary messenger RNAs (mRNA) to direct their degradation. Haploinsufficiency of two miRNA genes, *miR-145* (5q33.1) and *miR-146a* (5q33.3), in the distal CDR are responsible for the other key features of the 5q- syndrome, specifically thrombocytosis, hypolobulated megakaryocytes, neutropenia, and deregulation of the myddosome signaling complex [15, 31]. Starczynowski and colleagues identified two targets of *miR-145/146a*, tumor necrosis factor receptor-associated factor-6 (TRAF6) and Toll-interleukin-1 receptor domain-containing adaptor protein (TIRAP), which lies upstream of TRAF6 in Toll-receptor (TLR) signaling [31]. Elevation of TIRAP and TRAF6 activates the TLR-myddosome signaling axis and the downstream inflammatory transcription factor nuclear factor-kappa B (NF-kB), promoting cytokine generation and expansion of the del(5q) clone [32]. Enforced expression of *TRAF6* or *miR-145/146a* knockdown in murine models results in thrombocytosis, megakaryocytic dysplasia, and mild neutropenia [31]. Mice transplanted with *TRAF6*-expressing marrow progressed to either AML or marrow failure. In addition, Friend leukemia virus integration 1 (Fli-1) is a megakaryocyte and erythroid transcription factor that is normally repressed by *miR-145*. *Fli-1* overexpression preferentially expands megakaryocytic progenitors relative to erythroid cells, thereby contributing to thrombocytosis [33].

The epistatic interaction between neighboring haploinsufficient genes, specifically *miR-146a* and TRAF-interacting protein with forkhead-associated domain B (*TIFAB*), may further compound the neutropenia in del(5q) MDS [34]. In one animal study, deleting both *TIFAB* and *miR-146a* led to severe cytopenia mimicking a bone marrow failure-like state. The severity and frequency of leukopenia were less in mice with singular deficiency of either *TIFAB* or *miR-146a* [34]. Ribezzo and colleagues also recently described how combined insufficiency of *RPS14*, *CSNK1A1*, and *miR-145/146a* recapitulate the classic features of 5q- syndrome in a mouse

model [35]. Furthermore, they demonstrated that these deficiencies activate the innate immune response resulting in overexpression of S100A8, an endogenous Toll-like receptor 4 (TLR4) ligand that plays a role in inflammation, in the mesenchymal stem cell niche providing evidence that intrinsic defects of 5q- syndrome directly alters the microenvironment that contributes to ineffective hematopoiesis [35]. Figure 3.3 illustrates how allelic insufficiency drives aberrant innate immune signaling in del(5q) MDS. Collectively, these data indicate that the molecular pathogenesis of the del(5q) MDS hematologic phenotype is dictated by haploinsufficiency of genes encoded within the CDRs.

Haploinsufficiency Underlies Clonal Expansion

CSNK1A1 is a tumor suppressor gene located in the distal CDR (5q32) that encodes Casein Kinase I alpha (CKIα), a regulator of Wnt signaling and stem cell self-renewal [36]. Haploinsufficient *CSNK1A1* reduces levels of the CKIα protein, a component of the β-catenin destruction complex, that binds to and phosphorylates β-catenin. Conditional inactivation of *CSNK1A1* in a murine model showed that haplodeficiency induces hematopoietic stem cell expansion and a competitive repopulation advantage, whereas homozygous deletion induced hematopoietic stem cell failure [37]. Secreted protein acidic and rich in cysteine (*SPARC*) is a haplodeficient candidate tumor suppressor gene found in the CDR that has roles in proliferation and adhesion; however, the precise functional consequence of allelic insufficiency remains unclear [38, 39]. Other genes thought to promote del(5q)

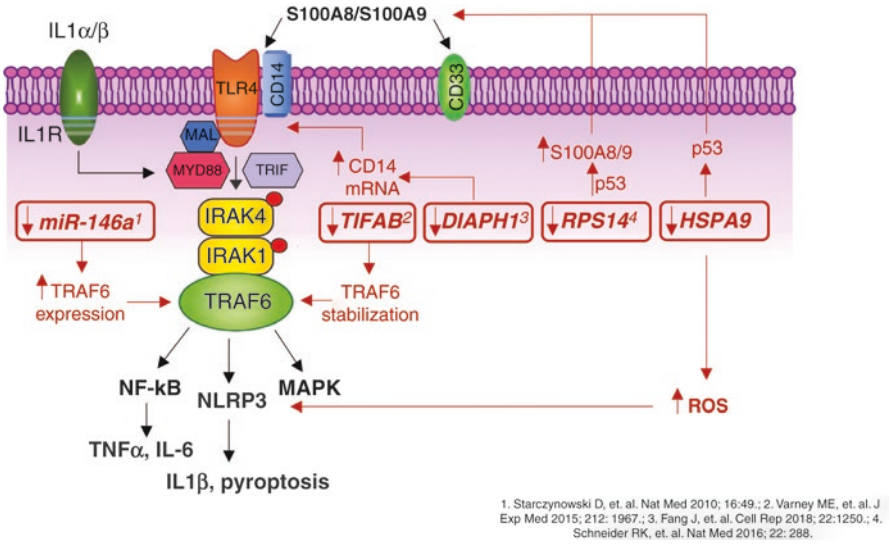

Fig. 3.3 Allelic insufficiency drives aberrant innate immune signaling in del(5q) MDS

clonal dominance include early growth response 1 (*EGR1*), located in the proximal CDR and adenomatous polyposis coli (*APC*) [40]. In one study, haploinsufficiency of EGR1 and the loss of TP53 in HSC compounded the rate of hematologic neoplasm development [41]. Similarly, deficiency in both EGR1 and APC cooperate in the presence of *TP53* deficiency to promote AML transformation [41].

Lenalidomide in Del(5q) MDS and Its Mechanism of Action

Besides the distinct clinical phenotype, MDS with del(5q) is unique among other subtypes of MDS because of its selective sensitivity to lenalidomide, a thalidomide analog. The karyotype-specific activity was first observed int he dose-finding study (MDS-001) where patients with del(5q) lesion had greater response rate (83%) compared to patients with other karyotypes (57% for patients with normal karyotype and 12% with other karyotypes, $p = 0.007$) and led to clonal suppression and cytogenetic response [42]. This led to the pivotal MDS-003 phase II clinical trial which included transfusion-dependent patients with a del(5q) lesion and low/intermediate-1 (int-1) risk disease according to IPSS [43]. Among 148 patients treated, 76% had a 50% or greater reduction in transfusion needs, and 67% achieved transfusion independence with a median rise in hemoglobin of 5.4 g/sl, providing the basis for its approval by the Food and Drug Administration (FDA) in 2005. At the time of drug approval, the mechanism of action of lenalidomide was not fully delineated; however, significant progress has been made since that time.

Lenalidomide is an immunomodulatory drug found to selectively inhibits del(5q) clones through several mechanisms. The dual specificity phosphatases, CDC25C (cell division cycle 25C) and PP2Acα (protein phosphatase 2A catalytic domain alpha) encoded within or adjacent to the proximal CDR are important co-regulators of the G2M checkpoint in the cell cycle [44]. Wei and colleagues showed that cells with reduced expression of *CDC25C* and *PP2Ac*α have enhanced sensitivity to lenalidomide, which causes G2M cell-cycle arrest and induction of apoptosis [45]. Lenalidomide inhibits phosphatase activity directly and indirectly. Lenalidomide inhibition of haplodeficient PP2A stabilizes MDM2 by hyperphosphorylating inhibitory residues, thereby promoting p53 degradation [46]. Kronke and colleagues in a proteomic study showed that haploinsufficient *CSNK1A1* cells are sensitized to lenalidomide, which makes these cells even more vulnerable due to additional degradation of CKIα [47]. Specifically, lenalidomide binds to cereblon (CRBN), the substrate receptor of the CRL4-CRBN E3 ubiquitin ligase and induces recruitment of the CKIα (substrate) [48]. Regulator of Cullin 1 (ROC1) serves to recruit the E2 enzyme that binds to ubiquitin. Ubiquitination and subsequent degradation of CKIα led to del(5q) progenitor cell arrest and death [49]. Furthermore, overexpression of *CSNK1A1* reduced the sensitivity of lenalidomide only in the HSC of patients with del(5q) MDS and not those with normal cytogenetics [50]. Taken together, the multipronged approach of lenalidomide in del(5q) MDS explains the high selectivity and efficacy in this karyotypically defined MDS subset (Fig. 3.4).

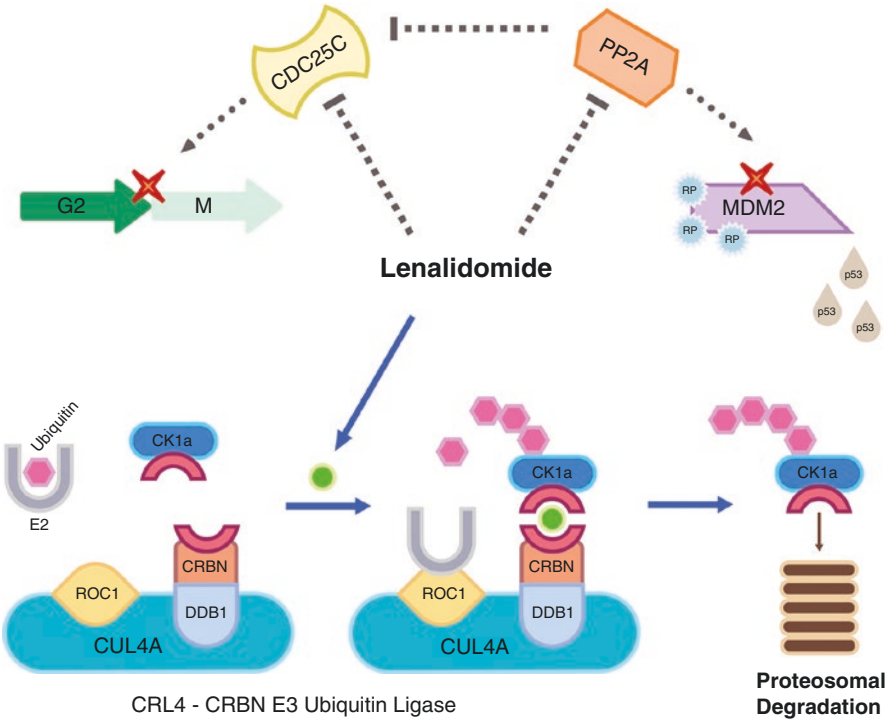

Fig. 3.4 Lenalidomide mechanism of action in del(5q) MDS. Reduced expression of cell division cycle 25C (CDC25C) and protein phosphatase *2A* catalytic domain alpha (PP2Ac*α*) enhances sensitivity to lenalidomide. Lenalidomide directly inhibits CDC25C inducing G2M cell cycle arrest and apoptosis in malignant cells (top left). It also inhibits PP2Ac*α* resulting in mouse double minute 2 protein (MDM2) stabilization and subsequently p53 degradation (top right). Recall it is the haploinsufficient ribosomal processing S14 (*RPS14*) that produces free ribosomal proteins (RP) that bind to MDM2 causing pathologic p53 accumulation. Inhibiting PP2A also causes CDC25C inactivation. Lenalidomide thereby selectively eliminate del(5q) cells and restore effective erythropoiesis. In addition, it binds to cereblon (CRBN), the substrate receptor of the CRL4-CRBN E3 ubiquitin ligase that is composed of damaged DNA-binding protein1 (DDB1), cullin 4a (CUL4A), and regulator of cullins 1 (ROC1) (bottom left). It induces recruitment of CKI*α* (substrate). Ubiquitin-conjugating enzyme (E2) functions with ROC1 to facilitate ubiquitin transfer to the substrate or ubiquitin chain (bottom middle) resulting in ubiquitination and degradation of CKI*α* (bottom right)

Mechanism of Lenalidomide Resistance in Del(5q) MDS

While most patients with lower-risk del(5q) MDS achieve remission with lenalidomide, the median duration of response is approximately 2.5 years [51, 52]. To determine if persistent malignant stem cells are responsible for relapse, Tehranchi and colleagues investigated specimens from 7 patients with del(5q) MDS who achieved a complete cytogenetic remission with lenalidomide [53]. Lenalidomide was able to

selectively and generally complete deplete del(5q) progenitor cells (CD34+, CD38+); however, a phenotypically distinct, quiescent group of del(5q) stem cells (CD34+,CD38−/low, CD90+) persisted. As the del(5q) clone expands over time under the selective pressure of lenalidomide, resistance develops and recurrence occurs [53]. In a separate study, *PP2Acα* overexpression induces resistance to lenalidomide, resulting in suppression of MDM2 and accumulation of p53 [46]. Strong p53 expression by immunohistochemistry (IHC) in turn is associated with *TP53* mutation, which can occur in about 18% of low-risk MDS del(5q) patients [54]. Furthermore, Saft and colleagues examined bone marrow specimens of 85 patients with IPSS low or int-1 risk del(5q) from the MDS-004 trial who were treated with lenalidomide and found strong p53 expression correlated with higher rates of AML transformation ($p = 0.0006$), lower rates of cytogenetic response ($p = 0.009$), and decreased overall survival ($p = 0.0175$) [55]. Cells with strong p53 expression were confirmed to have *TP53* mutation by pyrosequencing analysis. Developing novel medications targeting these defects is an area of active research and promising therapeutics such as cenersen (works to reduce cellular p53) and APR-246 (refold mutant p53 back to its wild-type conformation) is currently under investigation [56, 57].

Conclusion

Over the past decade, new insights into the pathogenesis of MDS with del(5q) have unveiled how allelic haploinsufficiency gives rise to the distinctive clinical phenotype. Haploinsufficient *RPS14*, *miR-145/146a*, and *CSNK1A1* located in the distal CDR and *HSPA9* and *EGR1* in the proximal CDR are epistatic molecular contributors to the hematologic manifestations of the disease. The selective sensitivity of lenalidomide in the del(5q) clone arises from CKI*α* degradation by binding to CRBN, and CDC25C and PP2A phosphatase inhibition. Understanding these mechanisms of lenalidomide action also sheds lights into the mechanisms of resistance. Much work is still needed to discern strategies to circumvent resistance and additional opportunities for therapeutic intervention.

References

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
2. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89(6):2079–88.
3. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Sole F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454–65.

4. Haase D, Germing U, Schanz J, Pfeilstocker M, Nosslinger T, Hildebrandt B, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. Blood. 2007;110(13):4385–95.
5. Bernasconi P, Klersy C, Boni M, Cavigliano PM, Calatroni S, Giardini I, et al. Incidence and prognostic significance of karyotype abnormalities in de novo primary myelodysplastic syndromes: a study on 331 patients from a single institution. Leukemia. 2005;19(8):1424–31.
6. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011;364(26):2496–506.
7. Bejar R, Stevenson KE, Caughey BA, Abdel-Wahab O, Steensma DP, Galili N, et al. Validation of a prognostic model and the impact of mutations in patients with lower-risk myelodysplastic syndromes. J Clin Oncol. 2012;30(27):3376–82.
8. Kulasekararaj AG, Smith AE, Mian SA, Mohamedali AM, Krishnamurthy P, Lea NC, et al. TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. Br J Haematol. 2013;160(5):660–72.
9. Van den Berghe H, Cassiman JJ, David G, Fryns JP, Michaux JL, Sokal G. Distinct haematological disorder with deletion of long arm of no. 5 chromosome. Nature. 1974;251(5474):437–8.
10. Zhao N, Stoffel A, Wang PW, Eisenbart JD, Espinosa R 3rd, Larson RA, et al. Molecular delineation of the smallest commonly deleted region of chromosome 5 in malignant myeloid diseases to 1-1.5 Mb and preparation of a PAC-based physical map. Proc Natl Acad Sci U S A. 1997;94(13):6948–53.
11. Boultwood J, Fidler C, Lewis S, Kelly S, Sheridan H, Littlewood TJ, et al. Molecular mapping of uncharacteristically small 5q deletions in two patients with the 5q- syndrome: delineation of the critical region on 5q and identification of a 5q- breakpoint. Genomics. 1994;19(3):425–32.
12. Boultwood J, Fidler C, Strickson AJ, Watkins F, Gama S, Kearney L, et al. Narrowing and genomic annotation of the commonly deleted region of the 5q- syndrome. Blood. 2002;99(12):4638–41.
13. Horrigan SK, Westbrook CA, Kim AH, Banerjee M, Stock W, Larson RA. Polymerase chain reaction-based diagnosis of del (5q) in acute myeloid leukemia and myelodysplastic syndrome identifies a minimal deletion interval. Blood. 1996;88(7):2665–70.
14. Douet-Guilbert N, De Braekeleer E, Basinko A, Herry A, Gueganic N, Bovo C, et al. Molecular characterization of deletions of the long arm of chromosome 5 (del(5q)) in 94 MDS/AML patients. Leukemia. 2012;26(7):1695–7.
15. Boultwood J, Pellagatti A, Cattan H, Lawrie CH, Giagounidis A, Malcovati L, et al. Gene expression profiling of CD34+ cells in patients with the 5q- syndrome. Br J Haematol. 2007;139(4):578–89.
16. Pellagatti A, Fernandez-Mercado M, Di Genua C, Larrayoz MJ, Killick S, Dolatshad H, et al. Whole-exome sequencing in del(5q) myelodysplastic syndromes in transformation to acute myeloid leukemia. Leukemia. 2014;28(5):1148–51.
17. Largaespada DA. Haploinsufficiency for tumor suppression: the hazards of being single and living a long time. J Exp Med. 2001;193(4):F15–8.
18. Komrokji RS, Padron E, Ebert BL, List AF. Deletion 5q MDS: molecular and therapeutic implications. Best Pract Res Clin Haematol. 2013;26(4):365–75.
19. Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N, et al. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. Nature. 2008;451(7176):335–9.
20. Gazda HT, Zhong R, Long L, Niewiadomska E, Lipton JM, Ploszynska A, et al. RNA and protein evidence for haplo-insufficiency in Diamond-Blackfan anaemia patients with RPS19 mutations. Br J Haematol. 2004;127(1):105–13.
21. Barlow JL, Drynan LF, Hewett DR, Holmes LR, Lorenzo-Abalde S, Lane AL, et al. A p53-dependent mechanism underlies macrocytic anemia in a mouse model of human 5q- syndrome. Nat Med. 2010;16(1):59–66.
22. Fumagalli S, Di Cara A, Neb-Gulati A, Natt F, Schwemberger S, Hall J, et al. Absence of nucleolar disruption after impairment of 40S ribosome biogenesis reveals an rpL11-translation-dependent mechanism of p53 induction. Nat Cell Biol. 2009;11(4):501–8.

23. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell. 1992;69(7):1237–45.
24. Peller S, Frenkel J, Lapidot T, Kahn J, Rahimi-Levene N, Yona R, et al. The onset of p53-dependent apoptosis plays a role in terminal differentiation of human normoblasts. Oncogene. 2003;22(30):4648–55.
25. Chaudhary AK, Chaudhary S, Ghosh K, Shanmukaiah C, Nadkarni AH. Secretion and expression of matrix metalloproteinase-2 and 9 from bone marrow mononuclear cells in myelodysplastic syndrome and acute myeloid leukemia. Asian Pac J Cancer Prev. 2016;17(3):1519–29.
26. Youn M, Huang H, Chen C, Kam S, Wilkes MC, Chae H-D, et al. MMP9 inhibition rescues the erythroid defect in RPS14-deficient del(5q) MDS models. Blood. 2018;132:756.. –.
27. Deocaris CC, Kaul SC, Wadhwa R. The versatile stress protein mortalin as a chaperone therapeutic agent. Protein Pept Lett. 2009;16(5):517–29.
28. Schmitz-Abe K, Ciesielski SJ, Schmidt PJ, Campagna DR, Rahimov F, Schilke BA, et al. Congenital sideroblastic anemia due to mutations in the mitochondrial HSP70 homologue HSPA9. Blood. 2015;126(25):2734–8.
29. Chen TH, Kambal A, Krysiak K, Walshauser MA, Raju G, Tibbitts JF, et al. Knockdown of Hspa9, a del(5q31.2) gene, results in a decrease in hematopoietic progenitors in mice. Blood. 2011;117(5):1530–9.
30. Liu T, Krysiak K, Shirai CL, Kim S, Shao J, Ndonwi M, et al. Knockdown of HSPA9 induces TP53-dependent apoptosis in human hematopoietic progenitor cells. PLoS One. 2017;12(2):e0170470.
31. Starczynowski DT, Kuchenbauer F, Argiropoulos B, Sung S, Morin R, Muranyi A, et al. Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype. Nat Med. 2010;16(1):49–58.
32. Gaballa MR, Besa EC. Myelodysplastic syndromes with 5q deletion: pathophysiology and role of lenalidomide. Ann Hematol. 2014;93(5):723–33.
33. Kumar MS, Narla A, Nonami A, Mullally A, Dimitrova N, Ball B, et al. Coordinate loss of a microRNA and protein-coding gene cooperate in the pathogenesis of 5q- syndrome. Blood. 2011;118(17):4666–73.
34. Varney ME, Choi K, Bolanos L, Christie S, Fang J, Grimes HL, et al. Epistasis between TIFAB and miR-146a: neighboring genes in del(5q) myelodysplastic syndrome. Leukemia. 2017;31(7):1659.
35. Ribezzo F, Snoeren IAM, Ziegler S, Stoelben J, Olofsen PA, Henic A, et al. Rps14, Csnk1a1 and miRNA145/miRNA146a deficiency cooperate in the clinical phenotype and activation of the innate immune system in the 5q- syndrome. Leukemia. 2019;33(7):1759–72.
36. Bello E, Pellagatti A, Shaw J, Mecucci C, Kusec R, Killick S, et al. CSNK1A1 mutations and gene expression analysis in myelodysplastic syndromes with del(5q). Br J Haematol. 2015;171(2):210–4.
37. Schneider RK, Adema V, Heckl D, Jaras M, Mallo M, Lord AM, et al. Role of casein kinase 1A1 in the biology and targeted therapy of del(5q) MDS. Cancer Cell. 2014;26(4):509–20.
38. Bradshaw AD, Sage EH. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J Clin Invest. 2001;107(9):1049–54.
39. Pellagatti A, Jadersten M, Forsblom AM, Cattan H, Christensson B, Emanuelsson EK, et al. Lenalidomide inhibits the malignant clone and up-regulates the SPARC gene mapping to the commonly deleted region in 5q- syndrome patients. Proc Natl Acad Sci U S A. 2007;104(27):11406–11.
40. Joslin JM, Fernald AA, Tennant TR, Davis EM, Kogan SC, Anastasi J, et al. Haploinsufficiency of EGR1, a candidate gene in the del(5q), leads to the development of myeloid disorders. Blood. 2007;110(2):719–26.
41. Stoddart A, Fernald AA, Wang J, Davis EM, Karrison T, Anastasi J, et al. Haploinsufficiency of del(5q) genes, Egr1 and Apc, cooperate with Tp53 loss to induce acute myeloid leukemia in mice. Blood. 2014;123(7):1069–78.

42. List A, Kurtin S, Roe DJ, Buresh A, Mahadevan D, Fuchs D, et al. Efficacy of lenalidomide in myelodysplastic syndromes. N Engl J Med. 2005;352(6):549–57.
43. List A, Dewald G, Bennett J, Giagounidis A, Raza A, Feldman E, et al. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med. 2006;355(14): 1456–65.
44. Sallman DA, Wei S, List A. PP2A: the achilles heal in MDS with 5q deletion. Front Oncol. 2014;4:264.
45. Wei S, Chen X, Rocha K, Epling-Burnette PK, Djeu JY, Liu Q, et al. A critical role for phosphatase haplodeficiency in the selective suppression of deletion 5q MDS by lenalidomide. Proc Natl Acad Sci U S A. 2009;106(31):12974–9.
46. Wei S, Chen X, McGraw K, Zhang L, Komrokji R, Clark J, et al. Lenalidomide promotes p53 degradation by inhibiting MDM2 auto-ubiquitination in myelodysplastic syndrome with chromosome 5q deletion. Oncogene. 2013;32(9):1110–20.
47. Kronke J, Fink EC, Hollenbach PW, MacBeth KJ, Hurst SN, Udeshi ND, et al. Lenalidomide induces ubiquitination and degradation of CK1alpha in del(5q) MDS. Nature. 2015;523(7559):183–8.
48. List A, Ebert BL, Fenaux P. A decade of progress in myelodysplastic syndrome with chromosome 5q deletion. Leukemia. 2018;32(7):1493–9.
49. Talati C, Sallman D, List A. Lenalidomide: myelodysplastic syndromes with del(5q) and beyond. Semin Hematol. 2017;54(3):159–66.
50. Fink EC, Ebert BL. The novel mechanism of lenalidomide activity. Blood. 2015;126(21): 2366–9.
51. Fenaux P, Giagounidis A, Selleslag D, Beyne-Rauzy O, Mufti G, Mittelman M, et al. A randomized phase 3 study of lenalidomide versus placebo in RBC transfusion-dependent patients with low-/intermediate-1-risk myelodysplastic syndromes with del5q. Blood. 2011;118(14):3765–76.
52. Zeidan AM, Gore SD, McNally DL, Baer MR, Hendrick F, Mahmoud D, et al. Lenalidomide performance in the real world: patterns of use and effectiveness in a Medicare population with myelodysplastic syndromes. Cancer. 2013;119(21):3870–8.
53. Tehranchi R, Woll PS, Anderson K, Buza-Vidas N, Mizukami T, Mead AJ, et al. Persistent malignant stem cells in del(5q) myelodysplasia in remission. N Engl J Med. 2010;363(11):1025–37.
54. Jadersten M, Saft L, Smith A, Kulasekararaj A, Pomplun S, Gohring G, et al. TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. J Clin Oncol. 2011;29(15):1971–9.
55. Saft L, Karimi M, Ghaderi M, Matolcsy A, Mufti GJ, Kulasekararaj A, et al. p53 protein expression independently predicts outcome in patients with lower-risk myelodysplastic syndromes with del(5q). Haematologica. 2014;99(6):1041–9.
56. Lee JH, List A, Sallman DA. Molecular pathogenesis of myelodysplastic syndromes with deletion 5q. Eur J Haematol. 2019;102(3):203–9.
57. Sallman D, Dezern A, Sweet K, Steensma D, Cluzeau T, Sekeres M, et al., editors. Phase 1B/2 combination study of APR-246 and azacitidine (AZA) in patients with TP53 mutant myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). 23rd Congress of the European Hematology Association; 17 June 2018; Stockholm, Sweden.

Chapter 4
MDS Stem Cell Biology

Matthew T. Villaume, P. Brent Ferrell, and Michael R. Savona

Introduction

Myelodysplastic syndromes (MDS) are clonal neoplasms characterized by cytopenias due to ineffective hematopoiesis, characteristic morphologic dysplasia, and risk for transformation into AML [1]. As over 50 recurring somatic gene mutations and chromosomal abnormalities contribute to the pathogenesis of MDS or its transformation into AML, recent focus has been on the molecular epidemiology of mutations, associated prognostication, and targeted therapy [1]. As the biologic consequences of these mutations, and the biologic conditions which give rise to these mutations, are explored, it is clear that commonly seen molecular aberrations in MDS affect energy metabolism, ineffective hematopoiesis, and risk for AML transformation. This story has grown more complicated as new research reveals the role of the stem cell niche in MDS, indicating that there are both mesenchymal and hematopoietic cell contributors to the disease [2]. Furthermore, increasing evidence illustrates pro-inflammatory programmed necrotic cell death (e.g., necroptosis or pyroptosis) drives cell death seen in the bone marrow (BM) of MDS patients [3]. This chapter will discuss the stem cell biology of MDS in the context of these rapidly growing areas of research: energy metabolism, the BM niche, and programmed necrosis.

M. T. Villaume · P. B. Ferrell · M. R. Savona (✉)
Department of Medicine and Program in Cancer Biology, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA
e-mail: michael.savona@vanderbilt.edu

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3_4

Stem Cells, Hematopoiesis, and Energy Metabolism

Stem Cell Differentiation and Quiescence

A hallmark of healthy hematopoietic stem cells (HSC) is the ability to produce multilineage hematopoiesis in the BM. The characteristic quantitative effects on hematopoiesis in lower- and higher-risk MDS have been well elucidated (Fig. 4.1)

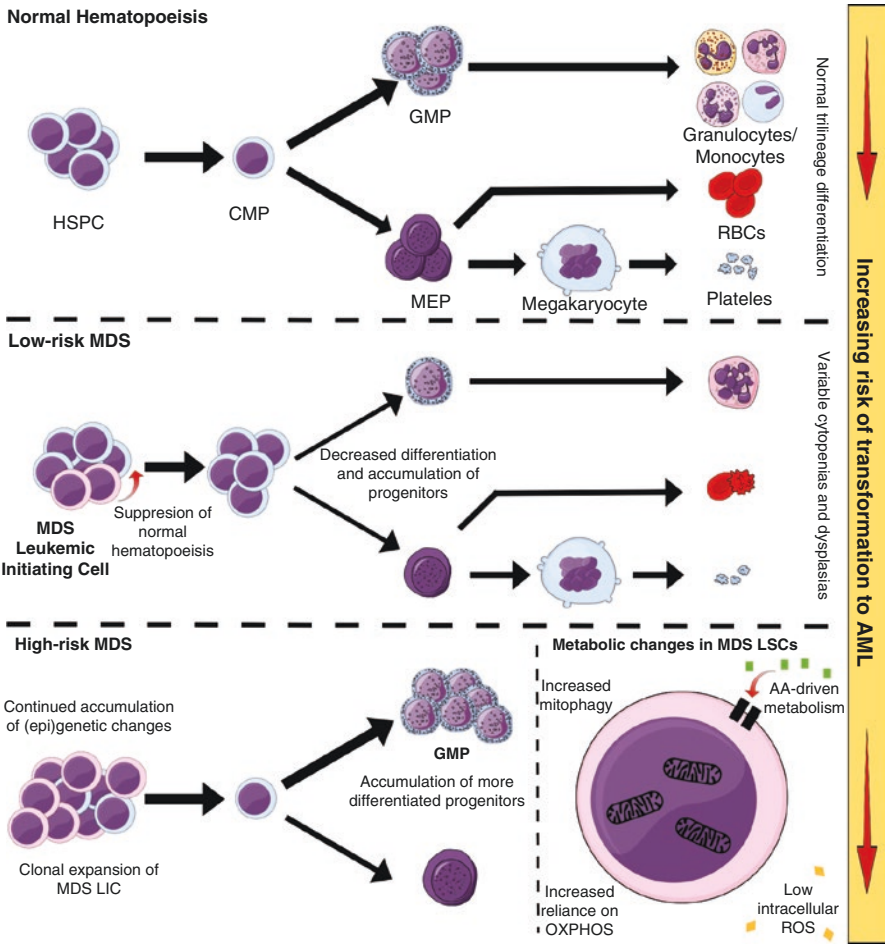

Fig. 4.1 Disordered stem cell differentiation and energy metabolism in MDS. The cytopenias that are characteristic of MDS have been attributed, in part, to decreased differentiation and accumulation of precursors such as common myeloid progenitors (CMPs) in lower-risk and granulocyte-macrophage progenitors (GMPs) in higher-risk MDS. Intrinsic epigenetic and genetic changes in leukemia-initiating cells (LICs) are correlated with these differentiation blocks but changes in stem cell energy metabolism represent another compelling, targetable, driver of disordered hematopoiesis in MDS

[4]. Lower-risk MDS is characterized by an increase in the common myeloid progenitor (CMP) population as well as a marked decrease in the megakaryocyte erythroid progenitor (MEP). These findings suggest dysfunction in differentiation that correlates well with the cytopenias seen clinically. Higher-risk MDS marks a transition to acute myeloid leukemia characterized by an increase in granulocyte monocyte progenitors (GMP) and significantly expanded long-term hematopoietic stem cell population (LT-HSC) as programs of proliferation emerge [5].

Most HSCs are in a quiescent state, where they cycle slowly or not at all but can expand or contract in response to environmental cues [6]. This quiescent state is thought to protect them from endogenous stresses and they have been shown to be more radioresistant and have decreased intracellular ROS than their more differentiated progenitors [7–9]. However, quiescence may be a double-edged sword, as mouse HSC DNA repair occurs predominantly through error-prone nonhomologous end joining (NHEJ), making them more prone to mutagenesis [10].

Stem Cell Immunophenotypes

The immunophenotype of human HSCs has been elucidated over the years and is characterized by the absence of terminally differentiated cell surface markers and a $CD34^{+}38^{-}$ immunophenotype [11]. Considerable effort to find immunophenotypic differences between leukemic initiating cells (LIC) and healthy HSCs led to the identification of upregulations of CD44, CD47, CD96, CD99, CD123, and Tim3 in LSCs of myeloid malignancies, but yielded no sine qua nonimmunophenotype to differentiate healthy HSCs and LICs [12–18]. Importantly, cell types throughout mouse hematopoietic ontogeny are better elucidated with stem cells classically identified by a $Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$ immunophenotype. Key differences exist between human and murine stem cell markers, adding complexity and challenge to translating work between the two. For example, there is profound enrichment of the signaling lymphocyte activation molecule family (SLAM) in the murine long-term (LT)-HSCs with the $CD150^{+}CD48^{-}$ immunophenotype mice but not in human LT-HSCs [19, 20]. $CD34^{+}38^{-}$ cells constitute roughly 0.01% of healthy BM but exist more frequently in acute myeloid leukemia (AML) [21]. The $CD34^{+}38^{-}$ immunophenotype was first used to isolate LSCs, and this same population in MDS samples has revealed an enrichment for known disease-driver mutations and an ability to persist even after clinical remission of the disease [5, 22]. This data suggests that MDS disease cells arise from a clone that exists at the HSC stage. These cells show a striking resemblance to healthy HSPCs and have a number of characteristics that make them resistant to chemotherapy, including quiescence, increased DNA repair, reduced apoptosis, increased neutralization of reactive oxygen species (ROS), enhanced drug efflux mechanisms, and reduced immune clearance [23–25]. The phenotypic similarities between normal HSCs and LICs present an obvious challenge for the investigation of selective treatments.

Leukemic Stem Cell Metabolism

Identifying targetable characteristics that are unique to MDS LICs is a growing area of research; however, efforts have been complicated by difficulties in establishing models of the disease and considerable heterogeneity between MDS cells and patients. Whereas challenges remain in this vein in MDS, the capacity to propagate leukemia in cell line-derived (CDX) or patient-derived xenograft (PDX) transplantation models has accelerated understanding of leukemogenesis [26]. Only recently have new immunocompromised murine strains allowed for MDS PDXs to successfully engraft [27]. Unique surface markers, signaling characteristics, and energy production phenotypes have helped to refine this line of experimentation [28, 29]. Traditionally, malignant cells were thought to rely on glycolysis rather than oxidative phosphorylation, a phenomenon referred to as the Warburg effect [30]. This is true in certain tumor populations and has been attributed to the larger cell machinery requirements of oxidative phosphorylation and the relative abundance of glucose available to cancer cells [31, 32]. However, more recent work has shown LSCs have lower rates of energy metabolism and low levels of reactive oxygen species (ROS) [33]. In fact, Jones et al. demonstrated that LSCs were dependent on cysteine-driven oxidative phosphorylation showing an increased resistance to glucose depletion [34, 35]. Further, while observing AML blasts from relapsed patients, acquired resistance to amino acid depletion and an upregulation of fatty acid metabolism was noted, demonstrating the complex ways metabolism changes can lead to a survival advantage of LSCs.

ROS-low $CD123^{+}$ MDS LICs also contain a significant upregulation of protein synthesis machinery, which has previously been shown to play an important role in stem cell survival [36–38]. Further, the protein synthesis was largely dedicated to energy metabolism rather than cell replication as may have been expected of a clonal neoplasm. The increased utilization of oxidative phosphorylation observed is consistent with previous reports on stem cells in AML as well as in melanoma, breast, and pancreatic cancer [39–41]. Most convincingly, MDS stem cells were selectively poisoned by the administration of small molecules targeting the oxidative phosphorylation pathway in xenograft models. This is, again, consistent with studies in AML from Schimmer et al. which showed the selective killing of stem cells with inhibition of the mitochondrial proteome [42, 43].

Work on AML stem cells has continued to advance the hypothesis that energy usage differences represent a distinguishing and targetable feature of LICs. LICs have been shown to decrease insulin sensitivity in peripheral tissue via insulin-like growth factor BP1 (IGFBP1) [44]. By isolating AML LSCs based on their low ROS phenotype, rather than by immunophenotype, LICs have been shown to be reliant on FIS1-mediated mitophagy for renewal and maintenance [45]. Oxygen metabolism via NADPH oxidases (NOX) in HSCs has been shown to play an increasingly diverse role with recent studies highlighting its ability to suppress differentiation and maintain LIC renewal [46, 47]. In each of these cases, inhibition of the dysregulated pathway leads to the eradication of LICs, often in a selective manner over

normal HSCs. Therapy targeting the metabolic liabilities of LICs has started to appear in the clinic. For example, the combination of the BCL-2 inhibitor, venetoclax, and azacitidine has been shown to disrupt amino acid metabolism, and selectively kill LSCs which cannot upregulate alternative energy use, and may explain the combination's selective activity against AML LICs [48]. More recently, Chen et al. demonstrated leukemic mitochondria may maintain and buttress cristae structure in the face of the blockade of oxidative phosphorylation with venetoclax as a mechanism of survival/resistance [49].

LICs also show increased expression levels of IL-1 receptor accessory protein (IL1RAP) in higher-risk MDS [50]. Its expression level was independently associated with poor overall survival and its inhibition lead to decreased viability and growth of AML cells. IL1RAP is involved in signaling through IL-1β, which is known to stimulate AML blast growth and increase resistance to apoptosis [36, 51–53]. However, this increase in signaling is not solely due to an increase in IL1RAP levels, opening questions as to the source of the increased inflammatory signaling in this stem cell population. Recent work elucidating the role of the BM niche and programmed necrotic cell death in inflammatory signaling could partially explain this discrepancy.

Inflammatory Signaling and Cell Death

There has long been an association between systemic inflammatory disease and MDS. Before a mechanism could be understood, reports highlighted that 10–20% of MDS patients had a concurrent autoimmune disease such as Crohn's disease or rheumatoid arthritis [54–56]. The causative mechanism between inflammation and MDS is still incompletely understood but significant headway has been made through increased understanding of both the dysregulated innate immune signaling pathway and the cell death mechanisms seen in MDS stem cells.

Disordered Innate Immune Signaling

There is a myriad of inflammatory cytokine and signaling changes in MDS patients, which are covered in more detail elsewhere [57]. Healthy HSCs have been shown to express Fas in response to increased levels of TNF-α or INF-γ, and apoptosis rates are correlated with levels of TNF-α in MDS samples [58, 59]. The pro-apoptotic TNF receptor 1 has been shown to predominate over the anti-apoptotic TNF receptor 2 in the stem cells of MDS with refractory anemia (MDS-SLD/MDS-MLD) [60]. This ratio of TNF receptors reverses in MDS with excessive blasts (MDS-EB), demonstrating the delicate way that innate immune signaling dysregulation can be reflected in the clinical environment.

Central to the disordered immune signaling in MDS stem cells are the toll-like receptors (TLR). These pattern recognition receptors activate the innate immune system in response to the pathogen (PAMP) or host-derived damage-associated molecular patterns (DAMPs), which are also known as alarmins. There are 10 TLRs and all serve distinct but overlapping roles [61]. TLR4 and subsequently TLR1, TLR2, and TLR6 have been found to be overexpressed in MDS LICs with TLR4 signaling found to be linked to HSC death [62, 63].

The primary downstream effector through which TLRs signal is the myeloid differentiation primary response gene 88 protein (MyD88). Cooperating with interleukin-1 receptor-associated kinases (IRAK) 1 and 4, in a collection known as the "Myddosome", it leads to increased activity of the transcription factor NF-κB via TNF receptor-associated factor 6 (TFAF6) and IκB kinase (IKK) [64]. Other mediators play important roles in TLR signaling and are reviewed elsewhere (TIR, p38 MAPK, JNK, AP-1) [61]. NF-κB plays a diverse role in MDS pathogenesis and its activation has been found to increase the production of DAMPs and inflammatory cytokines (IL-1β, IL-6, TNF-α) as well as anti-apoptotic proteins [65]. Modulators of the TLR pathway have been shown to play key roles in models of MDS, with loss of del(5q)-associated genes miRNA145, miRNA146a, and TIFAB all leading to upregulated TLR4 signaling and an MDS-like phenotype [66, 67].

Of the known DAMPs, the molecules S100A8 and S100A9, and their heterodimer calprotectin, have received the most attention in MDS stem cell biology. They are endogenous ligands for TLR4 and have been previously shown to play an important role in inflammatory conditions and more recently in malignancy [68]. S100A8/9 levels are known to be elevated in the serum of MDS patients and to induce the expression of the pro-survival programmed cell death protein 1 (PD-1) on MDS LICs [69, 70].

In addition to the increased production of DAMPs, S100A9-TLR4-mediated NF-κB activity also leads to the increased expression of the nucleotide-binding domain and leucine-rich repeat (NLR) pattern recognition receptor NLRP3, which has been shown to play a key role in pyroptosis, a pro-inflammatory cell death pathway discussed below [71]. Further elucidation of aberrant cell death types in MDS LICs has led to a greater understanding of the connection between stem cell biology and the inflammatory clinical phenotype.

Programmed Necrotic Cell Death

Pyroptosis and necroptosis are two examples of programmed necrotic cell death [72–74]. Their evolutionary purpose is thought to be protective against infectious microbes but the inappropriate activation of these pathways has been connected to both autoimmune disease and now myeloid neoplasia [75]. While the pathways use different machinery and signaling, they both result in a lytic cell death mechanism that releases DAMPs and pro-inflammatory cytokines such as IL-1ß and S100A9 via membrane-damaging pore formation. Traditionally, HSC cell death in MDS was

thought to occur via the immunologically silent apoptosis pathway. Previous studies have shown increased cell-extrinsic apoptosis in lower-risk and increased cell-intrinsic apoptosis in higher-risk MDS, and these pathways certainly still play a role in the disease [76]. However, many of these studies were conducted before an understanding of the molecular details of pyroptosis or necroptosis existed and the assays used could not distinguish between these different mechanisms of cell death [77]. An understanding of these two pathways and their relevance to the HSC BM niche is becoming increasingly crucial in understanding MDS stem cell biology.

Pyroptosis is driven by a collection of molecules collectively known as the "inflammasome" (Fig. 4.2) [3, 78]. Assembly of the inflammasome can be instigated by several cell-extrinsic signals including binding of DAMPs such as S100A9 to TLRs or by intracellular ROS. The inflammasome is composed of oligomers of NLRP3 and it recruits apoptosis-associated speck-like protein (ASC) into linear filaments termed ASC specks. These ASC specks trigger activation of caspase-1 which subsequently leads to the production of pro-inflammatory IL-1ß and IL-18. S100A9 and ROS are also generated in response to inflammasome activity and lead to activation of the pyroptotic cell death pathway and, paradoxically, the Wnt/ß--catenin pathway, which is known to promote LIC self-renewal [79]. Finally, these pro-inflammatory signals are released from cells via pores formed by another inflammasome product, gasdermin D. These pores simultaneously allow for the influx of cations, leading to cell swelling that is reminiscent of necrotic cell death. Evidence that the inflammasome is the driver of HSC cell death is compelling and exists regardless of the underlying genotype. Basiorka et al. demonstrated that pyroptosis is the predominant cell death mechanism in both a mouse model of MDS and human MDS samples [80]. Inhibition of components of the inflammasome pathway, such as S100A9 or NLRP3, attenuated pyroptosis and restored normal hematopoiesis in their mouse model. Additionally, released ASC specks have been shown to induce pyroptosis and serum levels of this inflammasome mediator have been demonstrated to be a biomarker of medullary pyroptotic cell death [81].

Necroptosis is a caspase-independent programmed cell death pathway that has only recently been shown to be increased in the BM of MDS patients by Wagner et al. [82] Traditionally, the pathway is activated by stimulation of TNF death receptors, which are usually associated with cell-extrinsic, apoptotic cell death. This signal triggers the formation of the necrosome, a cell death platform composed of receptor-interacting proteins (RIPK) 1 and 3 [72, 74]. This platform subsequently allows oligomerization of MLKL which forms membrane damaging pores, allowing the release of DAMPs and other cytokines, in a manner analogous to gasdermin D and pyroptosis. Importantly, traditional apoptotic pathway proteins, such as caspase-8 and Bid, have been found to be inhibitors of the necroptotic pathway through Ripk1 degradation and necroptosis does not predominate in the BM of mouse models until multiple members of the apoptotic pathway are inhibited [82, 83]. Necroptosis and pyroptosis are not likely mutually exclusive, as there is evidence existing for crosstalk between the two [74, 84].

These complex pathways paint the picture of a positive feedback cycle, where inflammatory cytokines trigger a lytic cell death in BM-resident cells which release further inflammatory cytokines to trigger pyroptosis or necroptosis in neighboring

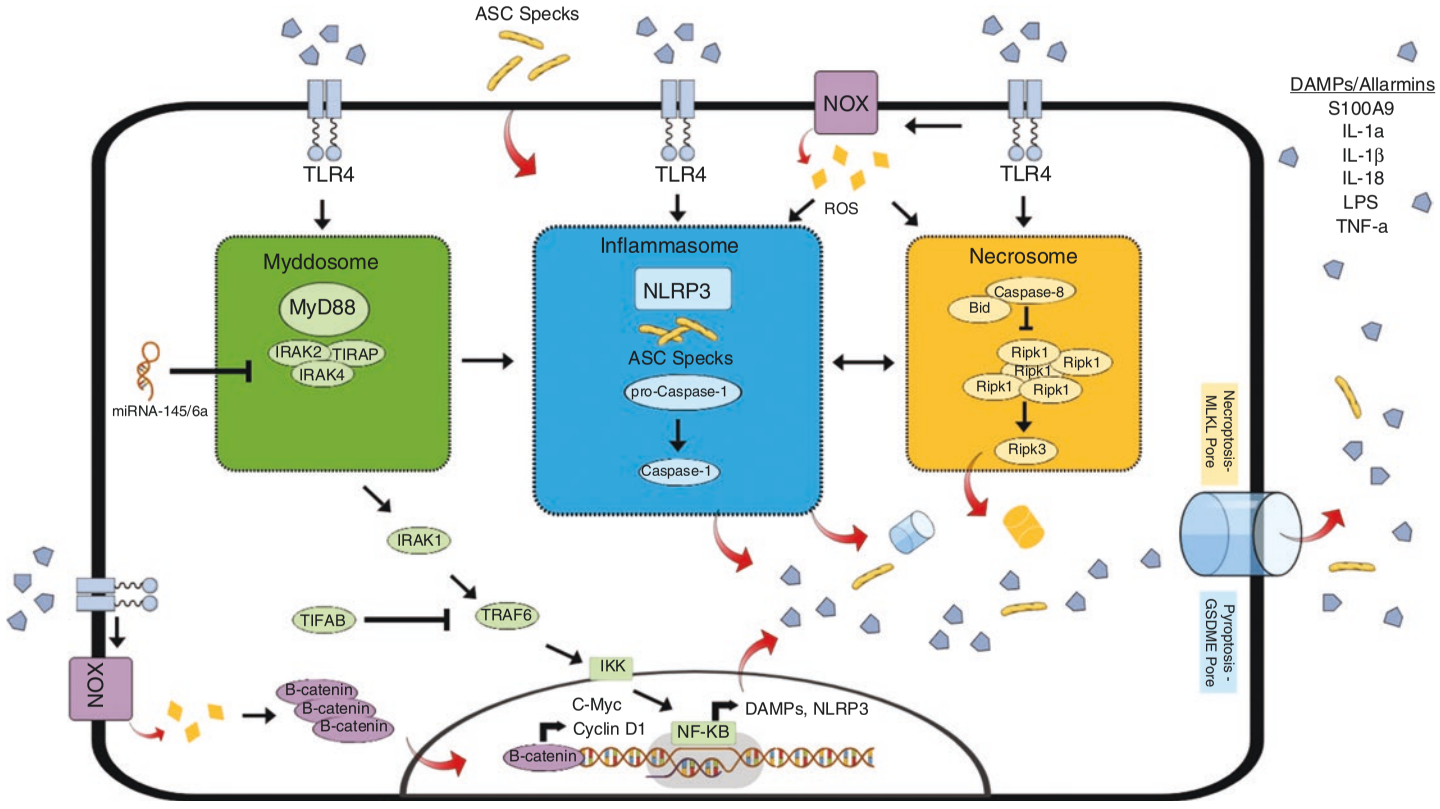

Fig. 4.2 Disordered inflammatory signaling in MDS. Disordered signaling in MDS occurs in multiple pro-inflammation signal transducers and effectors, including innate immune signaling through MyD88 (green), the NLRP3-driven inflammasome (blue), and the Ripk1-mediated necrosome (yellow). Complexity results from the fact that these pathways have considerable crosstalk and are variably triggered by extracellular signals (DAMP-TLR4 binding can trigger increased NLRP3 expression and Myd88 signaling) or intracellular signals (intracellular ROS can trigger inflammasome formation). This disordered signaling has been shown to increase NOX2 generation of ROS and inflammatory cell death via membrane permeabilization but also paradoxically increase cell survival via WNT/β-catenin signaling (purple)

cells. Cell death in more differentiated myeloid progenitor cells leads to clinical cytopenias observed in MDS patients. Whereas, the activation of pro-survival ß-catenin signaling in MDS LICs, in combination with the survival advantage offered by their unique oxidative and metabolic state, leads to a maintenance of the disease-driving stem cell. However, these stem cells exist in a complex environment composed of both mesenchymal stroma and immune cells that are affected by, and perhaps even perpetuate, this inflammatory milieu.

The Stem Cell Niche

The discussion thus far has centered on intrinsic changes in the MDS LIC, how its unique characteristics lead to characteristic cytopenias and dysplasias while preserving a growth advantage over neighboring healthy HSCs. The picture remains incomplete, however, as intrinsic LIC growth advantage has not been observed with all MDS driver mutations. The phenomenon of donor cell leukemia, where donor-derived HSCs undergo malignant transformation upon transplantation but do not become malignant in their original host, also invokes the possibility of a cell-extrinsic driver of leukemic transformation [85]. The answer to this question may lie in the myriad of cells that populate the BM stem cell niche.

This stem cell niche is composed of an HSPC-supportive stroma including endothelium, osteoblasts, osteoclasts, adipocytes, undifferentiated mesenchymal stem/stromal cells (MSCs), neuronal cells, and an array of immune cells [86–89]. Healthy HSCs have been shown to localize to trabecular-rich areas of the BM, specifically those rich in perivascular endothelial-lined sinusoids, which differs from more committed progenitors both by marrow location and surrounding cellular constituents [86]. For example, common lymphoid progenitors localize to areas rich with mature osteoblasts and require them to be maintained, while BM macrophages appear to play a unique role in erythroid maturation [90–92]. Mesenchymal-derived chemical mediators of this stem cell support have been identified and include stromal cell factor (SCF) and C-X-C motif chemokine 12 (CXCL12), with deletion of either resulting in decreased HSC number [93–95]. BM niche dysfunction has long been suspected of playing a role in MDS, with early studies showing a correlation between MDS and osteoporosis and histologic findings showing disrupted BM architecture [96–98]. Since then, research has continued to reveal the contributions of both the mesenchymal stroma and the immune microenvironment to MDS pathogenesis.

Mesenchymal Stroma

Early experimental evidence for niche disease contribution lies in the work from Medyouf et al. showing that transplanted human MDS HSCs engraft more efficiently if their donor's BM mesenchymal cells are co-transplanted [99]. Additionally,

there have been isolated examples where the transplantation of MDS HSCs into a normal BM stroma largely rescues these stem cells from leukemic transformation [100]. Mechanistic investigations into genetic drivers of this phenomenon have focused on producing mouse models with genetically altered stroma. A myelodysplastic phenotype and a predisposition for AML transformation were observed with deletions of *Dicer1* or *Sbds* in osteoprogenitor cells (Fig. 4.3) [101]. Osteoblasts with a deficient retinoic acid receptor (RARγ) produce a myeloproliferative phenotype even when wild-type HSCs are transplanted into the environment [102]. This

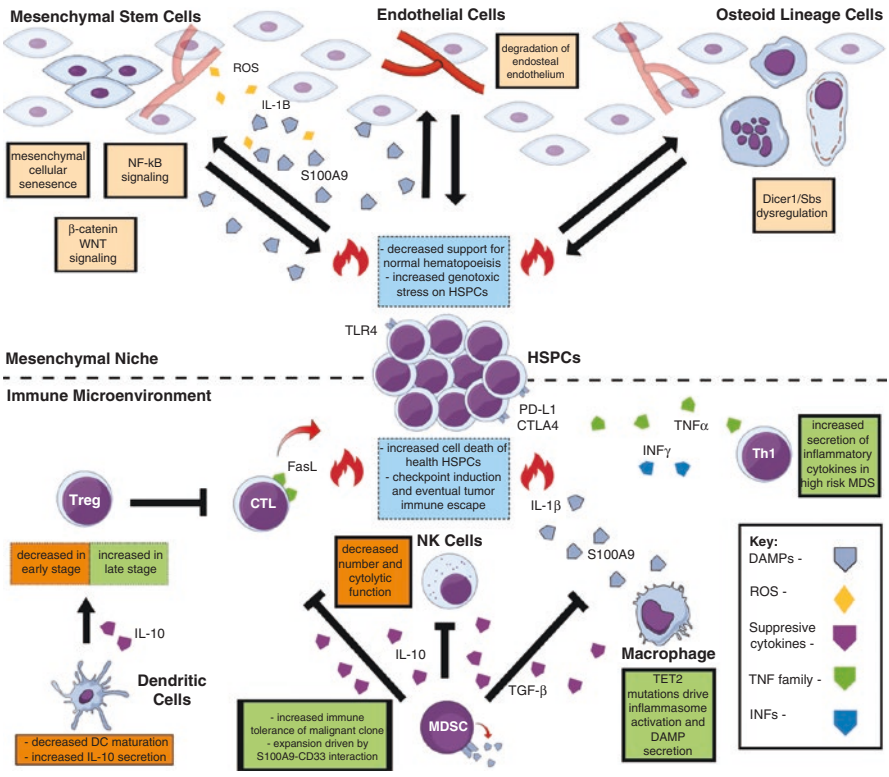

Fig. 4.3 Inflammation and immune tolerance in the MDS stem cell niche. Both mesenchymal and immune cells found in the bone marrow play key roles in hematopoiesis, with their ability to modulate the inflammatory microenvironment highlighted in this model. Bone marrow mesenchymal cells show a myriad of changes in MDS. Changes in NF-κB and WNT mesenchymal signaling have been tied to changes in the inflammatory signaling molecules found in the bone marrow microenvironment and could have implications for both healthy and diseased *HSPC*s. Dedicated immune cell changes also have important implications for HSPC health and function. A disordered immune microenvironment exists in the bone marrow of MDS patients driven by a paradoxical constellation of changes that include both immunosuppressive: increased Treg function, increased suppressive functions of dendritic cells and MDSCs, and also pro-inflammatory changes: increased inflammatory cytokine release by macrophages and Th1 cells and increased death-ligand expression by CTLs

phenotype was attributed to significantly increased BM TNF cytokine levels, implicating the type of disordered inflammatory signaling discussed previously. AML was induced via an activating mutation in β-catenin in mouse osteoblasts, which is of considerable interest in MDS as upregulated β-catenin has been observed in the BM of patients [103, 104]. In this case, the disordered stromal cells resulted in upregulated Notch1 signaling between the stromal environment and HSPCs. MSC mutations or deficiencies in Ptpn11 or Sipa1 have also been shown to give rise to myeloid neoplasms in mouse models [105, 106]. Cytogenetic alterations in MSCs from MDS patients have been identified that are unique from the malignant clone but have yet to be definitively tied to disease pathogenesis, let alone demonstrated to drive MDS de novo [107, 108]. Therefore, it is unclear whether the changes observed in MDS mesenchymal stroma are the cause of the malignancy, are caused by the LICs, or co-occur independently. Nonetheless, work is ongoing to understand the molecular basis for how the niche could facilitate or support a clonal hematopoietic neoplasm.

Pronk et al. recently summarized the existing molecular mechanistic proposals for a niche facilitated model of MDS, highlighting mesenchymal changes in inflammatory signaling, WNT activity, and decreased support for normal hematopoiesis as primary hypotheses (Fig. 4.3) [2]. Mesenchymal cells from MDS patients show reduced expression of supportive factors (CXCL12), increased senescence, and reduced ability to support HSCs ex vivo [109–111]. In this vein, *FLT3* expression-driven myeloproliferation has been proposed to suppress normal hematopoiesis through a cell-extrinsic mechanism involving degradation of BM endothelium via inflammatory cytokine release, resulting in a less supportive HSC niche [112]. The WNT pathway is overexpressed in MDS patient-derived mesenchymal cells and is thought to lead to β-catenin activation and disease progression in MDS [104]. This WNT hyperactivity was due to hypermethylation of WNT antagonists and could be reversed with the demethylating agent 5-azacytidine ex vivo. Disordered inflammatory signaling pathways in MDS HSCs have also been observed in mesenchymal cells. Pro-inflammatory S100A9 and NF-κB signaling, this time of stromal cell origin, play a role in the destruction of healthy HSCs and the promotion of further inflammatory cytokine production [104, 113].

Immune Microenvironment

The potential role of immune cells in a disease increasingly seen to be driven by dysregulated inflammatory processes is an area of active research [114]. An immune cell that has received considerable attention in MDS is the myeloid-derived suppressor cell (MDSC). These cells, largely of monocytic lineage, secrete immunosuppressive cytokines and their overabundance has been implicated in cancer immune evasion in several solid tumor malignancies, attributed to their ability to suppress cytotoxic T lymphocytes (CTL) and promote regulatory T

cells (Treg) [115]. While the existence and important role of MDSCs has been repeatedly demonstrated in other malignancies, questions remain about their characterization and role in MDS. Of note, increased MDSC quantity has been associated with decreased BM T-cell proliferation and overall prognosis [116, 117]. S100A9 may play yet another role in MDS pathogenesis here by inhibiting dendritic cell (DC) maturation and driving MDSC expansion through CD33 autocrine signaling [118]. Other studies have demonstrated that DC has a decreased ability to activate T cells in MDS patients, with a cytokine secretion profile favoring immunosuppression [119, 120]. The previously described decrease in the GMP population has been attributed to increased phagocytic activity in BM-resident macrophages [17].

There is a dysregulated balance between immune effector and regulatory cells, particularly in lower-risk MDS patients, with CTLs, Th17 helper cells, and natural killer cells being increased and Tregs being decreased [121]. In some cases of MDS, this pro-inflammatory cell environment likely leads to a growth advantage in MDS HSCs, as these disease cells have been shown to overexpress the immunosuppressive PDL-1, allowing them to escape immune-mediated destruction [122]. The induction of PD-L1 is thought to be induced by overstimulation of stem cells by TNF-α and IFN-γ secreted by BM T cells but S100A9 has recently been implicated as well [70]. The increasing levels of PD-L1 on MDS stem cells correlates well with the decreasing ratio of effector to regulatory T cells (CD8:CD4) seen throughout disease progression and why NK cells show decreased cytolytic function [123, 124]. These aberrancies of the stem cell niche paint a chaotic picture of the classic “bad seed in bad soil” phenomenon where a synergistically dysfunctional relationship exists between LICs and their environment.

Conclusion

Considerable efforts to understand intrinsic differences in the stem cells of MDS, specifically in metabolism and cell death mechanisms, have led to great strides in elucidating the pathogenesis of this disease. However, these advances simultaneously invited further questions as to the role of the hematopoietic niche in disease initiation and progression. The cross-talk between hematopoietic stem and progenitor cells, stromal cells, and immune cells is critical in MDS pathogenesis and propagation; and likewise, a wealth of new targets and the opportunity to rationally use existing therapies.

Financial Disclosure Statement Dr. Savona receives research funding from Astex, Incyte, Takeda and TG Therapeutics, has equity in Karyopharm, is a consultant for Karyopharm, Ryvu and Sierra Oncology, and serves on advisory or DSMBs for Abbvie, BMS, Celgene, Takeda, and TG Therapeutics. The other authors have no disclosures.

References

1. Steensma DP. Myelodysplastic syndromes: diagnosis and treatment. Mayo Clin Proc. 2015;90(7):969–83.
2. Pronk E, Raaijmakers MHGP. The mesenchymal niche in MDS. Blood. 2019;133(10):1031–8.
3. Sallman DA, Cluzeau T, Basiorka AA, et al. Unraveling the pathogenesis of MDS: the NLRP3 inflammasome and pyroptosis drive the MDS phenotype. Front Oncol. 2016;6:151.
4. Shastri A, Will B, Steidl U, et al. Stem and progenitor cell alterations in myelodysplastic syndromes. Blood. 2017;129(12):1586–94.
5. Will B, Zhou L, Vogler TO, et al. Stem and progenitor cells in myelodysplastic syndromes show aberrant stage-specific expansion and harbor genetic and epigenetic alterations. Blood. 2012;120(10):2076–86.
6. Pietras EM, Warr MR, Passegué E. Cell cycle regulation in hematopoietic stem cells. J Cell Biol. 2011;195(5):709–20.
7. Orford KW, Scadden DT. Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation. Nat Rev Genet. 2008;9(2):115–28.
8. Down JD, Boudewijn A, Van Os R, et al. Variations in radiation sensitivity and repair among different hematopoietic stem cell subsets following fractionated irradiation. Blood. 1995;86(1):122–7.
9. Tothova Z, Kollipara R, Huntly BJ, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell. 2007;128(2):235–339.
10. Mohrin M, Bourke E, Alexander D, et al. Hematopoietic stem cell quiescence promotes error-prone DNA repair and mutagenesis. Cell Stem Cell. 2010;7(2):174–85.
11. Terstappen LWM, Huang S, Safford M, et al. Sequential generations of hematopoietic colonies derived from single nonlineage-committed CD34+ CD38- progenitor cells. Blood. 1991;77(6):1218–27.
12. Jan M, Chao MP, Cha AC, et al. Prospective separation of normal and leukemic stem cells based on differential expression of TIM3, a human acute myeloid leukemia stem cell marker. Proc Natl Acad Sci U S A. 2011;108(12):5009–14.
13. Jin L, Hope KJ, Zhai Q, et al. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. Nat Med. 2006;12(10):1167–74.
14. Manz MG, Miyamoto T, Akashi K, et al. Prospective isolation of human clonogenic common myeloid progenitors. Proc Natl Acad Sci U S A. 2002;99(18):11872–7.
15. Chung SS, Eng WS, Hu W, et al. CD99 is a therapeutic target on disease stem cells in myeloid malignancies. Sci Transl Med. 2017;9(374):eaaj2025.
16. Jaiswal S, Jamieson CH, Pang WW, et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell. 2009;138(2):271–85.
17. Pang WW, Pluvinage JV, Price EA, et al. Hematopoietic stem cell and progenitor cell mechanisms in myelodysplastic syndromes. Proc Natl Acad Sci U S A. 2013;110(8):3011–6.
18. Hosen N, Park CY, Tatsumi N, et al. CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Proc Natl Acad Sci U S A. 2006;104(26):11008–13.
19. Kiel MJ, Yilmaz OH, Iwashita T, et al. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell. 2005;121(7):1109–21.
20. Larochelle A, Savona M, Wiggins M, et al. Human and rhesus macaque hematopoietic stem cells cannot be purified based only on SLAM family markers. Blood. 2011;117(5):1550–4.
21. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994;367(6464):645–8.
22. Woll PS, Kjällquist U, Chowdhury O, et al. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. Cancer Cell. 2014;25(6):794–808.
23. Costello RT, Mallet F, Sainty D, et al. Human acute myeloid leukemia CD34+/CD38− progenitor cells have decreased sensitivity to chemotherapy and Fas-induced apoptosis,

reduced immunogenicity, and impaired dendritic cell transformation capacities. Cancer Res. 2000;60(16):4403–11.
24. Ishikawa F, Yoshida S, Saito Y, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. Nat Biotechnol. 2007;25(11):1315–21.
25. Lytle NK, Barber AG, Reya T. Stem cell fate in cancer growth, progression and therapy resistance. Nat Rev Cancer. 2018;18:669–80.
26. Cook GJ, Pardee TS. Animal models of leukemia: any closer to the real thing? Cancer Metastasis Rev. 2013;32(0):63–76.
27. Song Y, Rongvaux A, Taylor A, et al. A highly efficient and faithful MDS patient-derived xenotransplantation model for pre-clinical studies. Nat Commun. 2019;10(1):1–14.
28. (a) Jordan CT, Upchurch D, Szilvassy SJ, et al. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. Leukemia. 2000;14(10):1777–84. (b) Gerber JM, Smith BD, Ngwang B, et al. A clinically relevant population of leukemic CD34(+)CD38(−) cells in acute myeloid leukemia. Blood. 2012;119(15):3571–7.
29. Xu Q, Simpson S-E, Scialla TJ, et al. Survival of acute myeloid leukemia cells requires PI3 kinase activation. Blood. 2003;102(3):972–80.
30. Warburg O. On the origin of cancer cells. Science. 1956;123(3191):309–14.
31. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324(5930):1029–33.
32. Vazquez A, Liu J, Zhou Y, et al. Catabolic efficiency of aerobic glycolysis: the Warburg effect revisited. BMC Syst Biol. 2010;4:58–67.
33. Lagadinou ED, Sach A, Callahan K, et al. BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. Cell Stem Cell. 2013;12(3):329–41.
34. Jones CL, Stevens BM, Alessandro AD, et al. Inhibition of amino acid metabolism selectively targets human leukemia stem cells. Cancer Cell. 2019;35(2):333–5.
35. Jones CL, Stevens BM, Alessandro AD, et al. Cysteine depletion targets leukemia stem cells through inhibition of electron transport complex II. Blood. 2019;134(4):389–94.
36. Stevens BM, Khan N, D'Alessandro A, et al. Characterization and targeting of malignant stem cells in patients with advanced myelodysplastic syndromes. Nat Commun. 2018;9(1):3694.
37. Li LJ, Tao JL, Fu R, et al. Increased CD34+CD38−CD123+ cells in myelodysplastic syndrome displaying malignant features similar to those in AML. Int J Hematol. 2014;100(1):60–9.
38. Signer RA, Magee JA, Salic A, et al. Haematopoietic stem cells require a highly regulated protein synthesis rate. Nature. 2014;509(7498):49–54.
39. Lee K-M, Giltnane JM, Balko JM, et al. MYC and MCL1 cooperatively promote chemotherapy-resistant breast cancer stem cells via regulation of mitochondrial oxidative phosphorylation. Cell Metab. 2017;26(4):633–47.
40. Roesch A, Vultur A, Bogeski I, et al. Cancer cell article overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B high cells. Cancer Cell. 2013;23(6):811–25.
41. Viale A, Pettazzoni P, Lyssiotis CA, et al. Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. Nature. 2014;514(7524):628–32.
42. Škrtić M, Sriskanthadevan S, Jhas B, et al. Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. Cancer Cell. 2011;20(5):674–88.
43. Cole A, Wang Z, Coyaud E, et al. Inhibition of the mitochondrial protease ClpP as a therapeutic strategy for human acute myeloid leukemia. Cancer Cell. 2015;27(6):864–76.
44. Ye H, Adane B, Serkova NJ, et al. Subversion of systemic glucose metabolism as a mechanism to support the growth of leukemia cells. Cancer Cell. 2018;34(4):659–673.e6.
45. Pei S, Minhajuddin M, Pollyea DA, et al. AMPK/FIS1-mediated mitophagy is required for self-renewal of human AML stem cells. Cell Stem Cell. 2018;23(1):86–100.e6.
46. Grauers Wiktorin H, Nilsson T, Aydin E, et al. Role of NOX2 for leukaemic expansion in a murine model of BCR-ABL1+ leukaemia. Br J Haematology. 2018;182(2):290–4.
47. Adane B, Ye H, Pollyea DA, et al. The hematopoietic oxidase NOX2 regulates self-renewal of leukemic stem cells. Cell Rep. 2019;27(1):238–54.

48. Pollyea DA, Stevens BM, Jones CL. Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia. Nat Med. 2018;24(12):1859–66.
49. Chen X, Glytsou C, Zhou H, et al. Targeting mitochondrial structure sensitizes acute myeloid leukemia to venetoclax treatment. Cancer Discov. 2019;9(7):890–909.
50. Barreyro L, Will B, Bartholdy B, et al. Overexpression of IL-1 receptor accessory protein in stem and progenitor cells and outcome correlation in AML and MDS. Blood. 2012;120(6):1290–8.
51. Turzanski J, Grundy M, Russell NH, et al. Interleukin-1beta maintains an apoptosis-resistant phenotype in the blast cells of acute myeloid leukaemia via multiple pathways. Leukemia. 2004;18(10):1662–70.
52. Cozzolino F, Rubartelli A, Aldinucci D, et al. Interleukin 1 as an autocrine growth factor for acute myeloid leukemia cells. Proc Natl Acad Sci U S A. 1989;86(7):2369–73.
53. Carey A, Edwards DK, Eide CA, et al. Identification of interleukin-1 by functional screening as a key mediator of cellular expansion and disease progression in acute myeloid leukemia. Cell Rep. 2017;18(13):3204–18.
54. Hebbar M, Kozlowski D, Wattel E, et al. Association between myelodysplastic syndromes and inflammatory bowel diseases. Report of seven new cases and review of the literature. Leukemia. 1997;11(12):2188–91.
55. De Hollanda A, Beucher A, Henrion D, et al. Systemic and immune manifestations in myelodysplasia: a multicenter retrospective study. Arthritis Care Res. 2011;63(8): 1188–94.
56. Mekinian A, Braun T, Decaux O, et al. Inflammatory arthritis in patients with myelodysplastic syndromes: a multicenter retrospective study and literature review of 68 cases. Medicine. 2014;93(1):1–10.
57. Ivy KS, Brent FP. Disordered immune regulation and its therapeutic targeting in myelodysplastic syndromes. Curr Hematol Malig Rep. 2018;13(4):244–55.
58. Maciejewski J, Selleri C, Anderson S, et al. Fas antigen expression on CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor α and potentiates cytokine-mediated hematopoietic suppression in vitro. Blood. 1995;85(11):3183–90.
59. Shetty V, Mundle S, Alvi S, et al. Measurement of apoptosis, proliferation and three cytokines in 46 patients with myelodysplastic syndromes. Leuk Res. 1996;20(11):891–900.
60. Sawanobori M, Yamaguchi S, Hasegawa M, et al. Expression of TNF receptors and related signaling molecules in the bone marrow from patients with myelodysplastic syndromes. Leuk Res. 2003;27(7):583–91.
61. Gañán-Gómez I, Wei Y, Starczynowski DT, et al. Deregulation of innate immune and inflammatory signaling in myelodysplastic syndromes. Leukemia. 2015;29(7):1458–69.
62. Maratheftis CI, Andreakos E, Moutsopoulos HM, et al. Toll-like receptor-4 is up-regulated in hematopoietic progenitor cells and contributes to increased apoptosis in myelodysplastic syndromes. Clin Cancer Res. 2007;13(4):1154–60.
63. Wei Y, Dimicoli S, Bueso-Ramos C, et al. Toll-like receptor alterations in myelodysplastic syndrome. Leukemia. 2013;27(9):1832–40.
64. Barreyro L, Chlon TM, Starczynowski DT. Chronic immune response dysregulation in MDS pathogenesis. Blood. 2018;132(15):1553–60.
65. Liu T, Zhang L, Joo D, et al. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2(1):e17023.
66. Starczynowski DT, Kuchenbauer F, Argiropoulos B, et al. Identification of miR-145 and miR-146a as mediators of the 5q-syndrome phenotype. Nat Med. 2010;16(1):49–58.
67. Varney ME, Niederkorn M, Konno H, et al. Loss of Tifab, a del(5q) MDS gene, alters hematopoiesis through derepression of Toll-like receptor–TRAF6 signaling. J Exp Med. 2015;212(11):1967–85.
68. Ehrchen JM, Sunderkötter C, Foell D, et al. The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. J Leukoc Biol. 2009;86(3):557–66.

69. Giudice V, Wu Z, Kajigaya S, et al. Circulating S100A8 and S100A9 protein levels in plasma of patients with acquired aplastic anemia and myelodysplastic syndromes. Cytokine. 2019;113:462–5.
70. Cheng P, Eksioglu EA, Chen X, et al. S100A9-induced overexpression of PD-1/PD-L1 contributes to ineffective hematopoiesis in myelodysplastic syndromes. Leukemia. 2019;33(8):2034–46.
71. Simard J-C, Cesaro A, Chapeton-Montes J, et al. S100A8 and S100A9 induce cytokine expression and regulate the NLRP3 Inflammasome via ROS-dependent activation of NF-κB1. PLoS One. 2013;8(8):e72138.
72. Weinlich R, Oberst A, Beere HM, et al. Necroptosis in development, inflammation and disease. Nat Rev Mol Cell Biol. 2017;18(2):127–36.
73. Liu X, Lieberman J. A mechanistic understanding of Pyroptosis: the fiery death triggered by invasive infection. Adv Immunol. 2017;135:81–117.
74. Frank D, Vince JE. Pyroptosis versus necroptosis: similarities, differences, and crosstalk. Cell Death Differ. 2019;26(1):99–114.
75. Menu P, Vince JE. The NLRP3 inflammasome in health and disease: the good, the bad and the ugly. Clin Exp Immunol. 2011;166(1):1–15.
76. Raza A, Gezer S, Mundle S, et al. Apoptosis in bone marrow biopsy samples involving stromal and hematopoietic cells in 50 patients with myelodysplastic syndromes. Blood. 1995;86(1):268–76.
77. Galluzzi L, Vitale I. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 2018;25(3):486–541.
78. Sallman DA, List A. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. Blood. 2019;133(10):1039–48.
79. Wang Y, Krivtsov AV, Sinha AU, et al. The Wnt/beta-catenin pathway is required for the development of leukemia stem cells in AML. Science. 2010;327(5973):1650–3.
80. Basiorka AA, McGraw KL, Eksioglu EA, et al. The NLRP3 inflammasome functions as a driver of the myelodysplastic syndrome phenotype. Blood. 2016;128(25):2960–75.
81. Basiorka AA, Mcgraw KL, Abbas-Aghababazadeh F, et al. Assessment of ASC specks as a putative biomarker of pyroptosis in myelodysplastic syndromes: an observational cohort study. Lancet Haematol. 2018;5(9):393–402.
82. Wagner PN, Shi Q, Salisbury-Ruf CT, et al. Increased Ripk1-mediated bone marrow necroptosis leads to myelodysplasia and bone marrow failure in mice. Blood. 2019;133(2):107–20.
83. Ch'en IL, Beisner DR, Degterev A, et al. Antigen-mediated T cell expansion regulated by parallel pathways of death. Proc Natl Acad Sci U S A. 2008;105(45):17463–8.
84. Lawlor KE, Khan N, Mildenhall A, et al. RIPK3 promotes cell death and NLRP3 inflammasome activation in the absence of MLKL. Nat Commun. 2015;6:6282.
85. Flynn CM, Kaufman DS, Debert C, et al. Donor cell leukemia: insight into cancer stem cells and the stem cell niche. Blood. 2007;109(7):2688–92.
86. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. Nature. 2014;505(7483):327–34.
87. Naveiras O, Nardi V, Wenzel PL, et al. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. Nature. 2009;460(7252):259–63.
88. Yamazaki S, Ema H, Ran Karlsson G, et al. Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. Cell. 2011;147(5):1146–58.
89. Butler JM, Nolan DJ, Vertes EL, et al. Endothelial cells are essential for the self-renewal and repopulation of notch-dependent hematopoietic stem cells. Cell Stem Cell. 2010;6(3):251–64.
90. Visnjic D, Kalajzic I, Gronowicz G, et al. Conditional ablation of the osteoblast lineage in Col2.3Δtk transgenic mice. J Bone Miner Res. 2001;16(12):2222–31.
91. Zhu J, Garrett R, Jung Y, et al. Osteoblasts support B-lymphocyte commitment and differentiation from hematopoietic stem cells. Blood. 2007;109(9):3706–12.
92. Chow A, Huggins M, Ahmed J, et al. CD169 + macrophages provide a niche promoting erythropoiesis under homeostasis and stress. Nat Med. 2013;19(4):429–36.

93. Sugiyama T, Kohara H, Noda M, et al. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity. 2006;25(6):977–88.
94. Ding L, Saunders TL, Enikolopov G, et al. Endothelial and perivascular cells maintain haematopoietic stem cells. Nature. 2012;481(7382):457–62.
95. Greenbaum A, Hsu YMS, Day RB, et al. CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. Nature. 2013;495(7440):227–30.
96. Mangi M, Mufti G. Primary myelodysplastic syndromes: diagnostic and prognostic significance of immunohistochemical assessment of bone marrow biopsies. Blood. 1992;79(1):198–205.
97. Mellibovsky L, Diez A, Serrano S, et al. Bone remodeling alterations in myelodysplastic syndrome. Bone. 1996;19(4):401–5.
98. Weidner H, Rauner M, Trautmann F, et al. Myelodysplastic syndromes and bone loss in mice and men. Leukemia. 2017;31(4):1003–7.
99. Medyouf H, Mossner M, Jann J-C, et al. Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. Cell Stem Cell. 2014;14(6):824–37.
100. Balderman SR, Li AJ, Hoffman CM, et al. Targeting of the bone marrow microenvironment improves outcome in a murine model of myelodysplastic syndrome. Blood. 2016;127(5):616–25.
101. Raaijmakers MHGP, Mukherjee S, Guo S, et al. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature. 2010;464(7290):852–7.
102. Walkley CR, Olsen GH, Dworkin S, et al. A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor γ deficiency. Cell. 2007;129(6):1097–110.
103. Kode A, Manavalan JS, Mosialou I, et al. Leukaemogenesis induced by an activating β-catenin mutation in osteoblasts. Nature. 2014;506(7487):240–4.
104. Bhagat TD, Chen S, Bartenstein M, et al. Epigenetically aberrant stroma in MDS propagates disease via Wnt/β-catenin activation. Cancer Res. 2017;77(18):4846–57.
105. Dong L, Yu W-M, Zheng H, et al. Leukaemogenic effects of Ptpn11 activating mutations in the stem cell microenvironment. Nature. 2016;539(7628):304–8.
106. Xiao P, Dolinska M, Sandhow L, et al. Sipa1 deficiency-induced bone marrow niche alterations lead to the initiation of myeloproliferative neoplasm. Blood Adv. 2018;2(5):534–48.
107. Lopez-Villar O, Garcia JL, Sanchez-Guijo FM, et al. Both expanded and uncultured mesenchymal stem cells from MDS patients are genomically abnormal, showing a specific genetic profile for the 5q- syndrome. Leukemia. 2009;23(4):664–72.
108. Ramakrishnan A, Awaya N, Bryant E, et al. The stromal component of the marrow microenvironment is not derived from the malignant clone in MDS. Blood. 2006;108(2):772–3.
109. Chen S, Zambetti NA, Bindels EMJ, et al. Massive parallel RNA sequencing of highly purified mesenchymal elements in low-risk MDS reveals tissue-context-dependent activation of inflammatory programs. Leukemia. 2016;30(9):1938–42.
110. Zhao Y, Wu D, Fei C, et al. Down-regulation of Dicer1 promotes cellular senescence and decreases the differentiation and stem cell-supporting capacities of mesenchymal stromal cells in patients with myelodysplastic syndrome. Haematologica. 2015;100(2):194–204.
111. Geyh S, Öz S, Cadeddu RP, et al. Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. Leukemia. 2013;27(9):1841–51.
112. Mead AJ, Neo WH, Barkas N, et al. Niche-mediated depletion of the normal hematopoietic stem cell reservoir by Flt3-ITD-induced myeloproliferation. J Exp Med. 2017;214(7):2005–21.
113. Zambetti NA, Ping Z, Chen S, et al. Mesenchymal inflammation drives genotoxic stress in hematopoietic stem cells and predicts disease evolution in human pre-leukemia. Cell Stem Cell. 2016;19(5):613–27.
114. Wang C, Yang Y, Gao S, et al. Immune dysregulation in myelodysplastic syndrome: clinical features, pathogenesis and therapeutic strategies. Crit Rev Oncol Hematol. 2018;122:123–32.

115. Hoechst B, Ormandy LA, Ballmaier M, et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4+CD25+Foxp3+ T cells. Gastroenterology. 2008;135(1):234–43.
116. Chen X, Eksioglu EA, Zhou J, et al. Induction of myelodysplasia by myeloid-derived suppressor cells. J Clin Invest. 2013;123(11):4595–611.
117. Kittang AO, Kordasti S, Sand KE, et al. Expansion of myeloid derived suppressor cells correlates with number of T regulatory cells and disease progression in myelodysplastic syndrome. Onco Targets Ther. 2015;5(2):e1062208.
118. Cheng P, Corzo CA, Luetteke N, et al. Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. J Exp Med. 2008;205(10):2235–49.
119. Matteo Rigolin G, Howard J, Buggins A, et al. Phenotypic and functional characteristics of monocyte-derived dendritic cells from patients with myelodysplastic syndromes. Br J Haematol. 1999;107(4):844–50.
120. Ma L, Ceuppens J, Kasran A, et al. Immature and mature monocyte-derived dendritic cells in myelodysplastic syndromes of subtypes refractory anemia or refractory anemia with ringed sideroblasts display an altered cytokine profile. Leuk Res. 2007;31(10):1373–82.
121. Fozza C, Contini S, Galleu A, et al. Patients with myelodysplastic syndromes display several T-cell expansions, which are mostly polyclonal in the CD4+ subset and oligoclonal in the CD8+ subset. Exp Hematol. 2009;37(8):947–55.
122. Yang H, Bueso-Ramos C, DiNardo C, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. Leukemia. 2014;28(6):1280–8.
123. Lopes MR, Traina F, de Melo Campos P, et al. IL10 inversely correlates with the percentage of CD8+ cells in MDS patients. Leuk Res. 2013;37(5):541–6.
124. Kiladjian JJ, Bourgeois E, Lobe I, et al. Cytolytic function and survival of natural killer cells are severely altered in myelodysplastic syndromes. Leukemia. 2006;20(3):463–70.

Chapter 5
Molecular Landscape of MDS

Torsten Haferlach and Ines Schmidts

Introduction

In the last decade, the advance of next-generation sequencing (NGS) has greatly expanded our insight into the underlying pathobiology of myelodysplastic syndromes (MDS). The contribution of cytogenetic aberrations to MDS was realized early on and has been implemented into classification, prognostication, and treatment planning [1–3]. However, only approximately half of MDS patients have a detectable cytogenetic aberration [4–8]. On the other hand, large-scale studies identified molecular genetic abnormalities in up to 80–90% of patients with de novo MDS [9, 10]. Mutations recurrent in MDS can also be found in other myeloid or – to a lesser extent – lymphoid neoplasms, albeit at varying frequencies [11].

Categorized according to their biological function, mutations can be assigned to one of seven major classes (compare Table 5.1).

Table 5.1 Overview of dysregulated pathways and biological processes in MDS

Class	Affected pathway	Effect
DNA methylation	Epigenetic regulation	Transcriptional dysregulation
Histone modification		
Transcription factors	Transcription	
Cohesin components	DNA looping	
Splicing factors	Splicing	Post-transcriptional dysregulation
Signaling factors	Signaling	Aberrant proliferation
p53 pathway factors	p53 pathway	Genetic instability Aberrant activation of DNA damage response Disruption of cell cycle control

T. Haferlach (✉) · I. Schmidts
MLL Munich Leukemia Laboratory, Munich, Germany
e-mail: torsten.haferlach@mll.com

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3_5

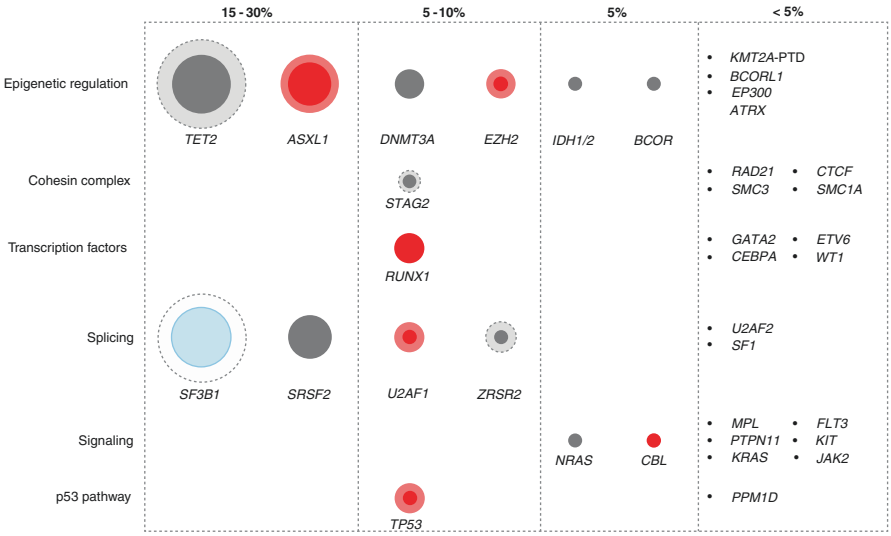

Fig. 5.1 Recurrently mutated genes in MDS, categorized according to biological function and mutation frequency. Circle size correlates with mutation frequency, light colored halos indicate the upper limit of frequency. Genes are mutated at frequencies ≥5% according to [12]. Genes mutated in less than 5% of cases are listed as bullet points, selection according to [9, 10]. Mutations that confer an IPSS-R-independent negative effect are colored in red/light red, mutations with no clear independent effect are displayed as gray/light gray circles. Only *SF3B1* mutations are associated with a favorable prognosis (light blue). Prognostic relevance according to [13]

Recurrently Mutated Genes in MDS

Figure 5.1 gives an overview of frequently mutated genes in MDS. Molecular aberrations with mutation frequencies ≥5% will be discussed in greater detail below.

Molecular Aberrations Contributing to Transcriptional Dysregulation in MDS

Epigenetic Regulation

Epigenetics is a major contributor to the regulation of gene expression. Based on the signature of epigenetic marks, which in human cells comprise DNA methylation and histone modifications, genes are either in a repressed or active state. However, this is no binary phenomenon, and the expression strength of active genes is tightly regulated. Setting epigenetic marks is an adaptive and reversible process and requires "writers" and "erasers." The epigenetic signature is recognized by "readers," which directly or indirectly mediate the biological outcome of the respective

epigenetic signature [14]. Epigenetic dysregulation is a hallmark of cancer since it allows tumor cells to silence tumor suppressor genes, activate or overexpress oncogenes, and to reset or halt cell differentiation [15, 16].

DNA Methylation

DNA methyltransferases (DNMT) can transfer a methyl group to the 5′ carbon of cytosine in CpG dinucleotides and thus belong to the class of epigenetic writers [17]. Erasure of DNA methylation is initiated by ten-eleven-translocation 2 (TET2), a methylcytosine dioxygenase. TET2 is thought to catalyze the first demethylation step, that is, the conversion of 5-methylcytosine to 5-hydroxymethylcytosine [18] (see Fig. 5.2). Both hypomethylation and hypermethylation phenotypes can contribute to pathobiology [17], the latter, however, can be pharmacologically antagonized with hypomethylating agents (HMA). The extensive methylation of promotor regions is strongly associated with gene silencing and malignant cells exploit this property to silence (putative) tumor suppressor genes, especially in high-risk MDS. The therapeutic effect of HMA appears to be greatly attributed to the reactivation of these genes [16].

DNMT3A *DNMT3A* mutations can be found in ~10% of MDS patients [12], however, they also represent the single most frequent aberration associated with age-related clonal hematopoiesis of indeterminate potential (CHIP) [19–21]. *DNMT3A* mutations themselves are not considered sufficient to drive MDS pathogenesis, but they contribute to gene expression deregulation by aberrant DNA methylation. Up

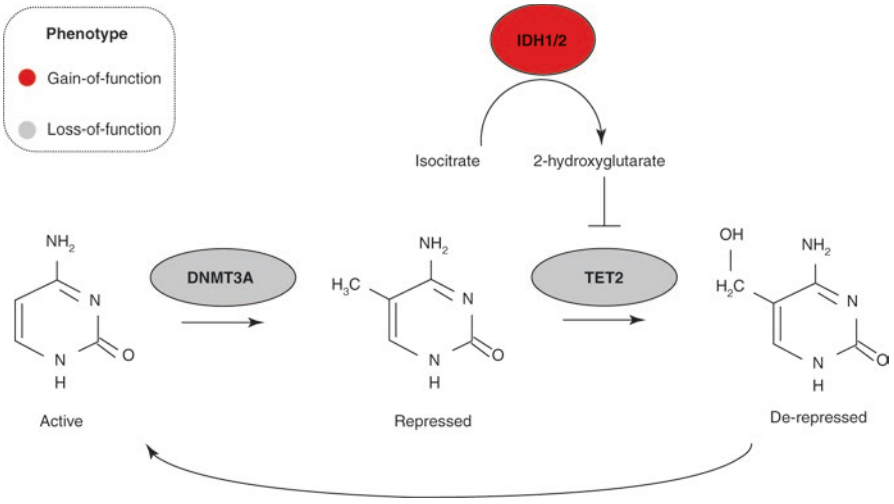

Fig. 5.2 Recurrent mutations in *DNMT3A*, *TET2*, and *IDH1/2* in MDS affect DNA methylation and contribute to an aberrant epigenome by causing a hypo- or a hypermethylation phenotype

to ~50% of *DNMT3A* mutations in MDS affect the arginine at position 882 [22–25]. A *DNMT3A*-R882H mutation leads to a loss-of-function phenotype and a decrease of catalytic activity by 80% [26]. Moreover DNMT3A-R882H exerts a dominant negative effect on wildtype DNMT3A, which adds to the loss-of-function phenotype [26]. *DNMT3A* mutations in MDS are associated with inferior overall survival and higher risk of transformation in many but not all studies [22–25, 27–30].

TET2 In accordance with the role of wildtype TET2 as eraser of DNA methylation, loss-of-function mutations of the *TET2* gene result in aberrant DNA hypermethylation [16, 31]. In MDS, up to 30% of patients have a detectable *TET2* mutation [12]. *TET2* mutations are also recurrently detected in other myeloid neoplasms as well as in CHIP, where the mutation frequency is approximately 10% [19–21]. While found associated with favorable outcome in one study [32], several other studies could not establish any influence of *TET2* mutations on prognosis [33–35].

IDH1/2 Isocitrate dehydrogenases IDH1 and IDH2 are predominantly known for their role in the tricarboxylic acid cycle, where they catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. Mutations of *IDH1* exclusively affect the arginine (R) at position 132, and in *IDH2* either codon R140 or R172 is found mutated [16]. Mutations in *IDH1/2* lead to a gain-of-function phenotype, since isocitrate is converted to 2-hydroxygluturate, instead of 2-oxoglutarate [36]. This aberrant metabolite competitively inhibits 2-oxoglutarate-dependent enzymes, including TET2 [16, 37, 38] (see Fig. 5.2). Accordingly, *IDH1/2* mutations are associated with a DNA hypermethylation phenotype [39]. The prognostic importance of *IDH1/2* mutations in MDS is unclear due to contradicting data [40, 41].

Histone Modifications

Histones, once thought of as merely "packaging material" for DNA, provide a versatile and highly dynamic platform for a myriad of different post-transcriptional modifications that fine-tune gene expression [15]. The concrete effect of histone modifications on transcription depends not only on the individual type of modification (e.g., acetylation, methylation, and ubiquitination) but also on the number and specific position of histone marks and the combinatorics of histone modifications (known as histone code) [15, 42]. Histone methylation, which can take the form of mono-, di-, and trimethylation, can represent either a repressive or an activating epigenetic mark. Here, a variety of "readers" are dedicated to interpret the respective methylation mark. For example, trimethylation of histone 3 lysine 4 is an "active" mark, while trimethylation of histone 3 lysine 27 (H3K27me3) is an "inactive/suppressive" epigenetic mark [15, 43].

H3K27me3 and its downstream effects are recurrently dysregulated in MDS. Under physiological conditions, H3K27me3 is "written" by the polycomb repressive complex 2 (PRC2), whose catalytic subunit is EZH2. Polycomb repressive complex 1 (PRC1) is both "reader" and "writer" at the same time. Upon recruitment

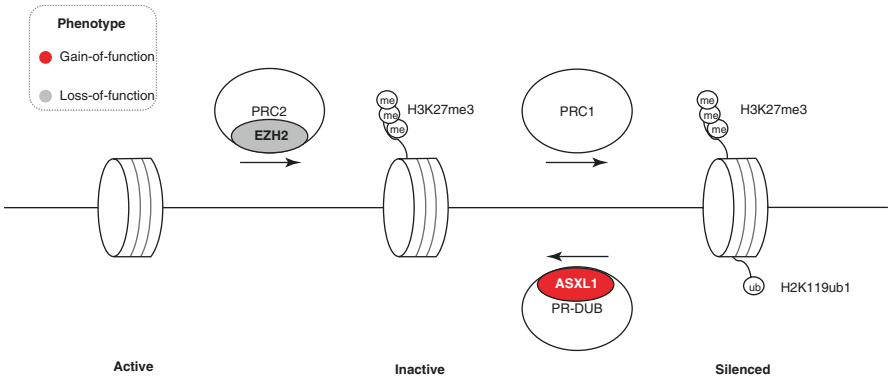

Fig. 5.3 Polycomb repressive complexes (PRC) contribute to transcriptional gene silencing by establishing the repressive epigenetic marks H3K27me3 (by PRC2) and H2K119ub1 (by PRC1). The PR-DUB complex can antagonize the action of PRC1. Mutations in *EZH2* and in *ASXL1* are found recurrently in MDS and result in an aberrant histone code

to H3K27me3, PRC1 marks histone H2A at lysine 119 with an ubiquitin molecule, and H2AK119 monoubiquitination (H2AK119ub1) results in further chromatin compaction and transcriptional silencing [43, 44]. The H2AK119ub1 mark can be erased by the polycomb repressive deubiquitinase (PR-DUB) complex [43, 44], in which ASXL1 functions as a chromatin binding subunit [45] (see Fig. 5.3).

EZH2 Mutations in the histone methyltransferase *enhancer of zeste 2* (*EZH2*) gene lead to loss of function by abrogating or strongly diminishing EZH2 catalytic activity and thus to impaired silencing by the PRC2 complex [46, 47]. Patients with *EZH2* mutations have a poor prognosis [13, 47], independent from IPSS-R [13].

ASXL1 Wildtype additional sex combs-like 1 (ASXL1) interacts with a variety of proteins; among other functions, it facilitates recruitment of PRC2 to target loci by protein–protein interactions with PRC2 subunits [48]. As mentioned above, ASXL1 is also part of the PR-DUB complex. The nonsense or frameshift mutations observed in myeloid neoplasms lead to truncated ASXL1 protein, which is thought to gain in function. Truncated ASXL1 hyperactivates the PR-DUB complex [49, 50], and in contrast to wildtype ASXL1 it interacts with BRD4, an epigenetic reader, which promotes transcriptional activation [51, 52]. Ultimately, mutations in *ASXL1* cause aberrant gene expression. *ASXL1* mutations are associated with a IPSS-R-independent poor prognosis [13, 53]. They are also found in ~9% of individuals with CHIP [19–21].

BCOR Aside to its name giving function as BCL6 corepressor (BCOR), BCOR is a subunit of a variant polycomb repressive complex 1, called PRC1.1. In contrast to the "canonical" PRC1 complex, PRC1.1 ubiquitinates loci independent from preset H3K27me3 marks [54]. The physiological function of the PRC1.1 complex appears to be the maintenance of a pluripotent state in stem cells. Mutations in *BCOR* thus lead to differentiation dysregulation and contribute to pathobiology [54, 55]. *BCOR* mutations are associated with a poor prognosis [56].

DNA Looping

Cohesins are named for their essential function in sister chromatid cohesion. A multiprotein ring-shaped complex consisting of STAG2, RAD21, SMC3, and SMC1A stabilizes the sister chromatids during metaphase and prevents replication fork collapse [11]. Moreover, the cohesin complex is now known to mediate interaction between distant genomic loci (e.g., promoter and its distant enhancer) by stabilization of DNA loops [57] (compare Fig. 5.4). It appears that dysregulation of cohesin-mediated DNA looping contributes to MDS pathogenesis through alteration of gene expression, since cohesin mutations in MDS are not associated with chromosomal aberrations [58]. In MDS, *stromal antigen 2* (*STAG2*) is the most frequently mutated cohesin [9, 10], and represents a poor prognostic marker [10, 58, 59].

Transcription Factors (TF)

Transcription is a well-orchestrated cellular process in which general transcription factors enable transcription and specific transcription factors regulate gene expression. Specific transcription factors themselves are tightly regulated by expression in a cell-type specific and/or temporal manner. Moreover, they are dedicated to the regulation of a specific set of target genes [60]. The core binding factor (CBF) family of proteins, for example, are master regulators of hematopoietic ontogeny and differentiation [61]. *Runt-related transcription factor 1* (*RUNX1*), which encodes the DNA-binding α-subunit of the heterodimeric CBF, is the most frequently mutated TF gene in MDS. *RUNX1* mutations are associated with a poor prognosis [10, 13, 62], independent from IPSS-R [13]. Moreover, individuals with a *RUNX1* germline mutation have an increased risk of developing myeloid neoplasms. This also holds true for germline mutations of the TF genes *CEBPA*, *ETV6*, and *GATA2*, which all define "myeloid neoplasms with germline predisposition" in the WHO classification [12]. Somatic mutations of *CEBPA*, *ETV6*, and *GATA2* are also found in MDS with mutation frequencies <5% [10].

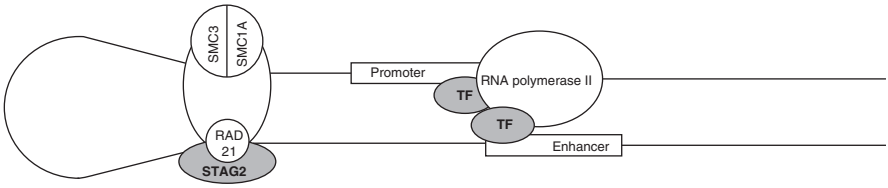

Fig. 5.4 Transcriptional dysregulation in MDS can result from abnormalities in cohesin complex-mediated DNA looping, which allows to bring distant gene regulatory elements (such as enhancer and promoter) into spatial proximity. Among the cohesins, *STAG2* is the most frequently mutated gene (gray). Transcription by RNA polymerase requires general transcription factors and is regulated by specific transcription factors that bind to regulatory elements (e.g., enhancer and promoter). Transcription factor (TF) mutations in MDS are found recurrently (gray) in master regulators of the hematopoietic cell differentiation program, for example, in the *RUNX1* gene

Molecular Aberrations Contributing to Dysregulation of Splicing

Following transcription, pre-mRNAs undergo a number of maturation steps, among them is splicing, that is, the removal of non-coding "intronic" sequences. The modular structure of metazoan pre-mRNAs, consisting of coding (exonic) and non-coding (intronic) sequences, is the prerequisite for alternative splicing, i.e., the selective inclusion or exclusion of a given exon. Due to alternative splicing, several protein isoforms can be generated from the same gene sequence, resulting in a complex proteome.

Splicing is a well-orchestrated, multi-step process catalyzed by the spliceosome, whose composition changes during the splicing process, making different subcomplexes distinguishable. Up to 60% of MDS patients carry a mutation in a splicing factor [63–66]. Most interestingly, splicing mutations in MDS mainly affect early spliceosome assembly at the 3′ splice site [11], see also Fig. 5.5.

Spliceosome formation is promoted by SR proteins, which are named after a protein domain that is enriched in serine (S) and arginine (R) and binds to exonic splicing enhancers. In MDS, *SRSF2*, which encodes such an SR protein, is found recurrently mutated [12, 67].

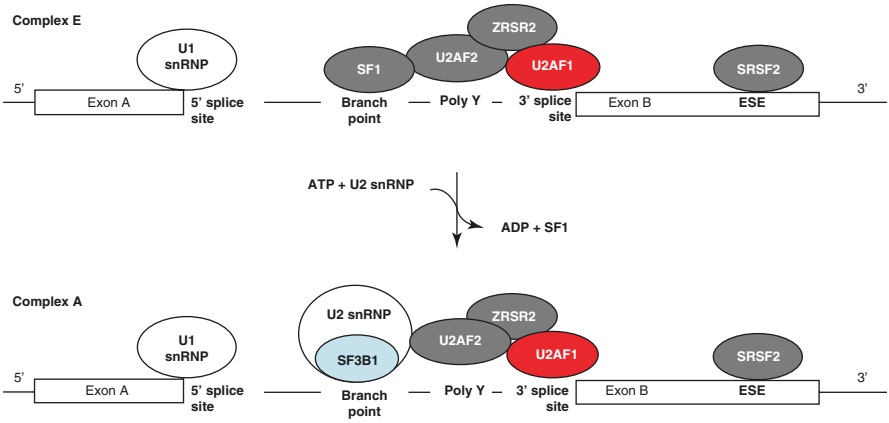

Fig. 5.5 Early spliceosome assembly is promoted by recognition of exonic splicing enhancers (ESE) by SR proteins. Correct positioning of splicing factors at regulatory intronic and exonic sequences is integral to the splicing process. U1 snRNP is required for the recognition of the 5′ splice site, while the 3′ spliceosome is composed of multiple factors. In complex E (commitment complex) splicing factor 1 (SF1) binds to the branch point region. The U2 auxiliary complex, comprised of U2AF1 and U2AF2, recognizes the 3′ splice site and the polypyrimidine tract, respectively (Y = pyrimidine). The transition to complex A (pre-spliceosome) is an energy-dependent step and leads to displacement of SF1 and the recognition of the branch point region by the U2 snRNP through its RNA binding subunit SF3B1. Factors found recurrently mutated in MDS are color-coded. Gray: factors without an independent prognostic value; red: *U2AF1* mutations are associated with a poor prognosis, independent from IPSS-R; light blue: mutations in *SF3B1* confer a favorable prognosis. Prognostic relevance according to [13]

Table 5.2 Interplay between *cis* (i.e., sequence) elements and *trans* (i.e., protein) factors to enable precise recognition and definition of exon-intron boundaries

Sequence	Recognized by
Complex E (commitment complex)	
5′ splice site	U1 snRNP
Branch point region	Splicing factor 1 (SF1)
Polypyrimidine tract	U2 auxiliary factor 2 (U2AF2)
3′ splice site	U2 auxiliary factor 1 (U2AF1)
Complex A (pre-spliceosome)	
5′ splice site	U1 snRNP
Branch point region	SF3B1 (as RNA binding subunit of U2 snRNP)
Polypyrimidine tract	U2 auxiliary factor 2 (U2AF2)
3′ splice site	U2 auxiliary factor 1 (U2AF1)

Correct splicing requires precise definition of exon-intron boundaries, which is facilitated by recognition of specific intronic and exonic sequences by dedicated factors (compare Table 5.2).

Mutations in *SF3B1*, *SRSF2*, and *U2AF1* alter the binding preferences of the respective encoded splicing factor, while mutations in *ZRSR2* result in complete loss of activity [67].

SF3B1 Mutations in *splicing factor 3b subunit 1 (SF3B1)* are strongly associated with a ring sideroblast (RS) phenotype, caused by aberrant accumulation of iron in mitochondria. The majority of MDS-RS patients carry a *SF3B1* mutation [63, 64, 68]. The *SF3B1* mutational status influences classification according to WHO (2017): in cases with wildtype *SF3B1*, ≥15% ring sideroblasts (as percentage of bone marrow erythroid elements) are required for the diagnosis of MDS-RS, however, if *SF3B1* is mutated, ring sideroblasts between 5% and 14% are sufficient [12]. Among splicing factors, it is also the only mutation that is associated with a favorable prognosis [13, 63, 68, 69].

SRSF2 Mutations in the *serine- and arginine-rich splicing factor 2* (*SRSF2*) gene are associated with a poor prognosis [70]. As is the case for *SF3B1* and *U2AF1* mutations, *SRSF2* mutations are heterozygous missense mutations and occur in distinct hotspots [11, 67, 71]. As a consequence, the binding preference of SRSF2 is altered, leading to an aberrant exonic enhancer site-binding pattern [67]. By this mechanism, mutations in *SRSF2* cause e.g. mis-splicing and aberrant degradation of *EZH2* transcripts, indirectly contributing to an aberrant epigenome [72].

U2AF1 *U2 small nuclear RNA auxiliary factor 1* (*U2AF1*) mutations confer an inferior prognosis independent from IPSS-R [13]. Given the importance of U2AF1 for the recognition of intron-exon boundaries, mutations that affect binding preferences result in increased exon skipping [11, 67].

ZRSR2 In "constitutive" splicing by the major spliceosome, ZRSR2 (zinc finger CCCH-type, RNA binding motif, and serine/arginine rich 2) interacts with the U2AF complex and stabilizes the formation of complex A [73]. However, a subset of transcripts of 700 to 800 genes are spliced by the "minor" spliceosome, in which ZRSR2 assumes the functional role of the U2AF complex [73–75]. *ZRSR2* mutations are thought to contribute to MDS disease biology by aberrant intron retention and mis-splicing in minor spliceosome-dependent transcripts [76]. In contrast to other splicing factors, mutations in *ZRSR2* do not occur in distinct hotspots [71]. The outcome and clinical course of patients with *ZRSR2* mutations is strongly dependent on *TET2* mutational status. Cases with mutated *ZRSR2* and wildtype *TET2* were observed to have a high AML transformation rate and a poor prognosis [71].

Molecular Aberrations Contributing to Dysregulation of Signaling

In comparison to other myeloid neoplasms, mutations in signaling factors are less common in MDS. Signaling factor mutations in AML are considered to represent late events and as such are often associated with progressive disease when found in MDS. Most frequently, the MAP kinase pathway is affected in ~10% of MDS patients [9–11]. *NRAS (neuroblastoma RAS viral oncogene homolog)*, which encodes one factor of this pathway, is found mutated in ~5% of MDS patients [12]. Mutations in *CBL (casitas B-lineage lymphoma)*, which are also detected in ~5% of MDS patients [12], are more prevalent in chronic myelomonocytic leukemia (CMML). Both gene mutations are linked to an inferior prognosis [9, 13, 53, 77–79], in case of *CBL* independent of IPSS-R [13]. Moreover, mutations in *CBL* are associated with aberrantly prolonged activation of other signaling factors, for example, FLT3 [80]. Mutations in the *FLT3* gene rarely occur in MDS; however, if present, they are associated with a very poor prognosis and progression to secondary AML [81–83].

Molecular Aberrations Contributing to Dysregulation of the p53 Pathway

Aberrations that affect the tumor protein p53, also often referred to as "guardian of the genome" are recurrently found in every cancer type. Its physiological function is to halt the cell cycle in case of cellular stresses or DNA damage and to promote, if necessary, apoptosis [11, 84]. Alterations in *TP53*, the gene encoding p53, are caused by deletion or gene mutation. *TP53* deletion is frequent among cases with

deletion of chromosome arm 17q and is commonly accompanied by *TP53* mutation of the other allele [85, 86], resulting in biallelic inactivation and a particularly inferior outcome [87]. *TP53* aberrations are associated with several predictors of poor clinical outcome, such as low platelet count, high blast count, high-risk disease, complex karyotype, and resistance to therapy [53, 88, 89]. The presence of *TP53* aberrations is a negative prognostic factor, independent from IPSS-R [13]. The negative prognostic impact is retained also in the setting of allogeneic stem cell transplantation [11, 29, 90].

In de novo MDS cases, *TP53* alterations are detected in ~5% of patients [12]. In the context of therapy-associated MDS, *TP53* aberrations are found in up to 33% [11, 91]. *TP53* and *PPM1D*, which encodes a phosphatase that negatively regulates p53, have been found mutated in CHIP, with frequencies of ~4% [20, 21]. This finding provides one possible explanation for the development of therapy-associated neoplasms (t-MN). Under the selective pressure of cytotoxic therapy, clones carrying aberrations of *TP53* and/or *PPM1D* gain selective advantage and can undergo clonal expansion. Screening patients for *TP53* and *PPM1D* aberrations prior to cytotoxic therapy could help identify individuals at risk to develop t-MN [92–95].

The Clinical Value of Molecular Genetic Characterization in MDS

Currently, only *SF3B1* mutations are considered as a diagnostic criterion in the WHO classification [12]. Given the diagnostic challenge of cytomorphological evaluation of (subtle) dysplastic features and the low reproducibility of blast count determination, it is likely that molecular genetics will gain in importance in classification in the future. Today, molecular characterization already plays a crucial role in state-of-the-art prognostic evaluation and therapeutic decision making.

Prognosis None of the prognostic models in MDS, discussed in depth in Chap. 7, takes molecular aberrations into account. However, mutations in several genes have been shown to have prognostic power independent from the revised IPSS score (IPSS-R). Aberrations of *ASXL1*, *CBL*, *EZH2*, *RUNX1*, *TP53*, and *U2AF1* have all been associated with significantly shortened overall survival in a study with >3000 MDS patients. Detection of a mutation in one of the six genes should warrant placing a case in the next unfavorable IPSS-R risk group [13].

Therapy Decisions The response to hypomethylating agents is strongly influenced by a patient's mutational landscape, especially in genes encoding epigenetic factors. Azacitidine resistance has been observed in the context of *DNMT3A*-R882 mutations as well as for mutations that affect the SKI domain of *SETBP1*, which also encodes an epigenetic regulator [96]. Another study found that mutations in *ASXL1* and *ETV6* are associated with short response duration [97]. In contrast, patients with *TET2* mutation (in the absence of a concomitant *ASXL1* mutation) showed a particu-

larly high sensitivity to azacitidine [34, 98–100]. However, there were no significant differences in overall survival and response duration between patients with mutated and wildtype *TET2* under azacitidine treatment [98, 100].

The mutation status of *TP53* should play a role in therapy planning in several respects. In general, patients with isolated 5q deletion benefit from treatment with lenalidomide. However, the presence of a concomitant *TP53* mutation poses the risk of faster disease progression [101], therefore *TP53* mutational status should be determined prior to lenalidomide therapy [102]. Moreover, AML and MDS patients with *TP53* mutation have been found to show a better initial response to a 10-day decitabine protocol than to conventional chemotherapy [103]; however, remission was eventually lost in all *TP53* mutated cases, including the nine MDS patients carrying *TP53* mutations. Although patients with *TP53* abnormalities should be considered for allogeneic stem cell transplantation [104], the negative prognostic effect persists post-transplant [11, 29, 90]. In patients eligible for allogeneic transplantation, the *TP53* mutation status should be taken into account for the selection of the conditioning scheme, since patients with *TP53* mutation do not benefit from myeloablative conditioning [105]. Therefore, whenever possible, alternative conditioning regimen should be considered for this patient group, possibly within a study setting [104].

From Clonal Hematopoiesis to Secondary AML – A Disease Continuum?

The advance of NGS led to the coincidental finding of leukemia-associated gene mutations as drivers of clonal hematopoiesis in the absence of hematological disease. CHIP is now known to be an age-related phenomenon [19–21, 106], whose clinical implications remain subject to discussion and research. Only 0.5–1% of individuals with CHIP develop myeloid neoplasms later on [19, 21]. As described above, mutations in three genes are strongly associated with CHIP: *DNMT3A*, *TET2*, and *ASXL1*.

Clonality has also been demonstrated in a major subset of patients with unexplained cytopenia [107, 108]. The presence of gene mutations as clonal drivers was associated with ~14-fold higher risk of progression to myeloid neoplasms compared to cases with idiopathic cytopenia [107]. Accordingly, clonal cytopenia of undetermined significance (CCUS) has been introduced as a pre-malignant condition [107–109].

The recognition of CHIP and CCUS as well as insight into the genetic landscape of MDS validates the multi-hit hypothesis in MDS pathogenesis (compare Fig. 5.6). In MDS, 3 mutations were detectable in the median [0–12 mutations] [10]. Mutations affecting DNA methylation and splicing factors show a higher mutational load than mutations in histone modifiers and signaling factors, which makes early and late mutational events in MDS pathogenesis distinguishable [9, 10].

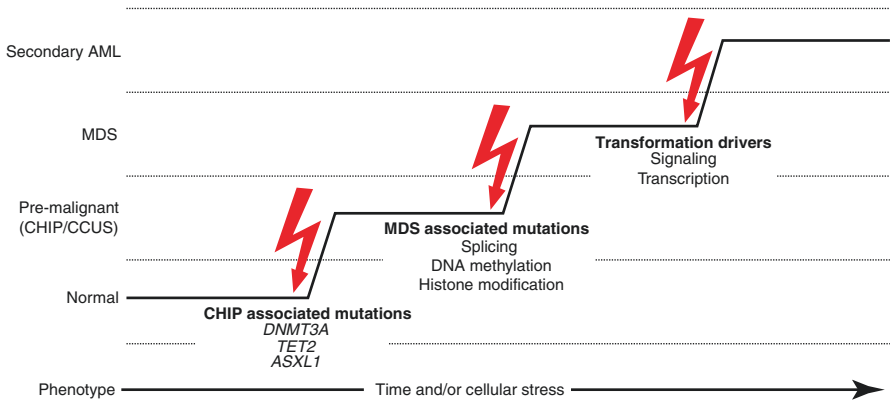

Fig. 5.6 Multi-hit hypothesis in the pathogenesis of myeloid neoplasms. Mutations found associated with CHIP and CCUS are not sufficient for MDS pathogenesis, however, they lay the foundation. Acquisition of additional mutations and/or selective pressure can cause clonal evolution and ultimately lead to the development of myeloid neoplasms

Progression of MDS to secondary AML is associated with abrogation of hematopoietic differentiation and/or uncontrolled proliferation [11]. Mutations in transcription factor genes such as *RUNX1*, *CEBPA*, and *GATA2* often herald disease progression [11, 110]. Same holds true for mutations affecting signaling, especially mutations of RAS pathway factors or *FLT3* are linked to progression to AML [11, 110, 111]. It is of clinical importance to distinguish between cases with sAML and de novo AML, since patients with sAML have an inferior prognosis and often are refractory to chemotherapy [11, 110].

In conclusion, NGS-based panel testing has paved the way for a comprehensive description of the molecular landscape in MDS within just a decade. Panel testing in MDS is increasingly used to support or exclude a diagnosis of MDS in cases of unclear cytopenia(s) and/or dysplasia. Several publications have demonstrated the clinical utility of NGS screening using a panel of genes whose mutation status can inform differential diagnostics, classification, and prognosis [104, 107, 112]. Particularly in light of the recently described pre-malignant conditions CHIP, ICUS, and CCUS, there is a need to further investigate the molecular landscape in MDS. Due to new technological advances, that is, whole exome sequencing (WES), whole genome sequencing (WGS), and whole transcriptome sequencing (WTS), it is now possible to gain a genome-wide molecular insight that not only tracks the mutational status but also measures gene expression and detects cytogenetic aberrations. In MDS, the implementation of gene mutations into the IPSS-M (molecular) represents the next step; this is currently underway driven by efforts of the International Working Group for Prognosis in MDS (IWG-PM). Since the clinical course in MDS is quite heterogeneous, the definition of “best treatment” and goals for outcome would most likely benefit from incorporation of cytogenetic and molecular genetic findings.

References

1. Jaffe ES, Harris NL, Vardiman JW. WHO classification of tumours. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. 3rd ed. Lyon: IARC Press; 2001.
2. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International Scoring System for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89(6):2079–88.
3. Bowen D, Culligan D, Jowitt S, Kelsey S, Mufti G, Oscier D, et al. Guidelines for the diagnosis and therapy of adult myelodysplastic syndromes. Br J Haematol. 2003;120(2):187–200.
4. Haase D, Germing U, Schanz J, Pfeilstöcker M, Nosslinger T, Hildebrandt B, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. Blood. 2007;110(13):4385–95.
5. Pozdnyakova O, Miron PM, Tang G, Walter O, Raza A, Woda B, et al. Cytogenetic abnormalities in a series of 1,029 patients with primary myelodysplastic syndromes: a report from the US with a focus on some undefined single chromosomal abnormalities. Cancer. 2008;113(12):3331–40.
6. Olney HJ, Le Beau MM. Evaluation of recurring cytogenetic abnormalities in the treatment of myelodysplastic syndromes. Leuk Res. 2007;31(4):427–34.
7. Steensma DP, List AF. Genetic testing in the myelodysplastic syndromes: molecular insights into hematologic diversity. Mayo Clin Proc. 2005;80(5):681–98.
8. Bacher U, Haferlach T, Kern W, Weiss T, Schnittger S, Haferlach C. The impact of cytomorphology, cytogenetics, molecular genetics, and immunophenotyping in a comprehensive diagnostic workup of myelodysplastic syndromes. Cancer. 2009;115(19):4524–32.
9. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122(22):3616–27.
10. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241–7.
11. Sperling AS, Gibson CJ, Ebert BL. The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia. Nat Rev Cancer. 2017;17(1):5–19.
12. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: International Agency for Research on Cancer (IARC); 2017.
13. Bejar R, Papaemmanuil E, Haferlach T, Garcia-Manero G, Maciejewski JP, Sekeres MA, et al. Somatic mutations in MDS patients are associated with clinical features and predict prognosis independent of the IPSS-R: analysis of combined datasets from the International Working Group for Prognosis in MDS-Molecular Committee. Blood (ASH Annual Meeting Abstracts). 2015;126(23):907.
14. Dawson MA. The cancer epigenome: concepts, challenges, and therapeutic opportunities. Science. 2017;355(6330):1147–52.
15. Esteller M. Epigenetics in cancer. N Engl J Med. 2008;358(11):1148–59.
16. Heuser M, Yun H, Thol F. Epigenetics in myelodysplastic syndromes. Semin Cancer Biol. 2018;51:170–9.
17. Wouters BJ, Delwel R. Epigenetics and approaches to targeted epigenetic therapy in acute myeloid leukemia. Blood. 2016;127(1):42–52.
18. Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature. 2010;468(7325):839–43.
19. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488–98.
20. Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med. 2014;20(12):1472–8.

21. Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371(26):2477–87.
22. Lin ME, Hou HA, Tsai CH, Wu SJ, Kuo YY, Tseng MH, et al. Dynamics of DNMT3A mutation and prognostic relevance in patients with primary myelodysplastic syndrome. Clin Epigenetics. 2018;10:42.
23. Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan M, et al. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. Leukemia. 2011;25(7):1153–8.
24. Thol F, Winschel C, Ludeking A, Yun H, Friesen I, Damm F, et al. Rare occurrence of DNMT3A mutations in myelodysplastic syndromes. Haematologica. 2011;96(12):1870–3.
25. Bejar R, Stevenson KE, Caughey BA, Abdel-Wahab O, Steensma DP, Galili N, et al. Validation of a prognostic model and the impact of mutations in patients with lower-risk myelodysplastic syndromes. J Clin Oncol. 2012;30(27):3376–82.
26. Russler-Germain DA, Spencer DH, Young MA, Lamprecht TL, Miller CA, Fulton R, et al. The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. Cancer Cell. 2014;25(4):442–54.
27. Xu F, Wu LY, He Q, Wu D, Zhang Z, Song LX, et al. Exploration of the role of gene mutations in myelodysplastic syndromes through a sequencing design involving a small number of target genes. Sci Rep. 2017;7:43113.
28. Jung SH, Kim YJ, Yim SH, Kim HJ, Kwon YR, Hur EH, et al. Somatic mutations predict outcomes of hypomethylating therapy in patients with myelodysplastic syndrome. Oncotarget. 2016;7(34):55264–75.
29. Bejar R, Stevenson KE, Caughey B, Lindsley RC, Mar BG, Stojanov P, et al. Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. J Clin Oncol. 2014;32(25):2691–8.
30. Liang S, Zhou X, Pan H, Yang Y, Shi L, Wang L. Prognostic value of DNMT3A mutations in myelodysplastic syndromes: a meta-analysis. Hematology. 2019;24(1):613–22.
31. Rasmussen KD, Jia G, Johansen JV, Pedersen MT, Rapin N, Bagger FO, et al. Loss of TET2 in hematopoietic cells leads to DNA hypermethylation of active enhancers and induction of leukemogenesis. Genes Dev. 2015;29(9):910–22.
32. Kosmider O, Gelsi-Boyer V, Cheok M, Grabar S, Della-Valle V, Picard F, et al. TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs). Blood. 2009;114(15):3285–91.
33. Haferlach C, Stengel A, Meggendorfer M, Kern W, Haferlach T. Characterization of MDS Harboring TET2 mutations and/or TET2 deletions. Blood. 2016;128(22):4288.
34. Lin Y, Lin Z, Cheng K, Fang Z, Li Z, Luo Y, et al. Prognostic role of TET2 deficiency in myelodysplastic syndromes: a meta-analysis. Oncotarget. 2017;8(26):43295–305.
35. Guo Z, Zhang SK, Zou Z, Fan RH, Lyu XD. Prognostic significance of TET2 mutations in myelodysplastic syndromes: a meta-analysis. Leuk Res. 2017;58:102–7.
36. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature. 2009;462(7274):739–44.
37. Rose NR, McDonough MA, King ON, Kawamura A, Schofield CJ. Inhibition of 2-oxoglutarate dependent oxygenases. Chem Soc Rev. 2011;40(8):4364–97.
38. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer Cell. 2011;19(1):17–30.
39. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell. 2010;18(6):553–67.
40. Thol F, Weissinger EM, Krauter J, Wagner K, Damm F, Wichmann M, et al. IDH1 mutations in patients with myelodysplastic syndromes are associated with an unfavorable prognosis. Haematologica. 2010;95(10):1668–74.

41. DiNardo CD, Jabbour E, Ravandi F, Takahashi K, Daver N, Routbort M, et al. IDH1 and IDH2 mutations in myelodysplastic syndromes and role in disease progression. Leukemia. 2016;30(4):980–4.
42. Jenuwein T, Allis CD. Translating the histone code. Science. 2001;293(5532):1074–80.
43. Itzykson R, Fenaux P. Epigenetics of myelodysplastic syndromes. Leukemia. 2014;28(3):497–506.
44. Chittock EC, Latwiel S, Miller TC, Muller CW. Molecular architecture of polycomb repressive complexes. Biochem Soc Trans. 2017;45(1):193–205.
45. Scheuermann JC, de Ayala Alonso AG, Oktaba K, Ly-Hartig N, McGinty RK, Fraterman S, et al. Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. Nature. 2010;465(7295):243–7.
46. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet. 2010;42(8):722–6.
47. Nikoloski G, Langemeijer SM, Kuiper RP, Knops RH, Massop M, Tonnissen ER, et al. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet. 2010;42(8):665–7.
48. Abdel-Wahab O, Adli M, Saunders L, Gao JG, Shih A, Pandey S, et al. ASXL1 mutations promote myeloid transformation through inhibition of PRC2-mediated gene repression. Blood. 2011;118(21):405a.
49. Balasubramani A, Larjo A, Bassein JA, Chang X, Hastie RB, Togher SM, et al. Cancer-associated ASXL1 mutations may act as gain-of-function mutations of the ASXL1-BAP1 complex. Nat Commun. 2015;6:7307.
50. Asada S, Goyama S, Inoue D, Shikata S, Takeda R, Fukushima T, et al. Mutant ASXL1 cooperates with BAP1 to promote myeloid leukaemogenesis. Nat Commun. 2018;9(1):2733.
51. Asada S, Fujino T, Goyama S, Kitamura T. The role of ASXL1 in hematopoiesis and myeloid malignancies. Cell Mol Life Sci. 2019;76(13):2511–23.
52. Yang H, Kurtenbach S, Guo Y, Lohse I, Durante MA, Li J, et al. Gain of function of ASXL1 truncating protein in the pathogenesis of myeloid malignancies. Blood. 2018;131(3):328–41.
53. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011;364(26):2496–506.
54. van den Boom V, Maat H, Geugien M, Rodriguez LA, Sotoca AM, Jaques J, et al. Non-canonical PRC1.1 targets active genes independent of H3K27me3 and is essential for leukemogenesis. Cell Rep. 2016;14(2):332–46.
55. Wang Z, Gearhart MD, Lee YW, Kumar I, Ramazanov B, Zhang Y, et al. A non-canonical BCOR-PRC1.1 complex represses differentiation programs in human ESCs. Cell Stem Cell. 2018;22(2):235–51.
56. Damm F, Chesnais V, Nagata Y, Yoshida K, Scourzic L, Okuno Y, et al. BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. Blood. 2013;122(18):3169–77.
57. Losada A. Cohesin in cancer: chromosome segregation and beyond. Nat Rev Cancer. 2014;14(6):389–93.
58. Thota S, Viny AD, Makishima H, Spitzer B, Radivoyevitch T, Przychodzen B, et al. Genetic alterations of the cohesin complex genes in myeloid malignancies. Blood. 2014;124:1790–8.
59. Montalban-Bravo G, Takahashi K, Patel K, Wang F, Xingzhi S, Nogueras GM, et al. Impact of the number of mutations in survival and response outcomes to hypomethylating agents in patients with myelodysplastic syndromes or myelodysplastic/myeloproliferative neoplasms. Oncotarget. 2018;9(11):9714–27.
60. Rosenbauer F, Tenen DG. Transcription factors in myeloid development: balancing differentiation with transformation. Nat Rev Immunol. 2007;7(2):105–17.
61. Speck NA, Gilliland DG. Core-binding factors in haematopoiesis and leukaemia. Nat Rev Cancer. 2002;2(7):502–13.

62. Chen CY, Lin LI, Tang JL, Ko BS, Tsay W, Chou WC, et al. RUNX1 gene mutation in primary myelodysplastic syndrome--the mutation can be detected early at diagnosis or acquired during disease progression and is associated with poor outcome. Br J Haematol. 2007;139(3):405–14.
63. Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med. 2011;365(15):1384–95.
64. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478(7367):64–9.
65. Graubert TA, Shen D, Ding L, Okeyo-Owuor T, Lunn CL, Shao J, et al. Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. Nat Genet. 2012;44(1):53–7.
66. Inoue D, Bradley RK, Abdel-Wahab O. Spliceosomal gene mutations in myelodysplasia: molecular links to clonal abnormalities of hematopoiesis. Genes Dev. 2016;30(9):989–1001.
67. Dvinge H, Kim E, Abdel-Wahab O, Bradley RK. RNA splicing factors as oncoproteins and tumour suppressors. Nat Rev Cancer. 2016;16(7):413–30.
68. Malcovati L, Karimi M, Papaemmanuil E, Ambaglio I, Jädersten M, Jansson M, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015;126(2):233–41.
69. Malcovati L, Papaemmanuil E, Bowen DT, Boultwood J, Della Porta MG, Pascutto C, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood. 2011;118(24):6239–46.
70. Gangat N, Mudireddy M, Lasho TL, Finke CM, Nicolosi M, Szuber N, et al. Mutations and prognosis in myelodysplastic syndromes: karyotype-adjusted analysis of targeted sequencing in 300 consecutive cases and development of a genetic risk model. Am J Hematol. 2018;93(5):691–7.
71. Damm F, Kosmider O, Gelsi-Boyer V, Renneville A, Carbuccia N, Hidalgo-Curtis CE, et al. Mutations affecting mRNA splicing define distinct clinical phenotypes and correlate with patient outcome in myelodysplastic syndromes. Blood. 2012;119(14):3211–8.
72. Kim E, Ilagan JO, Liang Y, Daubner GM, Lee SC, Ramakrishnan A, et al. SRSF2 mutations contribute to myelodysplasia by mutant-specific effects on exon recognition. Cancer Cell. 2015;27(5):617–30.
73. Larsson CA, Cote G, Quintas-Cardama A. The changing mutational landscape of acute myeloid leukemia and myelodysplastic syndrome. Mol Cancer Res. 2013;11(8):815–27.
74. Shen H, Zheng X, Luecke S, Green MR. The U2AF35-related protein Urp contacts the 3′ splice site to promote U12-type intron splicing and the second step of U2-type intron splicing. Genes Dev. 2010;24(21):2389–94.
75. Turunen JJ, Niemela EH, Verma B, Frilander MJ. The significant other: splicing by the minor spliceosome. Wiley Interdiscip Rev RNA. 2013;4(1):61–76.
76. Madan V, Kanojia D, Li J, Okamoto R, Sato-Otsubo A, Kohlmann A, et al. Aberrant splicing of U12-type introns is the hallmark of ZRSR2 mutant myelodysplastic syndrome. Nat Commun. 2015;6:6042.
77. Murphy DM, Bejar R, Stevenson K, Neuberg D, Shi Y, Cubrich C, et al. NRAS mutations with low allele burden have independent prognostic significance for patients with lower risk myelodysplastic syndromes. Leukemia. 2013;27(10):2077–81.
78. Makishima H, Cazzolli H, Szpurka H, Dunbar AJ, Tiu R, Huh J, et al. Mutations of e3 ubiquitin ligase cbl family members constitute a novel common pathogenic lesion in myeloid malignancies. J Clin Oncol. 2009;27(36):6109–16.
79. Bejar R, Levine R, Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. J Clin Oncol. 2011;29(5):504–15.
80. Sanada M, Suzuki T, Shih LY, Otsu M, Kato M, Yamazaki S, et al. Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. Nature. 2009;460(7257):904–8.

81. Shih LY, Huang CF, Wang PN, Wu JH, Lin TL, Dunn P, et al. Acquisition of FLT3 or N-ras mutations is frequently associated with progression of myelodysplastic syndrome to acute myeloid leukemia. Leukemia. 2004;18(3):466–75.
82. Georgiou G, Karali V, Zouvelou C, Kyriakou E, Dimou M, Chrisochoou S, et al. Serial determination of FLT3 mutations in myelodysplastic syndrome patients at diagnosis, follow up or acute myeloid leukaemia transformation: incidence and their prognostic significance. Br J Haematol. 2006;134(3):302–6.
83. Shih LY, Lin TL, Wang PN, Wu JH, Dunn P, Kuo MC, et al. Internal tandem duplication of fms-like tyrosine kinase 3 is associated with poor outcome in patients with myelodysplastic syndrome. Cancer. 2004;101(5):989–98.
84. Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. Nature. 2004;432(7015):307–15.
85. Soenen V, Preudhomme C, Roumier C, Daudignon A, Lai JL, Fenaux P. 17p deletions in acute myeloid leukemia and myelodysplastic syndrome. Analysis of breakpoints and deleted segments by fluorescence in situ. Blood. 1998;91(3):1008–15.
86. Lai JL, Preudhomme C, Zandecki M, Flactif M, Vanrumbeke M, Wattel E, et al. Myelodysplastic syndromes and acute myeloid leukemia with 17p deletion. An entity characterized by specific dysgranulopoiesis and a high incidence of p53 mutations. Leukemia. 1995;9:370.
87. Stengel A, Kern W, Haferlach T, Meggendorfer M, Fasan A, Haferlach C. The impact of TP53 mutations and TP53 deletions on survival varies between AML, ALL, MDS and CLL: an analysis of 3307 cases. Leukemia. 2017;31(3):705–11.
88. Wattel E, Preudhomme C, Hecquet B, Vanrumbeke M, Quesnel B, Dervite I, et al. p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. Blood. 1994;84(9):3148–57.
89. Kaneko H, Misawa S, Horiike S, Nakai H, Kashima K. TP53 mutations emerge at early phase of myelodysplastic syndrome and are associated with complex chromosomal abnormalities. Blood. 1995;85(8):2189–93.
90. Della Porta MG, Galli A, Bacigalupo A, Zibellini S, Bernardi M, Rizzo E, et al. Clinical effects of driver somatic mutations on the outcomes of patients with myelodysplastic syndromes treated with allogeneic hematopoietic stem-cell transplantation. J Clin Oncol. 2016;34(30):3627–37.
91. Wong TN, Ramsingh G, Young AL, Miller CA, Touma W, Welch JS, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature. 2015;518(7540):552–5.
92. Wong TN, Miller CA, Jotte MRM, Bagegni N, Baty JD, Schmidt AP, et al. Cellular stressors contribute to the expansion of hematopoietic clones of varying leukemic potential. Nat Commun. 2018;9(1):455.
93. Kindler T. CHIPing out PPM1D-mutant hematopoiesis. Blood. 2018;132(11):1087–8.
94. Kahn JD, Miller PG, Silver AJ, Sellar RS, Bhatt S, Gibson C, et al. PPM1D-truncating mutations confer resistance to chemotherapy and sensitivity to PPM1D inhibition in hematopoietic cells. Blood. 2018;132(11):1095–105.
95. Hsu JI, Dayaram T, Tovy A, De BE, Jeong M, Wang F, et al. PPM1D mutations drive clonal hematopoiesis in response to cytotoxic chemotherapy. Cell Stem Cell. 2018;23(5):700–13.
96. Falconi G, Fabiani E, Piciocchi A, Criscuolo M, Fianchi L, Lindfors Rossi EL, et al. Somatic mutations as markers of outcome after azacitidine and allogeneic stem cell transplantation in higher-risk myelodysplastic syndromes. Leukemia. 2019;33(3):785–90.
97. Craddock CF, Houlton AE, Quek LS, Ferguson P, Gbandi E, Roberts C, et al. Outcome of azacitidine therapy in acute myeloid leukemia is not improved by concurrent Vorinostat therapy but is predicted by a diagnostic molecular signature. Clin Cancer Res. 2017;23(21):6430–40.
98. Bejar R, Lord A, Stevenson K, Bar-Natan M, Perez-Ladaga A, Zaneveld J, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood. 2014;124(17):2705–12.

99. Shih AH, Meydan C, Shank K, Garrett-Bakelman FE, Ward PS, Intlekofer AM, et al. Combination targeted therapy to disrupt aberrant oncogenic signaling and reverse epigenetic dysfunction in IDH2- and TET2-mutant acute myeloid leukemia. Cancer Discov. 2017;7(5):494–505.
100. Itzykson R, Kosmider O, Cluzeau T, Mansat-De Mas V, Dreyfus F, Beyne-Rauzy O, et al. Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia. 2011;25(7):1147–52.
101. Jädersten M, Saft L, Smith A, Kulasekararaj AG, Pomplun S, Göhring G, et al. TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. J Clin Oncol. 2011;29(15):1971–9.
102. Hofmann WK, Platzbecker U, Gtze K, Haase D, Thol F, Stauder R, Passweg J, Germing U. Onkopedia Leitlinien: Myelodysplastische Syndrome (MDS). DGHO 2020. https://www.onkopedia.com/de/onkopedia/guidelines/myelodysplastische-syndrome-mds/@@guideline/html/index.html.
103. Welch JS, Petti AA, Miller CA, Fronick CC, O'Laughlin M, Fulton RS, et al. TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. N Engl J Med. 2016;375(21):2023–36.
104. Steensma DP. How I use molecular genetic tests to evaluate patients who have or may have myelodysplastic syndromes. Blood. 2018;132(16):1657–63.
105. Lindsley RC, Saber W, Mar BG, Redd R, Wang T, Haagenson MD, et al. Prognostic mutations in myelodysplastic syndrome after stem-cell transplantation. N Engl J Med. 2017;376(6):536–47.
106. McKerrell T, Park N, Moreno T, Grove CS, Ponstingl H, Stephens J, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. Cell Rep. 2015;10(8):1239–45.
107. Malcovati L, Galli A, Travaglino E, Ambaglio I, Rizzo E, Molteni E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. Blood. 2017;129(25):3371–8.
108. Kwok B, Hall JM, Witte JS, Xu Y, Reddy P, Lin K, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood. 2015;126(21):2355–61.
109. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9–16.
110. Lindsley RC, Mar BG, Mazzola E, Grauman PV, Shareef S, Allen SL, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood. 2015;125(9):1367–76.
111. Takahashi K, Jabbour E, Wang X, Luthra R, Bueso-Ramos C, Patel K, et al. Dynamic acquisition of FLT3 or RAS alterations drive a subset of patients with lower risk MDS to secondary AML. Leukemia. 2013;27(10):2081–3.
112. Baer C, Pohlkamp C, Haferlach C, Kern W, Haferlach T. Molecular patterns in cytopenia patients with our without evidence of myeloid neoplasm – a comparison of 756 cases. Leukemia. 2018;32(10):2295–8.

Chapter 6
MDS Mimics Including CHIP, ICUS, and CCUS

Rafael Bejar

Introduction

Medically speaking, the word "syndrome" refers to a collection of unrelated clinical signs and symptoms that are observed to frequently co-occur in patients without a requirement that all of those afflicted with a particular syndrome have a common cause for their condition. Myelodysplastic syndromes (MDS) are no exception, and have historically been defined almost exclusively by clinical and morphologic criteria. Without a clear understanding about the etiology of disorders described as MDS, even most MDS subtypes have similarly been defined by counting cells based on their appearance. As a consequence, the diagnostic criteria for MDS require potentially subjective morphologic interpretations and share many features with related conditions including benign disorders. Patients who appear to be mildly affected clinically may leave doubt as to whether their hematologic abnormalities are due to reactive or malignant causes, for example.

In practice, there exist several MDS "mimics" that share findings characteristic of MDS but are due to other causes (Fig. 6.1) [1]. These include a variety of benign conditions, other neoplasms, or even pre-malignant states that fall short of strict diagnostic boundaries. Several of these potential MDS mimics are covered in other chapters in this text including those describing germline predisposition states, diagnostic approaches, and clinical presentations of MDS. Here, we will touch on these briefly followed by a more in-depth examination of conditions that lay just outside the diagnostic boundary of MDS but which may not meet the criteria for other disorders. These can include pre-malignant states with varying potential for clonal progression and have important clinical consequences that go beyond the risk of developing a hematologic neoplasm.

R. Bejar (✉)
Moores Cancer Center, University of California San Diego, La Jolla, CA, USA
e-mail: rabejar@ucsd.edu

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3_6

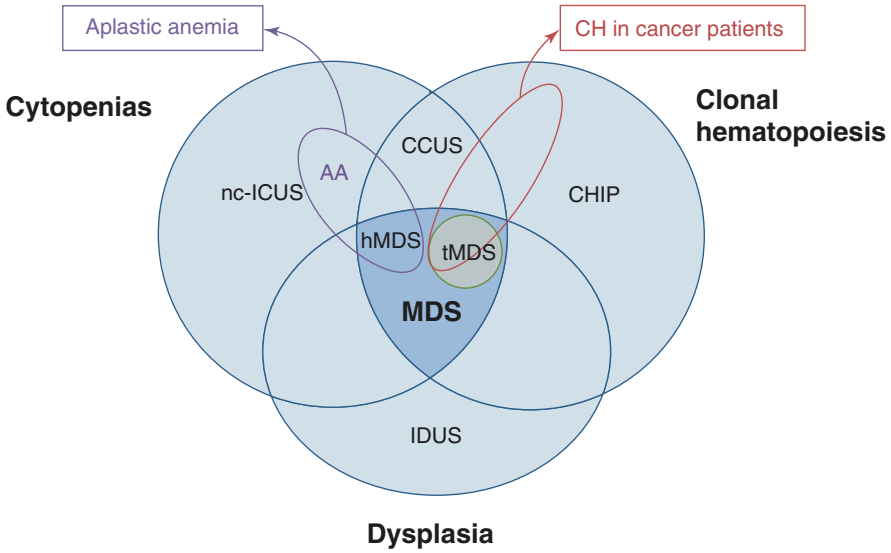

Fig. 6.1 Diagram showing the overlap of features characteristic of MDS and potential mimics that share some or more of these features. Abbreviations: AA aplastic anemia, nc-ICUS non-clonal idiopathic cytopenia of undetermined significance, CCUS clonal cytopenia of undetermined significance, CH clonal hematopoiesis, CHIP clonal hematopoiesis of indeterminate potential, MDS myelodysplastic syndrome, hMDS hypoplastic MDS, tMDS therapy-related MDS; IDUS idiopathic dysplasia of undetermined significance

MDS Diagnostic Criteria and Non-neoplastic Mimics

The most recent update to the World Health Organization (WHO) schema for the classification of myeloid neoplasms in 2016 changed little about the criteria used to diagnose MDS [2]. Patients are required to have a cytopenia in one or more mature myeloid cell lines (red blood cells, platelets, or neutrophils) defined as a value below the laboratory normal range for the population tested [2–4]. A cytopenia should not have another evident cause and should not be transient with a recommendation that it persists for 6 months in those with equivocal bone marrow findings [5]. In addition to a cytopenia, the bone marrow must show at least one of the following features to diagnose MDS: morphologic dysplasia in one or more myeloid lineages compromising at least 10% of cells in a lineage, the presence of 5–19% blast proportion, or one of several MDS-defining cytogenetic abnormalities. The only exception to these criteria are cases with 5–15% ring sideroblasts in the marrow in which a typical somatic mutation in *SF3B1* is also present [2, 6].

Patients with a high blast proportion, extensive dysplasia, and typical karyotype abnormalities are fairly straightforward to diagnose. However, most patients with MDS have less aggressive presentations with potentially more subtle findings. In particular, patients with no excess blasts or cytogenetic abnormalities often have less pronounced morphologic changes, the interpretation of which can be subject to

significant inter-observer variability [7]. And, in the minority of cases with a hypocellular bone marrow, accurately determining whether 10% of the available cells are affected can be difficult [8].

Adding to the potential ambiguity, many of the characteristic morphologic changes observed in MDS can also be found in non-malignant conditions which need to be considered before a diagnosis of MDS should be made. These include certain mineral and vitamin deficiencies, autoimmune conditions, viral infections, medications, liver disease, renal impairment, alcohol consumption, and several congenital syndromes (Table 6.1) [1, 9]. Most of these conditions will not reproduce all features common to MDS and many will have additional hematologic findings that are inconsistent with the diagnosis. For example, patients with chronic parvovirus B19 infection typically do not have cytopenias beyond their profound anemia and will have giant erythroblasts with characteristic nuclear viral inclusions in their marrow [10]. These patients are almost universally immunocompromised demonstrating how clinical context must be considered when trying to determine the cause of dysplastic hematopoiesis. Factors like nutritional state, family history, preexisting medical conditions, and exposure history will influence the probability that one of these "benign" mimics should be included in the differential diagnosis. Correctly identifying these disorders has important clinical implications as they are generally more reversible and amenable to treatment than MDS.

Congenital Syndromes with Predisposition to MDS

Several congenital disorders exhibit hematologic abnormalities that can resemble MDS including cytopenias and abnormal cellular morphology [11, 12]. Subsets of these disorders have the potential for clonal evolution and the development of a myeloid malignancy [13]. It can be challenging to determine when such a transformation has taken place versus when abnormalities are caused by the underlying predisposition syndrome. For example, patients with familial platelet disorder with associated myeloid malignancy (FPD/AMM) carry germline *RUNX1* mutations and can have thrombocytopenia decades before developing MDS or AML [11, 14]. Megakaryocytic dysplasia can be present long before evidence of clonal evolution arises and should not be used as the sole criterion for diagnosis MDS [14]. Making the distinction between syndromic features of the disorder and malignant progression is important as it affects when to consider an intervention like hematopoietic stem cell transplantation. Testing for somatic mutations indicative of clonal hematopoiesis may be useful to help predict when a patient is at an increased risk of malignant progression [13]. Other conditions like ANKRD26-related thrombocytopenia, Fanconi anemia, Shwachman-Diamond syndrome, and dyskeratosis congenita are subject to similar caveats as bone marrow failure often precedes the development of clonal neoplasm by a large margin [15–19].

In contrast, patients with germline *DDX41* variants typically do not have lifelong hematologic abnormalities [20–22]. They may, however, develop cytopenias and

Table 6.1 *MDS* mimics

Nutrient deficiencies	Common hematologic manifestations that can mimic MDS
Iron deficiency	Microcytic anemia, anisocytosis, hypochromia, thrombocytosis
Folate deficiency	Macrocytic anemia, megaloblastic changes, nuclear dysplasia, pancytopenia
B12 deficiency	Macrocytic anemia, megaloblastic changes, nuclear dysplasia, pancytopenia
Copper deficiency	Anemia, neutropenia, ring sideroblasts, erythrodysplasia, megaloblastic changes
Metabolic derangements	
Renal failure	Anemia, anisocytosis, poikilocytosis
Liver failure	Macrocytic anemia, leukopenia, thrombocytopenia (primary and from splenomegaly)
Hypothyroidism	Macrocytic anemia
Autoimmune conditions	
Aplastic anemia	Pancytopenia, marrow hypocellularity, clonal markers
ITP	Thrombocytopenia, micromegakaryocytes
HLH	Pancytopenia, dyserythropoiesis
SLE and RA	Anemia, leukopenia, thrombocytopenia, or pancytopenia
Viral infections	
HIV	Pancytopenia, bone marrow dysplasia, megaloblastic changes (often from treatment)
CMV, EBV	Monocytosis, anemia, thrombocytopenia
Hepatitis B or C	Neutropenia, thrombocytopenia, macrocytosis (also related to liver disease)
Parvovirus B19	Red cell aplasia, dyserythropoiesis
Medications and toxins	
Anti-metabolites	Macrocytosis, pancytopenia
Cytotoxic chemotherapy	Pancytopenia, hypogranulation, bone marrow dysplasia, and apoptosis
MMF	Pseudo-Pelger-Huet abnormality
Ethanol	Normo- or macrocytic anemia, erythrodysplasia, ring sideroblasts, dysmorphic granulocytes
Congenital disorders	
Sideroblastic anemias	Anemia, ring sideroblasts, erythrodysplasia
Diamond-Blackfan anemia	Anemia, erythroid hyperplasia, dyserythropoiesis
SBDS	Neutropenia, thrombocytopenia, anemia, myeloid dysplasia
Fanconi anemia	Pancytopenia, dysplasia
Severe congenital neutropenia	Neutropenia, granulocyte dysplasia
CDA	Anemia, erythroid hyperplasia, dyserythropoiesis
Other neoplastic conditions	
LGL	Neutropenia, anemia

Table 6.1 (continued)

Nutrient deficiencies	Common hematologic manifestations that can mimic MDS
MDS/MPN	Anemia, dyserythropoiesis, dysgranulopoiesis
AML	Myelodysplastic changes, increased blasts, Auer rods

ITP immune thrombocytopenic purpura, *HLH* hemophagocytic lymphohistiocytosis, *SLE* systemic lupus erythematosus, *RA* rheumatoid arthritis, *HIV* human immunodeficiency virus, *CMV* cytomegalovirus, *EBV* Epstein-Barr virus, *MMF* mycophenolate mofetil, *SBDS* Shwachman-Diamond syndrome, *CDA* congenital dyserythropoietic anemia, *LGL* large granular lymphocytic leukemia, *MDS/MPN* myelodysplastic syndrome/myeloproliferative neoplasms overlap syndrome, *AML* acute myelocytic anemia

bone marrow hypocellularity, months or years before they go on to meet diagnostic criteria for MDS or AML. In these cases, this non-diagnostic prodrome may not be a benign MDS mimic, but more of a harbinger of MDS that should be followed closely as clonal evolution has likely already occurred.

These topics are covered in greater detail in the chapter on familial and germline MDS.

Conditions at the Diagnostic Border of MDS

In clinical practice, we frequently encounter patients with cytopenias that remain difficult to explain even after thorough evaluation and examination of the bone marrow. In some patients, it is clear that a disorder is present, but there is ambiguity about which diagnosis to assign. In other cases, patients do not meet the criteria for any recognized condition and are left with a label of idiopathic cytopenia and uncertainty about their prognosis. With the advent of next-generation sequencing, it was hoped that identification of somatic mutation patterns in these conditions could aid in their assessment and proper diagnosis. This promise has partially been fulfilled, but surprising discoveries about the high incidence of clonal hematopoiesis in "benign" hematologic conditions, and even in normal individuals, can complicate the interpretation of somatic mutations.

Aplastic Anemia

For example, there exists a clinical overlap between patients with aplastic anemia (AA) and those with hypoplastic MDS (hMDS) [8, 23, 24]. Both conditions are associated with clinically significant cytopenias, bone marrow hypocellularity, and varying degrees of cellular dysplasia which may be challenging to quantify when few cells are present. In AA, it is assumed that bone marrow failure is a consequence of autoimmune destruction of stem cells and disruption of the normal microenvironment. Autoimmunity is not excluded in hMDS, but it is presumed that there is a

clonal, cell-intrinsic defect that contributes to the bone marrow failure whereas no such clonal outgrowth is required in AA. If an AA patient develops an MDS defining clonal abnormality, like monosomy 7, they would be considered to have progressed to hMDS even in the absence of increased blasts or dysplasia. However, careful sequencing and copy number analyses reveal that clonal outgrowth is remarkably common in AA even in the absence of clinical progression [23, 25–27]. Clonal chromosomal abnormalities were known to be present in 5–15% of AA cases and *PIGA* mutated paroxysmal nocturnal hemoglobinuria (PNH) clones detected in more than half of patients when sensitive methods are employed. Many of these lesions are not typical of MDS or associated with disease progression [28]. However, recent sequencing studies have identified somatic mutations in a third to a half of AA patients that include mutated genes common to MDS [26, 27, 29, 30]. Two major patterns of mutation appear to be present. The first is associated with immunologic escape and involves mutations or loss of heterozygosity (LOH) at the HLA loci on chromosome 6 [31, 32]. Mutations of *BCOR* and *BCORL1* may also fit in this category as they were not associated with resistance to immunosuppressive therapy (IST) and often had a decreased clone size after treatment [26]. In contrast, somatic mutations of *DNMT3A* and *ASXL1* were present in about 10% of cases, particularly older patients, and tended to show clonal expansion after IST. Mutations of splicing factor genes, cohesin genes, *TET2*, *RUNX1*, and *TP53* were much rarer in AA compared to MDS, but were associated with increased rates of malignant progression and inferior outcomes. Somatic mutations in AA also tended to involve fewer genes and have variant allele frequencies (VAF) of <10% compared to >30% VAF in MDS. These differences suggest metrics that may help distinguish AA with low potential for progression from hMDS and higher risk AA.

Clonal Hematopoiesis in Individuals with Clinically Normal Hematopoiesis

Clonal hematopoiesis defined by the presence of somatic mutations is not diagnostic of myeloid neoplasia (Fig. 6.1). Not only does it occur in AA, but it is also common in normal individuals who have no sign of a hematologic abnormality. Examination of blood specimens used in genome-wide association studies and as controls in cancer sequencing studies demonstrated a high prevalence of somatic mutations in DNA derived from blood cells [33–36]. The genes mutated in these individuals were typical of MDS and were dominated by epigenetic regulators like *DNMT3A*, *TET2*, and *ASXL1*, although several rarer myeloid malignancy mutations were identified in *JAK2*, *TP53*, splicing factors, and the *IDH1* and *IDH2* genes, among others. The prevalence of these mutations rises significantly with age ranging from 5% of persons at age 50 to over 20% at age 80 mirroring the age-related rise in the incidence of MDS. However, MDS and other myeloid malignancies occur at a rate more than 100-fold less often than the rate of clonal hematopoiesis in

normal individuals. Therefore, it was not surprising that the absolute risk of developing a hematologic malignancy in this population was low at 0.5–1.0% per year [37]. This is comparable to the rate of myeloma progression in persons with a monoclonal gammopathy of undetermined significance [38]. For this reason, the presence of a myeloid malignancy-associated driver mutation in a hematologically normal individual was termed clonal hematopoiesis of indeterminate potential (CHIP) [39].

A formal definition of CHIP has been proposed [39]. This requires the presence of a somatic mutation in a common myeloid malignancy driver gene with a variant allele frequency of 2% or greater. Most individuals with CHIP will harbor a single driver gene mutation with an average variant allele frequency of 9–12%. Larger CHIP clones appear to have a greater risk of malignant progression. Sensitive sequencing approaches have identified very small somatically mutated clones with VAFs well below 1% in a much greater proportion of individuals [40]. Nearly all women in their 50's sampled as part of the Nurses Health Study had one or more somatic mutations detectable in this range. As with CHIP, most of the mutations were in *DNMT3A*, but included other genes. A quarter of these clones could be detected in samples collected 10 years later (along with more new ones), typically with little change in VAF. Clearly, the almost universal nature of this "micro" CHIP in unselected individuals implies that it is not associated with malignancy risk. However, in specific clinical contexts CHIP can be highly relevant [41].

Despite its acronym, CHIP does not lack clinical significance. Otherwise normal individuals with CHIP were noted to have greater overall mortality ascribed primarily to cardiovascular disease [36, 42–44]. The degree of risk associated with CHIP was comparable to or greater than that of well-established risk factors such as hypertension and hypercholesterolemia. Whole-genome sequencing studies are capable of identifying clonal hematopoiesis even if myeloid malignancy driver genes are not present. In the study of an Icelandic population by Zink et al., the prevalence of clonal hematopoiesis was twice as high when non-driver gene mutations were used to identify patients with expanded clones [45]. These individuals had comparable increases in mortality risk to those with myeloid malignancy mutation-associated CHIP although not necessarily from cardiovascular causes, suggesting a direct role for myeloid malignancy mutations in this phenotype. The alternative to this interpretation is that CHIP is simply a marker of biologic age which is related to cardiovascular disease or other causes of mortality through an independent mechanism. However, the mutations responsible for CHIP can drive the development of atherosclerosis through inflammatory insults which have been modeled in mice [42, 46, 47]. The mediators of this process appear to be monocytes and macrophages derived from the mutant clone that activate the NLRP3 inflammasome resulting in increased levels of IL1β, IL6, and other pro-inflammatory molecules. Chemical inhibition of NLRP3 mitigates the atherogenic effects of Tet2 loss in a murine transplant model [46]. In patients with a history of cardiovascular disease and elevated C-reactive protein levels, inhibition of IL1β activity with the monoclonal antibody canakinumab was associated with a significant reduction in overall mortality only in those individuals later identified as having CHIP [48, 49].

Importantly, the NLRP3 inflammasome and IL1β signaling are now recognized as a major contributor to the MDS phenotype, particularly in lower-risk MDS patients [50, 51]. This connection between somatic mutations, CHIP, and MDS is examined in greater detail in the chapter on MDS Biology.

Without cytopenias, CHIP is unlikely to be confused clinically with MDS. However, the high background rate of typical mutations in older individuals complicates the interpretation of somatic mutations in persons suspected of having MDS [39]. It could be challenging, for example, to determine if a single *DNMT3A* mutation in an anemic patient is indicative of acquired bone marrow failure or simply an incidental finding unrelated to a benign cause for their disorder. It is therefore critical to consider the clinical context when interpreting sequencing results as their impact varies significantly based on the clinical scenario [41]. *Somatic mutations alone cannot be used as presumptive evidence of MDS absent other diagnostic criteria.*

Recent studies suggest that CHIP defined by mutations in particularly adverse genes may have significant malignant implications. First, pre-leukemic hematopoietic stem cells harboring *DNMT3A* mutations can be identified in AML patients in remission [52]. This indicates that clonal evolution from a preceding, clinically silent, clonal hematopoietic state led to the development of AML. The latency between clonal hematopoiesis and AML can be quite long. In a longitudinal health study, individuals who developed AML had DNA sequencing performed on samples collected months to years prior to their diagnosis [53]. These results were compared to sequencing from similarly aged individuals in the cohort who did not develop AML. While preexistent CHIP was more common in the AML cohort, mutations of *TP53*, *IDH1*, and *IDH2* were unique to this group, indicating a near-universal progression to AML, albeit with a median latency of 5–7 years. A similar case-control study by Abelson et al. confirmed the high risk of AML associated with *TP53* mutations and identified a comparable risk for *U2AF1* [54]. In both studies, time to AML diagnosis was inversely proportional to the VAF of adverse gene mutations and the total number of mutations identified.

CHIP is also frequently encountered in cancer patients where the implications of clonal hematopoiesis may be more severe than in unselected individuals [55]. This may be, in part, because of the selective effects of cancer treatments on pre-existing, somatically mutated clones. Clones with mutations that bestow chemo- or radioresistance may be selected for along with those that have a proliferative advantage during recovery from myelosuppression [56]. Studies of relapsed/refractory lymphoma patients destined for autologous stem cell transplantation identified a high rate of clonal hematopoiesis before the transplant was performed [57]. In contrast to unselected CHIP, or the micro-CHIP noted in normal individuals, treated lymphoma patients were much more likely to have mutations in *TP53* and *PPM1D*. These individuals had an increased likelihood of developing therapy-related myeloid neoplasms, but also had poor stem cell mobilization and early overall mortality compared to lymphoma patients without CHIP. Studies of solid tumor patients have confirmed a high frequency of blood cell-derived somatic mutations and the enrichment of mutations in *TP53* as well as an association with inferior outcomes [58–61].

There is evidence to suggest that germline mutations in DNA repair pathways may predispose to cancer-related CHIP and the development of t-MNs [62, 63]. Clearly, some forms of CHIP are not benign in this context, but absent other criteria, do not define a diagnosis of MDS.

Clonal and Idiopathic Cytopenias of Undetermined Significance (CCUS vs ICUS)

The most challenging diagnostic dilemmas arise when some features of MDS are present, but no alternative explanation can be identified (Fig. 6.1) [64]. Unexplained cytopenias in patients with insufficient bone marrow dysplasia, low blast percentage, and a normal karyotype have been described as idiopathic cytopenias of undetermined significance (ICUS) [1, 65–67]. Determining whether these individuals are at risk of developing a frank myeloid malignancy is difficult as longitudinal studies of ICUS patients are lacking. However, the advent of genetic sequencing has identified a high rate of clonal hematopoiesis in this population. MDS and AML patients who had a non-diagnostic biopsy prior to their eventual diagnosis were noted to have a high rate of somatic mutations in their initial sample [68]. While these patients did not meet marrow criteria for a myeloid disorder initially, more than 90% already carried a somatic mutation in one or more myeloid malignancy genes. Less than half of these individuals acquired a new driver mutation at the time of their eventual diagnosis, indicating that molecular evolution was not required for progression in many instances [68]. A similar study of patients with unexplained cytopenias who underwent a routine bone marrow evaluation found that many either had insufficient dysplasia to diagnose MDS or no dysplasia at all, labeling them as having ICUS [69]. Of those patients that were diagnosed with MDS, more than 90% carried at least one clonal chromosomal abnormality or gene mutation. However, more than 70% of ICUS patients with some dysplasia had a clonal marker as did a quarter of those with no dysplasia. This rate of clonal hematopoiesis was much higher than the 10–15% of CHIP identified in comparably aged normal populations, suggesting that clonality and cytopenias might be related in these cases. The types of mutations identified were somewhat different from CHIP in that they included a higher rate of splicing factors, *RUNX1*, and *TP53*, were more likely to have multiple mutations, and had greater VAFs than is typical for CHIP (Table 6.2). Therefore, ICUS patients with clonal markers were described as having clonal cytopenias of undetermined significance (CCUS) to distinguish them from non-clonal ICUS (ncICUS) and to indicate that the risk of progression in these cases remained unclear [70].

A subsequent study by Malcovati et al. confirmed the high rate of clonal hematopoiesis in ICUS [71]. About 40% of patients suspected of having a myeloid disorder, but who fell short of diagnostic criteria, were found to have somatic mutations indicative of clonal hematopoiesis. The predicted probability of subsequent progression to a myeloid malignancy was significant, approaching 50% at 4 years for

Table 6.2 Distinctions among *MDS* mimics

Disease features	Aplastic anemia	CHIP	nc-ICUS	CCUS	MDS
Cytopenias	Present	Absent	Present	Present	Present
Bone marrow cellularity	Hypocellular	Normal	Mostly normal	Hypo- to hypercellular	Mostly hypercellular
Somatic mutations	20%	5–20%*	0	35–40%	90+%
Most commonly mutated genes	*PIGA, BCOR, BCORL1,* HLA alleles and/or *DNMT3A, ASXL1*	*DNMT3A, TET2, ASXL1, JAK2, PPM1D, TP53*	None	*TET2, DNMT3A, ASXL1,* splicing factors, *TP53*	*TET2, SF3B1, ASXL1, SRSF2, DNMT3A, RUNX1, U2AF1, TP53*
Number of driver mutations	1–2	1	0	1–2	2–3+
Typical variant allele frequency	2–10%	2–12%	0	30–40%	30–40%
Karyotype abnormalities	20%	<2%	0	33%	50%

*The prevalence of CHIP is highly age dependent

patients with isolated mutations in *DNMT3A*, *TET2*, or *ASXL1*, and nearly 100% at 5 years for patients with splicing factor mutations, *RUNX1* mutations, *JAK2* V617F, or mutations in multiple genes. More importantly, ICUS patients *without* somatic mutations on a multi-gene panel (i.e., presumed to have nc-ICUS), had an extremely low rate of malignant progression, approximated at 1% per year. This strong negative predictive value supports the use of multi-gene panel testing at diagnosis for patients suspected of having MDS as even an absence of mutations has important clinical significance. A larger study by Baer et al. examined clonal hematopoiesis in 756 patients with unexplained cytopenias, comparing them to patients with MDS and AML [72]. As before, AML-like mutations (i.e., *NPM1*, *NRAS*, *RUNX1*, and *IDH1/2*) were rare in CCUS as were mutations of *SF3B1* which were seen most often in MDS, suggesting diagnostic associations with some gene mutations [73].

Current guidelines would consider even high-risk CCUS patients to lie just shy of the diagnostic border with MDS, yet their overall survival appeared comparable to that of lower risk MDS patients [71]. This is also the case for the ~60% patients with unexplained monocytosis who do not meet diagnostic criteria for chronic myelomonocytic leukemia, but harbor mutations typical of the disease [74]. Larger studies may demonstrate that certain mutations should be considered presumptive evidence of MDS when unexplained cytopenias are present, much the same way that specific cytogenetic abnormalities do today. The umbrella of WHO-defined MDS will likely expand to include some forms of CCUS just as it did in 2016 when *SF3B1* mutant patients with as few as 5% ring sideroblasts were included in the diagnostic classification [2].

MDS Can Sometimes Be Considered an AML Mimic

On the other side of the spectrum of clinical severity lays the diagnostic boundary between MDS and AML [75]. Patients with fewer than 20% bone marrow blasts are considered to have MDS, while those with 20% or more have acute leukemia. In the past, patients with 20–29% blasts were described as having a form of MDS then called refractory anemia with excess blasts in transformation (RAEB-T) [76]. This category was eliminated in 2008 when the boundary was reduced to 20% blasts as outcomes were comparable between RAEB-T and AML patients of similar age [5, 77]. However, the 20% cut-off is somewhat arbitrary [78]. Outcomes for MDS patients with 10–19% blasts are more like that of patients with oligoblastic leukemia than lower-risk MDS. In particular, there appear to be some forms of AML that are more MDS-like and others that resemble examples of de novo AML that are seen more often in younger individuals [79]. Not surprisingly, secondary AML arising after MDS often shares morphologic and molecular features with MDS. In particular, mutations of splicing factors and chromatin modifiers such as *ASXL1* and *EZH2* were highly specific for secondary AML. Patients presumed to have de novo AML who harbored mutations in these genes were more likely to be older and had response rates and outcomes comparable to those with known secondary AML [79]. In contrast, AML patients with *FLT3* abnormalities, isolated biallelic mutations of *CEBPA*, or frameshifts in *NPM1* tended to be younger and were more likely to achieve a complete remission. These gene mutations appear to identify patients with disease that is fairly sensitive to chemotherapy, even when they have not yet passed the 20% blast threshold [80–82]. Finally, there is growing evidence that certain mutated genes (e.g., *FLT3*, *PTPN11*, *WT1*, *IDH1*, *IDH2, NPM1*, and *NRAS*) and patterns of gene expression can predict transformation to AML in patients with MDS [83, 84]. These patients might be considered examples of MDS mimicking AML. Different molecular profiles of patients in this oligoblastic area of overlap between MDS and AML may identify groups that respond better to different therapeutic approaches regardless of which diagnosis is playing the mimic.

Summary

Establishing a diagnosis of MDS in a cytopenic patient can be challenging as it relies on the presence of largely morphologic criteria and exclusion of alternative diagnoses that include both benign and neoplastic conditions. Many MDS mimics can cause dysplasia or harbor karyotype abnormalities that are not specific enough to establish an MDS diagnosis. Somatic mutations of MDS-associated genes can help clarify the picture, but the interpretation of molecular findings is highly dependent on the clinical context in which they are found. Future revisions to MDS diagnostic criteria are likely to incorporate certain somatic events as evidence of disease leading to a more standardized definition of MDS. In the meantime, we must recognize

areas of ambiguity, such as CCUS, clonality in aplastic anemia, and oligoblastic leukemia that may not only mimic MDS, but might share therapeutic benefit from treatment options available to patients on each side of these diagnostic boundaries.

Conflict of Interest Dr. Bejar has received research funding from Celgene and Takeda and served as an advisor to Celgene, Genoptix (NeoGenomics), AbbVie, Daiichi-Sankyo, Forty Seven, and Astex. He is employed by Aptose Biosciences.

References

1. Steensma DP. Dysplasia has a differential diagnosis: distinguishing genuine myelodysplastic syndromes (MDS) from mimics, imitators, copycats and impostors. Curr Hematol Malig Rep. 2012;7:310–20.
2. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–405.
3. Greenberg PL, Tuechler H, Schanz J, et al. Cytopenia levels for aiding establishment of the diagnosis of myelodysplastic syndromes. Blood. 2016;128:2096–7.
4. Malcovati L, Hellstrom-Lindberg E, Bowen D, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood. 2013;122:2943–64.
5. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114:937–51.
6. Malcovati L, Karimi M, Papaemmanuil E, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015;126:233–41.
7. Font P, Loscertales J, Benavente C, et al. Inter-observer variance with the diagnosis of myelodysplastic syndromes (MDS) following the 2008 WHO classification. Ann Hematol. 2013;92:19–24.
8. Bono E, McLornan D, Travaglino E, et al. Clinical, histopathological and molecular characterization of hypoplastic myelodysplastic syndrome. Leukemia. 2019;33:2495–505.
9. DeZern AE, Sekeres MA. The challenging world of cytopenias: distinguishing myelodysplastic syndromes from other disorders of marrow failure. Oncologist. 2014;19:735–45.
10. Servey JT, Reamy BV, Hodge J. Clinical presentations of parvovirus B19 infection. Am Fam Physician. 2007;75:373–6.
11. University of Chicago Hematopoietic Malignancies Cancer Risk T. How I diagnose and manage individuals at risk for inherited myeloid malignancies. Blood. 2016;128:1800–13.
12. DiNardo CD, Bannon SA, Routbort M, et al. Evaluation of patients and families with concern for predispositions to hematologic malignancies within the hereditary hematologic malignancy clinic (HHMC). Clin Lymphoma Myeloma Leuk. 2016;16:417–28.. e2
13. Churpek JE, Pyrtel K, Kanchi KL, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. Blood. 2015;126:2484–90.
14. Chisholm KM, Denton C, Keel S, et al. Bone marrow morphology associated with germline RUNX1 mutations in patients with familial platelet disorder with associated myeloid malignancy. Pediatr Dev Pathol. 2019;22:315–28.
15. Marquez R, Hantel A, Lorenz R, et al. A new family with a germline ANKRD26 mutation and predisposition to myeloid malignancies. Leuk Lymphoma. 2014;55:2945–6.
16. Yoshimi A, Toya T, Nannya Y, et al. Spectrum of clinical and genetic features of patients with inherited platelet disorder with suspected predisposition to hematological malignancies: a nationwide survey in Japan. Ann Oncol. 2016;27:887–95.

17. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. Blood. 2003;101:822–6.
18. Mourad S, Bilodeau M, Roussy M, et al. IDH1 as a cooperating mutation in AML arising in the context of Shwachman-Diamond syndrome. Front Oncol. 2019;9:772.
19. Yamaguchi H, Baerlocher GM, Lansdorp PM, et al. Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. Blood. 2003;102:916–8.
20. Polprasert C, Schulze I, Sekeres MA, et al. Inherited and somatic defects in DDX41 in myeloid neoplasms. Cancer Cell. 2015;27:658–70.
21. Lewinsohn M, Brown AL, Weinel LM, et al. Novel germ line DDX41 mutations define families with a lower age of MDS/AML onset and lymphoid malignancies. Blood. 2016;127:1017–23.
22. Sebert M, Passet M, Raimbault A, et al. Germline DDX41 mutations define a significant entity within adult MDS/AML patients. Blood. 2019;134(17):1441–4.
23. Afable MG 2nd, Wlodarski M, Makishima H, et al. SNP array-based karyotyping: differences and similarities between aplastic anemia and hypocellular myelodysplastic syndromes. Blood. 2011;117:6876–84.
24. Nazha A, Seastone D, Radivoyevitch T, et al. Genomic patterns associated with hypoplastic compared to hyperplastic myelodysplastic syndromes. Haematologica. 2015;100:e434–7.
25. Ogawa S. Clonal hematopoiesis in acquired aplastic anemia. Blood. 2016;128:337–47.
26. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic mutations and clonal hematopoiesis in aplastic Anemia. N Engl J Med. 2015;373:35–47.
27. Kulasekararaj AG, Jiang J, Smith AE, et al. Somatic mutations identify a sub-group of aplastic anemia patients that progress to myelodysplastic syndrome. Blood. 2014;18:2014–05.
28. Maciejewski JP, Risitano A, Sloand EM, Nunez O, Young NS. Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia. Blood. 2002;99:3129–35.
29. Babushok DV, Olson TS, Bessler M. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med. 2015;373:1673.
30. Babushok DV, Perdigones N, Perin JC, et al. Emergence of clonal hematopoiesis in the majority of patients with acquired aplastic anemia. Cancer Genet. 2015;208:115–28.
31. Zaimoku Y, Takamatsu H, Hosomichi K, et al. Identification of an HLA class I allele closely involved in the autoantigen presentation in acquired aplastic anemia. Blood. 2017;129:2908–16.
32. Katagiri T, Sato-Otsubo A, Kashiwase K, et al. Frequent loss of HLA alleles associated with copy number-neutral 6pLOH in acquired aplastic anemia. Blood. 2011;118:6601–9.
33. Laurie CC, Laurie CA, Rice K, et al. Detectable clonal mosaicism from birth to old age and its relationship to cancer. Nat Genet. 2012;44:642–50.
34. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med. 2014;20:1472–8.
35. Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371:2477–87.
36. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371:2488–98.
37. Genovese G, Jaiswal S, Ebert BL, McCarroll SA. Clonal hematopoiesis and blood-cancer risk. N Engl J Med. 2015;372:1071–2.
38. Kyle RA, Larson DR, Therneau TM, et al. Long-term follow-up of monoclonal gammopathy of undetermined significance. N Engl J Med. 2018;378:241–9.
39. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126:9–16.
40. Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. Nat Commun. 2016;7:12484.
41. Bejar R. CHIP, ICUS, CCUS and other four-letter words. Leukemia. 2017;31:1869–71.
42. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med. 2017;377:111–21.
43. Dorsheimer L, Assmus B, Rasper T, et al. Association of mutations contributing to clonal hematopoiesis with prognosis in chronic ischemic heart failure. JAMA Cardiol. 2019;4:25–33.

44. Sano S, Wang Y, Walsh K. Clonal hematopoiesis and its impact on cardiovascular disease. Circ J. 2018;83:2–11.
45. Zink F, Stacey SN, Norddahl GL, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. Blood. 2017;130:742–52.
46. Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science. 2017;355:842–7.
47. Sano S, Oshima K, Wang Y, Katanasaka Y, Sano M, Walsh K. CRISPR-mediated gene editing to assess the roles of Tet2 and Dnmt3a in clonal hematopoiesis and cardiovascular disease. Circ Res. 2018;123:335–41.
48. Svensson EC, Madar A, Campbell CD, et al. Abstract 15111: TET2-driven clonal hematopoiesis predicts enhanced response to canakinumab in the CANTOS Trial: an exploratory analysis. Circulation. 2018;138:A15111–A.
49. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med. 2017;377:1119–31.
50. Basiorka AA, McGraw KL, Eksioglu EA, et al. The NLRP3 inflammasome functions as a driver of the myelodysplastic syndrome phenotype. Blood. 2016;128:2960–75.
51. Sallman DA, List A. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. Blood. 2019;133:1039–48.
52. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506:328–33.
53. Desai P, Mencia-Trinchant N, Savenkov O, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. Nat Med. 2018;24:1015–23.
54. Abelson S, Collord G, Ng SWK, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature. 2018;559:400–4.
55. Bolton KL, Gillis NK, Coombs CC, et al. Managing clonal hematopoiesis in patients with solid tumors. J Clin Oncol. 2019;37:7–11.
56. Kahn JD, Miller PG, Silver AJ, et al. PPM1D-truncating mutations confer resistance to chemotherapy and sensitivity to PPM1D inhibition in hematopoietic cells. Blood. 2018;132:1095–105.
57. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. J Clin Oncol. 2017;35:1598–605.
58. Coombs CC, Zehir A, Devlin SM, et al. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. Cell Stem Cell. 2017;21:374–82.. e4
59. Coombs CC, Gillis NK, Tan X, et al. Identification of clonal hematopoiesis mutations in solid tumor patients undergoing unpaired next-generation sequencing assays. Clin Cancer Res. 2018;24:5918–24.
60. Swisher EM, Harrell MI, Norquist BM, et al. Somatic mosaic mutations in PPM1D and TP53 in the blood of women with ovarian carcinoma. JAMA Oncol. 2016;2:370–2.
61. Zajkowicz A, Butkiewicz D, Drosik A, Giglok M, Suwinski R, Rusin M. Truncating mutations of PPM1D are found in blood DNA samples of lung cancer patients. Br J Cancer. 2015;112:1114–20.
62. Churpek JE, Marquez R, Neistadt B, et al. Inherited mutations in cancer susceptibility genes are common among survivors of breast cancer who develop therapy-related leukemia. Cancer. 2016;122:304–11.
63. McNerney ME, Godley LA, Le Beau MM. Therapy-related myeloid neoplasms: when genetics and environment collide. Nat Rev Cancer. 2017;17:513–27.
64. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood. 2004;104:2263–8.
65. Valent P, Horny HP, Bennett JM, et al. Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: consensus statements and report from a working conference. Leuk Res. 2007;31:727–36.

66. Valent P, Bain BJ, Bennett JM, et al. Idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of uncertain significance (IDUS), and their distinction from low risk MDS. Leuk Res. 2012;36:1–5.
67. Malcovati L, Cazzola M. The shadowlands of MDS: idiopathic cytopenias of undetermined significance (ICUS) and clonal hematopoiesis of indeterminate potential (CHIP). Hematology Am Soc Hematol Educ Program. 2015;2015:299–307.
68. Cargo CA, Rowbotham N, Evans PA, et al. Targeted sequencing identifies patients with preclinical MDS at high risk of disease progression. Blood. 2015;126:2362–5.
69. Kwok B, Hall JM, Witte JS, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood. 2015;126:2355–61.
70. DeZern AE, Malcovati L, Ebert BL. CHIP, CCUS, and other acronyms: definition, implications, and impact on practice. Am Soc Clin Oncol Educ Book. 2019;39:400–10.
71. Malcovati L, Galli A, Travaglino E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. Blood. 2017;129:3371–8.
72. Baer C, Pohlkamp C, Haferlach C, Kern W, Haferlach T. Molecular patterns in cytopenia patients with or without evidence of myeloid neoplasm-a comparison of 756 cases. Leukemia. 2018;32:2295–8.
73. Steensma DP. How I use molecular genetic tests to evaluate patients who have or may have myelodysplastic syndromes. Blood. 2018;132:1657–63.
74. Cargo C, Cullen M, Taylor J, et al. The use of targeted sequencing and flow cytometry to identify patients with a clinically significant monocytosis. Blood. 2019;133:1325–34.
75. Tanaka TN, Bejar R. MDS overlap disorders and diagnostic boundaries. Blood. 2019;133:1086–95.
76. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100:2292–302.
77. Pleyer L, Burgstaller S, Stauder R, et al. Azacitidine front-line in 339 patients with myelodysplastic syndromes and acute myeloid leukaemia: comparison of French-American-British and World Health Organization classifications. J Hematol Oncol. 2016;9:39.
78. Lichtman MA. Does a diagnosis of myelogenous leukemia require 20% marrow myeloblasts, and does <5% marrow myeloblasts represent a remission? The history and ambiguity of arbitrary diagnostic boundaries in the understanding of myelodysplasia. Oncologist. 2013;18:973–80.
79. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood. 2015;125:1367–76.
80. Montalban-Bravo G, Kanagal-Shamanna R, Sasaki K, et al. NPM1 mutations define a specific subgroup of MDS and MDS/MPN patients with favorable outcomes with intensive chemotherapy. Blood Adv. 2019;3:922–33.
81. Konstandin NP, Pastore F, Herold T, et al. Genetic heterogeneity of cytogenetically normal AML with mutations of CEBPA. Blood Adv. 2018;2:2724–31.
82. Strickland SA, Shaver AC, Byrne M, et al. Genotypic and clinical heterogeneity within NCCN favorable-risk acute myeloid leukemia. Leuk Res. 2018;65:67–73.
83. Makishima H, Yoshizato T, Yoshida K, et al. Dynamics of clonal evolution in myelodysplastic syndromes. Nat Genet. 2017;49:204–12.
84. Shiozawa Y, Malcovati L, Galli A, et al. Gene expression and risk of leukemic transformation in myelodysplasia. Blood. 2017;130:2642–53.

Part II
Prognosis

Chapter 7
Prognostic Models in Myelodysplastic Syndromes

Jan Philipp Bewersdorf and Amer M. Zeidan

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell neoplasms that are characterized by peripheral blood cytopenias, dysplastic features in one or more cell lineages, and a variable risk of progression to acute myeloid leukemia (AML) [1–3]. Given the heterogeneity of the disease pathobiology and the highly variable disease course, tools to risk stratify patients are essential for appropriate counseling and individualization of treatment decisions [1, 4]. Figure 7.1 illustrates the various factors influencing the prognosis of MDS patients. Understanding the prognostic picture for individual patients is important as the goals of care and treatment options vary significantly. For example, the treatment of lower-risk MDS may entail watchful waiting, transfusions, erythropoiesis-stimulating agents, lenalidomide, and immunosuppressive therapy, while patients with higher-risk MDS are typically treated with therapies that aim to change the natural history of the disease such as hypomethylating agents (HMA) and allogeneic hematopoietic cell transplant (allo-HCT) for eligible patients [4–10].

Several risk stratification tools have been developed over the last decades and will be reviewed herein. While the earlier scoring systems such as the International Prognostic Scoring System (IPSS) are based on the extent of peripheral blood cytopenias, bone marrow blast count, and certain cytogenetic features, recent advances in technology, mainly genetic testing, have shown that the incorporation of this information may provide a more accurate assessment of overall prognosis and potentially serve as a predictive marker for response to certain treatment modalities [7, 11–14]. Furthermore, MDS disease risk may change over the disease course, and reassessment under specific circumstances such as HMA failure or prior to

J. P. Bewersdorf · A. M. Zeidan (✉)
Department of Internal Medicine, Section of Hematology, Yale School of Medicine, Yale University, New Haven, CT, USA
e-mail: amer.zeidan@yale.edu

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3_7

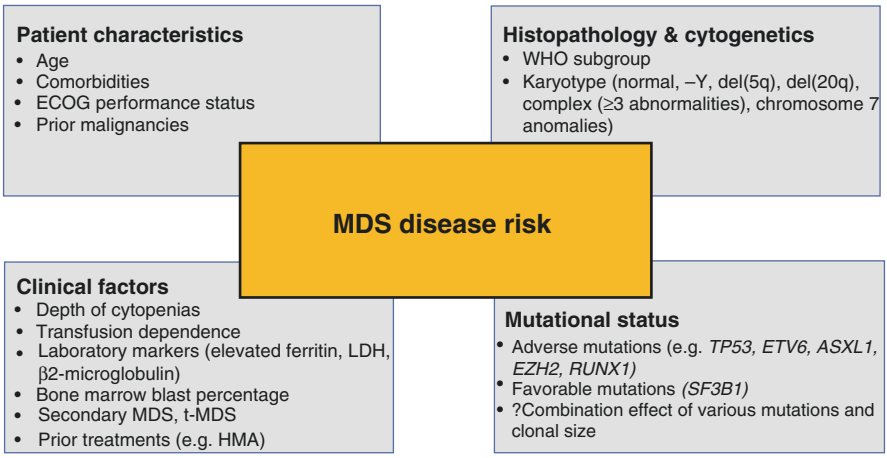

Fig. 7.1 Factors influencing the prognosis of MDS patients: The prognosis of the individual MDS patient depends on various factors and interactions thereof. These include patient characteristics (age, comorbidities, ECOG performance status, a history of prior malignancies), clinical and laboratory features (depth of cytopenias, transfusion dependence, selected laboratory markers [e.g., elevated ferritin, LDH, β2-microglobulin], bone marrow blast percentage, secondary MDS, therapy-related MDS [t-MDS], prior MDS-specific treatments [e.g., hypomethylating agents], histopathologic and cytogenetic factors (WHO subgroup. Karyotype [normal, −Y, del(5q), del(20q), complex (≥3 abnormalities), chromosome 7 anomalies])), and mutational status. However, none of the established risk stratification tools includes all of these factors leaving some residual uncertainty when counseling MDS patients and warranting further research to derive better prediction models

allo-HCT by the same tools used at the time of initial diagnosis has limitations. Herein, we review established scoring systems, describe novel tools that incorporate genetic characteristics as well as tools focusing on specific patient subgroups and disease stages, and venture into the future with an emphasis on dynamic scores incorporating more genetic information to further individualize treatments and predict responses.

Established Scoring Systems

The first MDS classification system subdividing MDS into five categories was developed by the French-American-British (FAB) group based on morphologic changes, blast percentage in the blood and bone marrow, as well as peripheral blood monocyte count [15]. While the FAB classification is foremost a diagnostic classification, several studies have shown that it also has prognostic utility [16–18]. For example, refractory anemia with excess blast in transformation (RAEB-T) was behaving more like AML, while other MDS subforms were associated with a more indolent disease course [16, 17].

In 1997, the International MDS Risk Analysis Workshop derived the International Prognostic Scoring System (IPSS) that classifies MDS patients as low, intermediate-1, intermediate-2, and high risk based on a combination of cytogenetic features that define a specific cytogenetic subgroup, the number of peripheral blood cytopenias, and the percentage of bone marrow blasts, Table 7.1 [11]. Based on these features the authors showed a wide spectrum of 25%-AML progression risk and median overall survival (OS) that ranged from 9.4 years and 5.7 years in the low-risk patients to 0.2 years and 0.4 years in the high-risk patient population, respectively [11]. While the IPSS was developed as a risk stratification tool at the time of diagnosis and for untreated patients, it has subsequently been externally validated in various settings including allo-HCT and has been the standard tool for treatment decision-making and clinical trials in MDS for many years [19, 20]. However, despite its ease and widespread use IPSS has several limitations, for example, in patients with previously treated or secondary MDS, its emphasis on the prognostic impact of blast percentage over the cytogenetics, and the fact that it included only a limited number of cytogenetic abnormalities while not accounting for the depth of cytopenias and other prognostic markers such as patient age, performance status, and laboratory values (e.g., lactate dehydrogenase [LDH], ferritin) [19, 21–23]. These limitations have led to the development of various subsequent scoring systems that are compared in Table 7.1.

Based on the 2001 classification of MDS by the World Health Organization (WHO), the WHO classification-based Prognostic Scoring System (WPSS) has been derived that includes WHO subgroups, red blood cell (RBC) transfusion requirements, and the IPSS karyotype [24]. The WPSS has subsequently been refined to include the absolute hemoglobin level instead of transfusion dependency [25]. A major strength of the WPSS is that it is a dynamic tool with applicability at various time points during the disease course as well as its prognostic value in the post-allo-HCT setting [24, 26]. However, the many limitations hampering IPSS are also applicable to WPSS such as not accounting for the depth of cytopenias and the limited number of cytogenetic subgroups. Furthermore, many pathologists in the USA do not report the MDS WHO subtype in their reports.

The Global MD Anderson Risk Model Score for MDS (MDAPSS) has been developed using patient (Eastern Cooperative Oncology Group (ECOG) performance status $\geq$2, age $\geq$65 years) and disease factors (anemia, thrombocytopenia, increased bone marrow blast percentage, leukocytosis, chromosome 7 of complex cytogenetic abnormalities, prior transfusions) to derive a prediction rule that includes patients with secondary MDS and chronic myelomonocytic leukemia (CMML) who were not included in IPSS and WPSS [27].

Finally, the revised version of the IPSS was published in 2012 (IPSS-R) [28]. Based on 7012 MDS patients, prognostic variables were identified and the five most impactful covariates (cytogenetic risk group, bone marrow blast percentage, depth of cytopenias [hemoglobin, platelets, and absolute neutrophil count]) were included in the model. Conversely to the original IPSS, IPSS-R contains 5 prognostic cytogenetic risk groups with updated and more extensive cytogenetic aberrations, splits bone marrow blast percentage into 4 subcategories, and takes the depth of cytopenias into account, which had previously been shown to be a valuable prognostic

Table 7.1 Comparison of selected established MDS risk stratification tools

Score (Ref.)	Bone marrow blast %	Cytogenetics	Cytopenias	Other parameters	Prognostic relevance
IPSS [11]	0 points: <5% 0.5 points: 5–10% 1.5 points: 11–20% 2.0 points: 21–30%	0 points: good (normal, −Y, del(5q), del(20q)) 0.5 points: intermediate (other) 1.0 points: poor (complex (≥3 abnormalities)) or chromosome 7 anomalies	0 points: 0 or 1 cell lineages 0.5 points: 2/3 lineages	None	Low risk: median OS 5.7 years, 25% AML progression: 9.4 years Int-1: median OS 3.5 years, 25% AML progression: 3.3 years Int-2: median OS 1.2 years, 25% AML progression: 1.1 years High: median OS 0.4 years, 25% AML progression: 0.2 years
IPSS-R [28]	0 points: ≤2% 1 point: 2–5% 2 points: 5–10% 3 points: >10%	0 points: very good (del(11q), −Y) 1 point: good (normal, del(5q), del(12p), del(20q), double including del(5q)) 2 points: intermediate (del(7q), +8, +19, i(17q), any other single or double independent clones) 3 points: poor (−7, inv(3)/t(3q)/del(3q), double including −7/del(7q), complex: 3 abnormalities) 4 points: very poor (complex (>3 abnormalities))	Hemoglobin: 0 points: ≥10 1 point: 8–10 2 points: <8 Platelets: 0 points: ≥100 1 point: 50–100 2 points: <50 ANC: 0 points: ≥0.8 20.5 points: <0.8	None	Very good: median OS 5.4 years, 25% AML progression: not reached Good: median OS 4.8 years, 25% AML progression: 9.4 years Intermediate: median OS 2.7 years, 25% AML progression: 2.5 years Poor: median OS 1.5 years, 25% AML progression: 1.7 years Very poor: median OS 0.7 years, 25% AML progression: 0.7 years

MDAPSS [27]	1 point: 5–10% 2 points: 11–29%	3 points: complex (≥3 abnormalities) or chromosome 7 abnormalities	Hemoglobin: 2 points: <12 Platelets: 1 point: 50–199 2 points: 30–49 3 points: <30	ECOG performance status: 2 points Age: 1 point: 60–64 years 2 points: ≥65 years WBC >20 × 10^9/L: 2 points Prior transfusions: 1 point	Score 0–4: median OS 54 months Score 5–6: median OS 25 months Score 7–8: median OS 14 months Score ≥9: median OS 6 months
WPSS [24]	None	0 points: good (normal, −Y, del(5q), del(20q)) 1 point: intermediate (other) 2 points: poor (complex (≥3 abnormalities)) or chromosome 7 anomalies	None	WHO subgroups: 0 points: RA, RARS 1 point: RCMD, RCMD-RS 2 points: RAEB-1 3 points: RAEB-2 RBC transfusion dependency (1 pRBC every 8 weeks): 1 point	Median OS, 5-year AML progression Very low (0 points): 103 months, 6% Low (1 point): 72 months, 24% Intermediate (2 points): 40 months, 48% High (3–4 points): 21 months, 63% Very high (5–6 points): 12 months, 100%

factor [28]. The major advantages of the IPSS-R are the better stratification of patients with 27% of IPSS lower-risk patients being "upstaged" and 18% of higher-risk patients being "downstaged" in IPSS-R, respectively, which enables a more individualized therapeutic approach given the different rates of progression to AML. While other predictors of adverse survival such as patient age, serum LDH, ferritin, and β2-microglobulin were also validated in this large patient cohort, they did not have any prognostic impact on progression to AML and were therefore omitted from the final IPSS-R [28]. The IPSS-R has been externally validated and is increasingly used as the standard risk stratification tool for both research and routine clinical practice [4, 29–31]. However, especially in the intermediate risk group, the prognosis is variable and a further division into intermediate-favorable and intermediate-adverse risk based on patients age ≥66 years, peripheral blood blasts ≥2%, and history of RBC transfusions has been proposed [3, 32–34]. An IPSS-R score of 3.5 points has been suggested as a cutoff for differentiation between lower- and higher-risk MDS patients and has been demonstrated to retain prognostic power over time [33].

Scoring Systems Dedicated to Specific Patient Populations and Clinical Circumstances

However, several limitations affecting these scoring systems exist and individual aspects of these have been addressed by the development of dedicated scores. Table 7.2 provides an overview of selected risk stratification tools for designated patient subpopulations.

Therapy-Related and Secondary MDS

IPSS and IPSS-R were derived from patient populations with de novo MDS and excluded patients who had subsequent initiation of MDS-directed treatment [11, 28]. While these scores have subsequently been validated in patients treated with azacitidine (AZA) and lenalidomide [35–37], there are concerns about their predictive potential in patients with therapy-related or secondary MDS [38, 39]. This led to the development of a dedicated prognostic model for therapy-related MDS (t-MDS) which identified 7 prognostic factors (age ≥65 years, ECOG performance status ≥2, poor cytogenetics, WHO MDS subtype, hemoglobin <11 g/dl, platelets $<50 \times 10^9$ /dl, and transfusion dependence) as predictors for OS and leukemia-free survival [40]. This t-MDS Prognostic Scoring System (TPSS) score has been validated in only one retrospective study of 50 patients with t-MDS treated with AZA and identified patient subgroups with different OS and leukemia-free survival [41].

Table 7.2 Selected risk stratification tools applicable to specific patient subpopulations

Patient subpopulation	Parameters	Outcomes	Reference
Therapy-related MDS (281 patients)	1 point each for: Age ≥ 65 years, cytogenetics (−7 and/or complex), WHO classification (RARS, RAEB-1/2), hemoglobin (Hgb) <11 g/dl, platelets <50 mg/L, transfusion dependence, ECOG performance status (PS) ≥2	Good risk (0–2 risk factors): median OS: 26 months Intermediate risk (3–4 risk factors): median OS 13 months Poor risk (5–7 risk factors): median OS 7 months	[40]
Lower-risk MDS (856 patients with low or intermediate-1 risk)	Unfavorable cytogenetics (all but diploid and 5q): 1 point Age >60 years: 2 points Hgb <10 g/dl: 1 point Platelet <50 × 10^9 /L: 2 points Platelets 50–200 × 10^9 /L: 1 point Bone marrow blasts ≥4%: 1 point	Score 0–2 points: median OS 80 months, 65% 4-year OS Score 3–4 points: median OS 27 months, 33% 4-year OS Score ≥5 points: median OS 14 months, 7% 4-year OS	[65]
Higher-risk MDS treated with HMA (282 patients with intermediate-2 and high risk MDS)	1 point each for ECOG PS ≥ 2, presence of circulating blasts, and RBC transfusion dependence ≥4 RBC units/8 weeks, intermediate-risk cytogenetics 2 points for poor-risk cytogenetics	Low risk (0 points): median OS not reached Intermediate risk (1–3 points): median OS 15.0 months High risk (4–5 points): median OS 6.1 months	[52]
Higher-risk MDS at time of HMA failure (310 patients treated with AZA, 140 with DEC)	0.75 points: bone marrow blasts >20%, transfusion dependence 1 point: ECOG PS >1, very poor cytogenetics (>3 karyotype abnormalities), age 75–84 years, platelets <30 2 points: age > 84 years	Low risk (≤2.25 points): median OS 11.0 months (95% CI: 8.8–13.6 months) High risk (>2.25 points): median OS 4.5 months (95% CI: 3.9–5.3 months)	[62]
Hypocellular (<20% bone marrow cellularity) MDS (253 patients)	1 point each for hemoglobin <10 g/dl, ECOG PS ≥2, unfavorable cytogenetics, bone marrow blasts ≥5%, serum LDH >600 IU/l	0 points: median OS not reached, 2-/3-year OS 71/61% 1 point: median OS 27 months, 2-/3-year OS 59/38% 2 points: median OS 19.4 months, 2-/3-year OS 43/20% 3 points: median OS 9.3 months, 2-/3-year OS 14/7% 4 points: median OS 4.7 months, 2-/3-year OS 12/6% 5 points: median OS 2 months, 2-/3-year OS: 0%	[73]

However, the TPSS is rarely used in clinical practice. In a large cohort of 370 patients with t-MDS, several well-established models (WPSS, IPSS, IPSS-R, MDAPSS) were shown to retain their diagnostic utility in this setting [42]. In this study, Zeidan et al. also showed that overall prognosis of patients with t-MDS was worse compared to de novo MDS (19 months vs 46 months; $p = 0.005$) independent of the prior malignancy and treatment strategy [42]. However, these results suggest that factors in t-MDS patients not captured by standard risk stratification tools such as comorbidities or specific genetic alterations have important prognostic implications [42, 43]. Given the heterogeneity of t-MDS disease courses, appropriate risk stratification in t-MDS is important [44, 45]. While patients with t-MDS and higher risk by IPSS-R tend to have adverse survival compared to de novo MDS, OS in patients with lower-risk disease was comparable for t-MDS and de novo MDS which emphasizes that risk stratification tools should also be applied in this setting and standard tools such as IPSS-R are valid options [42]. However, further challenges remain such as the impact of specific mutations or the presence of preexisting clonal hematopoiesis of indeterminate potential (CHIP), and the lack of a unifying definition of t-MDS needs to be addressed to develop better prognostic scores for t-MDS patients [43, 46].

Prediction Rules for the Response to Hypomethylating Agents and for Prognostication in HMA-Refractory Patients

The HMAs AZA and decitabine (DEC) are both approved in the USA for the treatment of MDS. The overall response rate (ORR) ranges between 40% and 50% and studies have shown a significant reduction of the risk of progression to AML and substantial mortality benefit for AZA in patients with higher-risk MDS in comparison with conventional care options [47–51]. However, none of the general risk stratification tools had been developed to predict the response to a specific treatment.

The Groupe Francophone des Myélodysplasies (GFM) studied factors associated with survival and response to AZA [52]. In a multivariate analysis, ECOG performance status ≥2, intermediate- and poor-risk cytogenetics by IPSS, presence of circulating blasts, and RBC transfusion dependency ≥4 units/8 weeks were predictive of an adverse OS and were used to construct a prognostic score that successfully discriminated the intermediate-2 and high-risk MDS patients into 3 groups with statistically significant differences in OS (median OS not reached, 15.0 and 6.1 months, respectively [$P < 10^{-4}$]) [52]. Furthermore, an abnormal karyotype, bone marrow blast percentage >15%, and prior treatment with low-dose cytarabine were associated with a lower response rate to AZA [52]. These covariates have been validated in subsequent studies by the same group and others [53, 54]. The addition of platelet doubling time, an independent marker of response to AZA, did not improve the predictive value of the IPSS and French Prognostic Scoring System (FPSS) [55, 56].

However, in an external validation study, the FPSS failed to provide a superior OS prediction compared to IPSS-R [57]. Zeidan et al. subsequently compared various risk stratification tools as outcome predictors for higher-risk MDS patients treated with HMA and showed that all 5 studied scoring systems performed well in predicting OS [58]. However, none of those scores was able to predict the objective responses to HMA treatment and the FPSS did not outperform the other predictive models [58].

It is important to note that responses to HMA are often transient and the prognosis after HMA failure is dismal with a median OS of 14–17 months and 4–5.6 months for lower-risk and higher-risk MDS patients based on IPSS/IPSS-R, respectively [59–61]. Although IPSS and IPSS-R have been derived from a population of de novo MDS patients and their applicability to the HMA-refractory setting is questionable, they are frequently used as the basis for clinical trial enrollment [38]. This is exemplified by the fact that up to 77% of lower-risk MDS patients treated with HMAs remain in the lower-risk MDS strata by IPSS, which would predict a median OS of 5.7 years while the observed median OS in lower-risk, HMA-refractory patients is only 14–17 months [11, 59]. Acknowledging this discrepancy, a dedicated risk stratification tool for HMA failure patients has been developed that includes 6 covariants: patient age, ECOG performance status >1, complex cytogenetics (>3 abnormalities), bone marrow blast percentage >20%, platelet count <30, and RBC transfusion dependency [62]. This score dichotomizes patients into low-risk and high-risk categories with a median OS of 11.0 months and 4.5 months, respectively, which is helpful for appropriate counseling of patients and treatment decision-making. It has been validated in two separate cohorts and may be used as a stratification tool for clinical trial eligibility after HMA-failure [62–64].

Scores in Patients with Lower-Risk MDS

The prognosis of patients within one IPSS risk category can vary substantially which is of clinical relevance as patients with lower-risk IPSS but otherwise poor prognostic features may benefit from a more aggressive upfront treatment [65–67]. For example, there are IPSS low-risk patients with poor prognostic features such as advanced age and low hemoglobin and platelet levels requiring supportive transfusions that are not sufficiently reflected by IPSS alone. As discussed above, this discrepancy is reflected by the difference in predicted and real-world OS for *low*-risk IPSS and IPSS-R patients who had been treated with HMAs with a predicted median OS of several years but a real-world median OS of 4.3–5.6 months in higher-risk and 15 months in lower-risk patients, respectively [59, 60, 62].

While the IPSS and other risk tools estimate risk of progression to AML, it has become apparent that most patients with lower-risk MDS die rather with MDS or from bone marrow failure or therapy complications rather than from progression to AML [65, 68]. The realization that a substantial minority of patients with

LR-MDS die within 2 years of diagnosis led to the derivation of a prognostic score for low and intermediate-1 risk MDS that stratifies patients based on cytogenetic features, age, bone marrow blast percentage, and peripheral blood hemoglobin and platelet levels [65, 68]. Although elevated ferritin and β2-microglobulin levels were associated with an adverse prognosis, they were not included in this score [65]. However, especially in the IPSS-R intermediate-risk group, the addition of additional parameters such as LDH, elevated ferritin, age, and poor performance status might be helpful to better risk stratify these patients and aid in clinical decision-making.

Additionally, IPSS and IPSS-R are validated to predict the risk of disease at the time of diagnosis and are not dynamic. As shown in a large study from the MDS International Working Group for Prognosis database including 7212 MDS patients, hazards for OS and transformation to AML become similar among various IPSS-R subgroups after about 5 years [33]. The authors showed that lower-risk patients remained at lower risk while the risk for patients in the higher-risk strata at the time of diagnosis was decreasing over time. This loss of prognostic power over time might be due to the selective loss of higher-risk patients to MDS-related death, the larger effect of comorbidities in lower-risk MDS patients, and the exclusion of patients who received MDS-directed treatment, which was more likely in the higher-risk group [33]. This suggests that the time since diagnosis should be taken into consideration and that for patients with an initial high risk who remain stable without treatment re-classification as lower-risk can be considered.

Hypocellular MDS

While MDS mostly presents with a normo- or hypercellular bone marrow, 10–20% of patients have a hypocellular bone marrow which has been shown to have different cytogenetic features and prognostic implications compared to normo- or hypercellular MDS [69–72]. Although hypocellular MDS is not considered a separate subentity of MDS, studies have identified a hypocellular bone marrow as an independent positive prognostic factor and showed that IPSS was insufficient to discriminate hypocellular MDS into prognostic subgroups [71, 73]. In order to improve risk stratification for this patient population, Tong et al. developed a dedicated score using a cohort of 253 patients at MD Anderson Cancer Center with hypocellular MDS that included ECOG ≥2, hemoglobin <10 g/dl, unfavorable cytogenetics (−7/7q or complex), ≥5% bone marrow blasts, and high serum LDH (>600 IU/l) as unfavorable prognostic factors [73].

However, this score lacks external validation and subsequent studies showed that IPSS and IPSS-R were sufficient for risk stratification in hypocellular MDS [70].

A New Era: Risk Stratification Tools Including Somatic Mutations

Thanks to the increasing availability of new diagnostic techniques such as next-generation sequencing (NGS) various studies have shown that up to 90% of MDS patients harbor recurrent somatic mutations in at least one gene [12, 74–76]. However, the role of genetic testing in diagnosis, treatment, and prognostication of MDS remains controversial [12, 38, 77].

In 2011, Bejar et al. analyzed 18 genes in over 400 MDS patients by Sanger sequencing and showed that mutations in *TP53, ETV6, ASXL1, EZH2, and RUNX1* were adverse prognostic markers independent of IPSS, and upshifted the risk to the next higher IPSS category [76]. These general findings have been confirmed in several studies but nuances exist that necessitate a highly individualized approach to genetic testing [75, 78, 79]. For example, while mutations in *U2AF1*, *SRSF2*, *SF3B1*, and *ASXL1* have been identified as adverse prognostic features, they lost their independent prognostic value in patients with >5% bone marrow blasts [79].

Given the prognostic relevance of somatic mutations in MDS, various risk stratification tools that incorporate genetic testing have been proposed (Table 7.3).

Nazha et al. studied 508 MDS patients treated at Cleveland Clinic and derived a predictive model that included patient age, IPSS-R score, and somatic mutations in *EZH2, SF3B1,* and *TP53* [80]. By using paired samples, the authors showed that their model retained its prognostic strength throughout the disease course and was applicable to both primary and secondary MDS [80]. Such a dynamic model that is not impacted by initial or subsequent treatment is important as the clonal architecture of the disease changes and a significant proportion of patients acquire additional mutations along the disease course [80]. In a subsequent study, the same group showed that the addition of the binary assessment (i.e., mutation present or absent) of *EZH2, SF3B1*, and *TP53* increased the predictive value of various established scores (IPSS, IPSS-R, WPSS but not MDAPSS) for median OS (not for progression to AML) and led to the upstaging of 53% and 58% of patients with intermediate-1 to intermediate-2 by IPSS and from intermediate to high-risk by IPSS-R, respectively [81]. This reclassification of patients has potential implications on the therapeutic approach but needs additional external validation.

By means of a 104 gene panel in 944 patients with various MDS subtypes, Haferlach et al. identified 25 genes that were significantly associated with survival in univariate analyses [75]. Using a Cox regression in a proportional hazards model, the authors developed a score that included mutations in 14 genes, age, gender, and IPSS-R categories to stratify patients into four risk groups: low, intermediate, high, and very high risk with 3-year survival rates of 95.2%, 69.3%, 32.8%, and 5.3%, respectively [75]. While this combined model of genetic and clinical parameters outperformed IPSS-R, a gene-only model was inferior to the combined model [75].

Finally, Xu et al. identified mutations in *TP53, STAG2, DNMT3A, EZH2, RUNX1, ROBO1/2, SRSF2*, *and WT1* as being associated with adverse prognosis and higher

Table 7.3 Selected scores incorporating mutational testing

Patient population	Parameters	Outcomes	Reference
944 MDS patients across all WHO subtypes and IPSS/IPSS-R risk groups	Combined model: mutational analysis of 14 genes (*ASXL1, CBL, ETV6, EZH2, KRAS, LAMB4, NCOR2, NF1, NPM1, NRAS, PRPF8, RUNX1, TET2, TP53*), age, male gender, hemoglobin (8–10 g/dl, <8 g/dl), platelets (50–100 × 10^9 /L, <50 × 10^9 /L), blasts (2–5%, 5–10%, >10%)	Combined model: 3-year OS of 95.2%, 69.3%, 32.8%, and 5.3% in low-, intermediate-, high-, and very-high-risk groups, respectively Combined model superior to IPSS-R and genetic model alone	[75]
508 MDS patients (all IPSS-R risk categories, 15% patients with chronic myelomonocytic leukemia)	Age, IPSS-R score, mutations in *EZH2 SF3B1* (improved survival), *TP53*	Low risk: median OS 37.4 months Intermediate-1 risk: median 23.2 months Intermediate-2 risk: median 19.9 months High risk: median OS 12.2 months	[80]
320 MDS patients	Presence of mutation in any of 28 tested genes (mutation in *TP53, STAG2, DNMT3A, EZH2, RUNX1, ROBO1/2, SRSF2,* and *WT1* associated with poor prognosis), IPSS/IPSS-R	Low: 0–1 mutations (except for *DNMT3A, TP53, WT1, SRSF2, IDH1/2, STAG2,* and *ROBO1/2);* Intermediate: 1 driving mutation (*DNMT3A, TP53, WT1, SRSF2, IDH1/2, STAG2,* and *ROBO1/2*) or 2 other mutations High: ≥2 poor prognosis mutations or ≥3 other mutations Addition of mutation analysis increased prognostic value of IPSS and IPSS-R	[82]

rates of progression to AML in 324 Chinese MDS patients [82]. Unlike other scores, this model subdivided patients into 4 genetic risk groups: low (no mutations), intermediate-1 (≥1 mutation in any gene except those previously specified genes associated with adverse prognosis), intermediate-2 (2–3 mutations in any genes other than those associated with worse outcomes or 1 mutation in genes associated with worse outcomes with 0–2 mutations in any other MDS-related genes), and high (presence of at least 2 mutations in genes associated with worse outcomes) [82]. Integration of genetic information into IPSS and IPSS-R led to an increased predictive value of both scores for OS and AML transformation [82].

Although further validation of these combined genetic-clinical risk stratification tools is necessary prior to routine clinical use, they seem to have higher predictive value for OS than previous scores. However, as of now, genetic scores should be regarded as a complementary tool rather than a replacement for established scores but may provide important patient-level information that allows individualization of treatment decisions.

Future Directions and Conclusion

Although significant progress has been made since the introduction of IPSS, several challenges remain especially at the dawn of a new era in which a more detailed understanding of the disease pathobiology, the increasing use of genetic testing, and the availability of novel targeted therapeutic options are paving the way to a personalized treatment concept for the individual MDS patient.

Especially in the realm of genetic testing, further studies are needed to elucidate the prognostic relevance of parameters such as the size of the clonal population, the interaction of various different genetic alterations, and whether these mutations have the same or variable impact on patients within different conventionally determined risk groups. Machine learning models are a promising tool to develop personalized geno-clinical models that allow individualized treatment concepts and prognostication. These models randomly include clinical and molecular variables and appear to better account for the complex interactions between those than classic stepwise Cox regression models that had been used to derive conventional risk stratification tools. Nazha et al. recently presented such a model derived from MDS patients that outperformed other models in predicting OS and AML progression and similar models have been used for AML patients and to distinguish patients with MDS and chronic myelomonocytic leukemia [83–85]. However, these models need additional validation in larger cohorts before being used in clinical practice. Furthermore, the risk of MDS patients is dynamic over time as a significant proportion of patients acquires new mutations during the disease course and patients often receive multiple sequential lines of therapy [80]. Tools such as IPSS-R lose their prognostic power over time [33], emphasizing the need for better, dynamic tools in clinical decision-making. The machine-learning model developed by Nazha et al. has been derived from treated MDS patients and seems to be applicable to different time points during the disease course [85].

Despite the great potential of genetic testing, the risk stratification of MDS patients by conventional clinical-pathologic tools such as IPSS-R remains the mainstay for prognostication and clinical decision-making in routine clinical practice. However, dynamic tools that combine genetic and clinical parameters have been developed and appear to provide a better risk prediction of the individual patient, which is essential for appropriate patient counseling and holds promise for clinical trial enrollment and potentially treatment selection.

Declaration of Conflicts of Interest AMZ received research funding (institutional) from Celgene, Abbvie, Astex, Pfizer, Medimmune/AstraZeneca, Boehringer-Ingelheim, Trovagene, Incyte, Takeda, Novartis, Aprea, and ADC Therapeutics. AMZ had a consultancy with and received honoraria from AbbVie, Otsuka, Pfizer, Celgene, Jazz, Incyte, Agios, Boehringer-Ingelheim, Novartis, Acceleron, Astellas, Daiichi Sankyo, Cardinal Health, Taiho, Seattle Genetics, BeyondSpring, Trovagene, Takeda, Ionis, and Epizyme. AMZ received travel support for meetings from Pfizer, Novartis, and Trovagene. None of these relationships were related to the development of this manuscript. JPB has no conflicts of interest to declare.

Acknowledgments AMZ is a Leukemia and Lymphoma Society Scholar in Clinical Research and is also supported by a NCI's Cancer Clinical Investigator Team Leadership Award (CCITLA).

References

1. Steensma DP. Myelodysplastic syndromes current treatment algorithm 2018. Blood Cancer J. 2018;8(5):47.
2. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
3. Zeidan AM, Shallis RM, Wang R, Davidoff A, Ma X. Epidemiology of myelodysplastic syndromes: why characterizing the beast is a prerequisite to taming it. Blood Rev. 2019;34:1–15.
4. Network NCC. NCCN guidelines version 2.2019: myelodysplastic syndromes 2019 [cited 20 Dec 2018]. Available from: https://www.nccn.org/store/login/login.aspx?ReturnURL=https%3a%2f%2fwww.nccn.org%2fprofessionals%2fphysician_gls%2fPDF%2fmds.pdf.
5. Stahl M, Bewersdorf JP, Giri S, Wang R, Zeidan AM. Use of immunosuppressive therapy for management of myelodysplastic syndromes: a systematic review and meta-analysis. Haematologica. 2019;105:102.
6. Bewersdorf JP, Zeidan AM. Transforming growth factor (TGF)-beta pathway as a therapeutic target in lower risk myelodysplastic syndromes. Leukemia. 2019;33:1303.
7. Platzbecker U. Treatment of MDS. Blood. 2019;133:1096. https://doi.org/10.1182/blood-2018-10-844696.
8. Santini V. Treatment of low-risk myelodysplastic syndromes. Hematology Am Soc Hematol Educ Program. 2016;2016(1):462–9.
9. Santini V. How I treat MDS after hypomethylating agent failure. Blood. 2019;133(6):521–9.
10. Faltas B, Zeidan A, Gergis U. Myelodysplastic syndromes: toward a risk-adapted treatment approach. Expert Rev Hematol. 2013;6:611.
11. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89(6):2079–88.
12. Chokr N, Pine AB, Bewersdorf JP, Shallis RM, Stahl M, Zeidan AM. Getting personal with myelodysplastic syndromes: is now the right time? Expert Rev Hematol. 2019;12(4):215–24.
13. Lee EJ, Podoltsev N, Gore SD, Zeidan AM. The evolving field of prognostication and risk stratification in MDS: recent developments and future directions. Blood Rev. 2016;30(1):1–10.
14. Zeidan AM, Gore SD, Padron E, Komrokji RS. Current state of prognostication and risk stratification in myelodysplastic syndromes. Curr Opin Hematol. 2015;22(2):146–54.

15. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol. 1982;51(2):189–99.
16. Varela BL, Chuang C, Woll JE, Bennett JM. Modifications in the classification of primary myelodysplastic syndromes: the addition of a scoring system. Hematol Oncol. 1985;3(1):55–63.
17. Rubio-Felix D, Giraldo P, Franco E, Gimeno J, Giralt M. Prognostic factors in myelodysplastic syndromes: analysis of five scoring systems. Hematol Oncol. 1995;13(3):139–52.
18. Zeidan AM, Komrokji RS. There's risk, and then there's risk: the latest clinical prognostic risk stratification models in myelodysplastic syndromes. Curr Hematol Malig Rep. 2013;8(4):351–60.
19. Jonas BA, Greenberg PL. MDS prognostic scoring systems – past, present, and future. Best Pract Res Clin Haematol. 2015;28(1):3–13.
20. Cutler CS, Lee SJ, Greenberg P, Deeg HJ, Perez WS, Anasetti C, et al. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. Blood. 2004;104(2):579–85.
21. Della Porta MG, Malcovati L, Strupp C, Ambaglio I, Kuendgen A, Zipperer E, et al. Risk stratification based on both disease status and extra-hematologic comorbidities in patients with myelodysplastic syndrome. Haematologica. 2011;96(3):441–9.
22. Schanz J, Tüchler H, Solé F, Mallo M, Luño E, Cervera J, et al. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. J Clin Oncol Off J Am Soc Clin Oncol. 2012;30(8):820–9.
23. Zeidan AM, Smith BD, Komrokji RS, Gore SD. Prognostication in myelodysplastic syndromes: beyond the International Prognostic Scoring System (IPSS). Am J Med. 2013;126(4):e25.
24. Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R, et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol. 2007;25(23):3503–10.
25. Malcovati L, Della Porta MG, Strupp C, Ambaglio I, Kuendgen A, Nachtkamp K, et al. Impact of the degree of anemia on the outcome of patients with myelodysplastic syndrome and its integration into the WHO classification-based Prognostic Scoring System (WPSS). Haematologica. 2011;96(10):1433–40.
26. Alessandrino EP, Della Porta MG, Bacigalupo A, Van Lint MT, Falda M, Onida F, et al. WHO classification and WPSS predict posttransplantation outcome in patients with myelodysplastic syndrome: a study from the Gruppo Italiano Trapianto di Midollo Osseo (GITMO). Blood. 2008;112(3):895–902.
27. Kantarjian H, O'Brien S, Ravandi F, Cortes J, Shan J, Bennett JM, et al. Proposal for a new risk model in myelodysplastic syndrome that accounts for events not considered in the original International Prognostic Scoring System. Cancer. 2008;113(6):1351–61.
28. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Sole F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454–65.
29. Della Porta MG, Alessandrino EP, Bacigalupo A, van Lint MT, Malcovati L, Pascutto C, et al. Predictive factors for the outcome of allogeneic transplantation in patients with MDS stratified according to the revised IPSS-R. Blood. 2014;123(15):2333–42.
30. Voso MT, Fenu S, Latagliata R, Buccisano F, Piciocchi A, Aloe-Spiriti MA, et al. Revised International Prognostic Scoring System (IPSS) predicts survival and leukemic evolution of myelodysplastic syndromes significantly better than IPSS and WHO Prognostic Scoring System: validation by the Gruppo Romano Mielodisplasie Italian Regional Database. J Clin Oncol. 2013;31(21):2671–7.
31. Neukirchen J, Lauseker M, Blum S, Giagounidis A, Lubbert M, Martino S, et al. Validation of the revised international prognostic scoring system (IPSS-R) in patients with myelodysplastic syndrome: a multicenter study. Leuk Res. 2014;38(1):57–64.

32. Benton CB, Khan M, Sallman D, Nazha A, Nogueras Gonzalez GM, Piao J, et al. Prognosis of patients with intermediate risk IPSS-R myelodysplastic syndrome indicates variable outcomes and need for models beyond IPSS-R. Am J Hematol. 2018;93(10):1245–53.
33. Pfeilstöcker M, Tuechler H, Sanz G, Schanz J, Garcia-Manero G, Solé F, et al. Time-dependent changes in mortality and transformation risk in MDS. Blood. 2016;128(7):902–10.
34. Bejar R. Clinical and genetic predictors of prognosis in myelodysplastic syndromes. Haematologica. 2014;99(6):956–64.
35. Mishra A, Corrales-Yepez M, Ali NA, Kharfan-Dabaja M, Padron E, Zhang L, et al. Validation of the revised International Prognostic Scoring System in treated patients with myelodysplastic syndromes. Am J Hematol. 2013;88(7):566–70.
36. Lamarque M, Raynaud S, Itzykson R, Thepot S, Quesnel B, Dreyfus F, et al. The revised IPSS is a powerful tool to evaluate the outcome of MDS patients treated with azacitidine: the GFM experience. Blood. 2012;120(25):5084–5.
37. Sekeres MA, Swern AS, Fenaux P, Greenberg PL, Sanz GF, Bennett JM, et al. Validation of the IPSS-R in lenalidomide-treated, lower-risk myelodysplastic syndrome patients with del(5q). Blood Cancer J. 2014;4(8):e242-e.
38. Nazha A. The MDS genomics-prognosis symbiosis. Hematology Am Soc Hematol Educ Program. 2018;2018(1):270–6.
39. Nazha A, Seastone DP, Keng M, Hobson S, Kalaycio M, Maciejewski JP, et al. The Revised International Prognostic Scoring System (IPSS-R) is not predictive of survival in patients with secondary myelodysplastic syndromes. Leuk Lymphoma. 2015;56(12):3437–9.
40. Quintas-Cardama A, Daver N, Kim H, Dinardo C, Jabbour E, Kadia T, et al. A prognostic model of therapy-related myelodysplastic syndrome for predicting survival and transformation to acute myeloid leukemia. Clin Lymphoma Myeloma Leuk. 2014;14(5):401–10.
41. Breccia M, Fianchi L, Lunghi M, Gaidano G, Levis A, Finelli C, et al. Newly proposed therapy-related myelodysplastic syndrome prognostic score predicts significant differences in overall survival and leukemia-free survival in patients treated with azacitidine. Leuk Lymphoma. 2013;54(8):1786–7.
42. Zeidan AM, Al Ali N, Barnard J, Padron E, Lancet JE, Sekeres MA, et al. Comparison of clinical outcomes and prognostic utility of risk stratification tools in patients with therapy-related vs de novo myelodysplastic syndromes: a report on behalf of the MDS Clinical Research Consortium. Leukemia. 2017;31(6):1391–7.
43. Zeidan AM, Sekeres MA, Barnard J, Steensma DP, Komrokji R. Therapy-related myelodysplastic syndromes-specific risk stratification: are we putting the cart before the horse? Leukemia. 2017;31(11):2539–41.
44. Abou Zahr A, Bernabe Ramirez C, Wozney J, Prebet T, Zeidan AM. New insights into the pathogenesis of MDS and the rational therapeutic opportunities. Expert Rev Hematol. 2016;9(4):377–88.
45. Abou Zahr A, Kavi AM, Mukherjee S, Zeidan AM. Therapy-related myelodysplastic syndromes, or are they? Blood Rev. 2017;31(3):119–28.
46. Calvo X, Florensa L, Arenillas L. Is there a pressing need for improving prognostication strategies in therapy-related myelodysplastic syndromes? Leukemia. 2017;31:2538.
47. Voso MT, Leone G, Piciocchi A, Fianchi L, Santarone S, Candoni A, et al. Feasibility of allogeneic stem-cell transplantation after azacitidine bridge in higher-risk myelodysplastic syndromes and low blast count acute myeloid leukemia: results of the BMT-AZA prospective study. Ann Oncol. 2017;28(7):1547–53.
48. de Witte T, Bowen D, Robin M, Malcovati L, Niederwieser D, Yakoub-Agha I, et al. Allogeneic hematopoietic stem cell transplantation for MDS and CMML: recommendations from an international expert panel. Blood. 2017;129(13):1753–62.
49. Kantarjian H, Beran M, Cortes J, O'Brien S, Giles F, Pierce S, et al. Long-term follow-up results of the combination of topotecan and cytarabine and other intensive chemotherapy regimens in myelodysplastic syndrome. Cancer. 2006;106(5):1099–109.

50. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009;10(3):223–32.
51. Lubbert M, Suciu S, Baila L, Ruter BH, Platzbecker U, Giagounidis A, et al. Low-dose decitabine versus best supportive care in elderly patients with intermediate- or high-risk myelodysplastic syndrome (MDS) ineligible for intensive chemotherapy: final results of the randomized phase III study of the European Organisation for Research and Treatment of Cancer Leukemia Group and the German MDS Study Group. J Clin Oncol. 2011;29(15):1987–96.
52. Itzykson R, Thepot S, Quesnel B, Dreyfus F, Beyne-Rauzy O, Turlure P, et al. Prognostic factors for response and overall survival in 282 patients with higher-risk myelodysplastic syndromes treated with azacitidine. Blood. 2011;117(2):403–11.
53. Itzykson R, Thépot S, Quesnel B, Dreyfus F, Recher C, Wattel E, et al. Long-term outcome of higher-risk MDS patients treated with azacitidine: an update of the GFM compassionate program cohort. Blood. 2012;119(25):6172–3.
54. Breccia M, Loglisci G, Cannella L, Finsinger P, Mancini M, Serrao A, et al. Application of French prognostic score to patients with International Prognostic Scoring System intermediate-2 or high risk myelodysplastic syndromes treated with 5-azacitidine is able to predict overall survival and rate of response. Leuk Lymphoma. 2012;53(5):985–6.
55. van der Helm LH, Alhan C, Wijermans PW, van Marwijk Kooy M, Schaafsma R, Biemond BJ, et al. Platelet doubling after the first azacitidine cycle is a promising predictor for response in myelodysplastic syndromes (MDS), chronic myelomonocytic leukaemia (CMML) and acute myeloid leukaemia (AML) patients in the Dutch azacitidine compassionate named patient programme. Br J Haematol. 2011;155(5):599–606.
56. Zeidan AM, Lee J-W, Prebet T, Greenberg P, Sun Z, Juckett M, et al. Platelet count doubling after the first cycle of azacitidine therapy predicts eventual response and survival in patients with myelodysplastic syndromes and oligoblastic acute myeloid leukaemia but does not add to prognostic utility of the revised IPSS. Br J Haematol. 2014;167(1):62–8.
57. Zeidan AM, Lee JW, Prebet T, Greenberg P, Sun Z, Juckett M, et al. Comparison of the prognostic utility of the revised International Prognostic Scoring System and the French Prognostic Scoring System in azacitidine-treated patients with myelodysplastic syndromes. Br J Haematol. 2014;166(3):352–9.
58. Zeidan AM, Sekeres MA, Garcia-Manero G, Steensma DP, Zell K, Barnard J, et al. Comparison of risk stratification tools in predicting outcomes of patients with higher-risk myelodysplastic syndromes treated with azanucleosides. Leukemia. 2016;30(3):649–57.
59. Jabbour EJ, Garcia-Manero G, Strati P, Mishra A, Al Ali NH, Padron E, et al. Outcome of patients with low-risk and intermediate-1-risk myelodysplastic syndrome after hypomethylating agent failure: a report on behalf of the MDS Clinical Research Consortium. Cancer. 2015;121(6):876–82.
60. Prebet T, Gore SD, Esterni B, Gardin C, Itzykson R, Thepot S, et al. Outcome of high-risk myelodysplastic syndrome after azacitidine treatment failure. J Clin Oncol. 2011;29(24):3322–7.
61. Jabbour E, Garcia-Manero G, Batty N, Shan J, O'Brien S, Cortes J, et al. Outcome of patients with myelodysplastic syndrome after failure of decitabine therapy. Cancer. 2010;116(16):3830–4.
62. Nazha A, Komrokji RS, Garcia-Manero G, Barnard J, Roboz GJ, Steensma DP, et al. The efficacy of current prognostic models in predicting outcome of patients with myelodysplastic syndromes at the time of hypomethylating agent failure. Haematologica. 2016;101(6):e224–e7.
63. Prebet T, Fenaux P, Vey N, Groupe Francophone des Myelodysplasies. Predicting outcome of patients with myelodysplastic syndromes after failure of azacitidine: validation of the North American MDS consortium scoring system. Haematologica. 2016;101(10):e427–e8.
64. Nazha A, Sekeres MA, Komrokji R, Steensma DP, Kantarjian H, Roboz G, et al. Validation of a post-hypomethylating agent failure prognostic model in myelodysplastic syndromes patients

treated in a randomized controlled phase III trial of rigosertib vs. best supportive care. Blood Cancer J. 2017;7(12):644.
65. Garcia-Manero G, Shan J, Faderl S, Cortes J, Ravandi F, Borthakur G, et al. A prognostic score for patients with lower risk myelodysplastic syndrome. Leukemia. 2007;22:538.
66. Belli C, Acevedo S, Bengio R, Arrossagaray G, Watman N, Rossi N, et al. Detection of risk groups in myelodysplastic syndromes. A multicenter study. Haematologica. 2002;87(1):9–16.
67. Al-Issa K, Madanat YF, Mukherjee S, Hirsch CM, Gerds AT, Abuhadra N, et al. Model heterogeneity in predicting outcomes of patients with Myelodysplastic syndromes (MDS). Blood. 2017;130(Suppl 1):2972.
68. Zeidan AM, Sekeres MA, Wang XF, Al Ali N, Garcia-Manero G, Steensma DP, et al. Comparing the prognostic value of risk stratifying models for patients with lower-risk myelodysplastic syndromes: is one model better? Am J Hematol. 2015;90(11):1036–40.
69. Huang TC, Ko BS, Tang JL, Hsu C, Chen CY, Tsay W, et al. Comparison of hypoplastic myelodysplastic syndrome (MDS) with normo−/hypercellular MDS by International Prognostic Scoring System, cytogenetic and genetic studies. Leukemia. 2008;22(3):544–50.
70. Schemenau J, Baldus S, Anlauf M, Reinecke P, Braunstein S, Blum S, et al. Cellularity, characteristics of hematopoietic parameters and prognosis in myelodysplastic syndromes. Eur J Haematol. 2015;95(3):181–9.
71. Yue G, Hao S, Fadare O, Baker S, Pozdnyakova O, Galili N, et al. Hypocellularity in myelodysplastic syndrome is an independent factor which predicts a favorable outcome. Leuk Res. 2008;32(4):553–8.
72. Yao CY, Hou HA, Lin TY, Lin CC, Chou WC, Tseng MH, et al. Distinct mutation profile and prognostic relevance in patients with hypoplastic myelodysplastic syndromes (h-MDS). Oncotarget. 2016;7(39):63177–88.
73. Tong W-G, Quintás-Cardama A, Kadia T, Borthakur G, Jabbour E, Ravandi F, et al. Predicting survival of patients with hypocellular myelodysplastic syndrome: development of a disease-specific prognostic score system. Cancer. 2012;118(18):4462–70.
74. Bejar R. Myelodysplastic syndromes diagnosis: what is the role of molecular testing? Curr Hematol Malig Rep. 2015;10(3):282–91.
75. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241–7.
76. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011;364(26):2496–506.
77. Nazha A, Sekeres MA, Gore SD, Zeidan AM. Molecular testing in myelodysplastic syndromes for the practicing oncologist: will the progress fulfill the promise? Oncologist. 2015;20(9):1069–76.
78. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122(22):3616–27; quiz 99.
79. Bejar R, Papaemmanuil E, Haferlach T, Garcia-Manero G, Maciejewski JP, Sekeres MA, et al. Somatic mutations in MDS patients are associated with clinical features and predict prognosis independent of the IPSS-R: analysis of combined datasets from the International Working Group for Prognosis in MDS-Molecular Committee. Blood. 2015;126(23):907.
80. Nazha A, Narkhede M, Radivoyevitch T, Seastone DJ, Patel BJ, Gerds AT, et al. Incorporation of molecular data into the Revised International Prognostic Scoring System in treated patients with myelodysplastic syndromes. Leukemia. 2016;30(11):2214–20.
81. Nazha A, Al-Issa K, Hamilton BK, Radivoyevitch T, Gerds AT, Mukherjee S, et al. Adding molecular data to prognostic models can improve predictive power in treated patients with myelodysplastic syndromes. Leukemia. 2017;31:2848.
82. Xu F, Wu LY, He Q, Wu D, Zhang Z, Song LX, et al. Exploration of the role of gene mutations in myelodysplastic syndromes through a sequencing design involving a small number of target genes. Sci Rep. 2017;7:43113.

83. Nazha A, Mukherjee S, Al-Issa K, Makhoul A, Adema V, Hirsch CM, et al. A personalized prediction model to risk stratify patients with acute myeloid leukemia (AML). Blood. 2017;130(Suppl 1):1408.
84. Hilton CB, Sekeres MA, Meggendorfer M, Walter W, Hutter S, Padron E, et al. Geno-clinical model to aid in the diagnosis of myelodysplastic syndrome (MDS) versus chronic myelomonocytic leukemia (CMML). Blood. 2018;132(Suppl 1):1813.
85. Nazha A, Komrokji RS, Barnard J, Al-Issa K, Padron E, Madanat YF, et al. A personalized prediction model to risk stratify patients with myelodysplastic syndromes (MDS). Blood. 2017;130(Suppl 1):160.

Part III
Treatment

Chapter 8
Treatment Algorithms for Lower-Risk Myelodysplastic Syndrome

Pierre Fenaux and Lionel Adès

Lower-risk myelodysplastic syndromes (MDS), as defined by the international prognostic scoring system (IPSS) or the revised IPSS (IPSS-R), have a low risk of progression to AML, and patients mainly suffer from cytopenias, principally anemia, whose treatment is generally the major aim [1].

Definition and Limits of Lower-Risk MDS

Since 1997, MDS patients are classified according to prognostic scores, i.e., the (classical) International Prognostic Scoring System (IPSS), recently revised (IPSS-R), both based on marrow blast percentage, number/extent of blood cytopenias, and marrow cell karyotype (Table 8.1). Those scores separate patients into 4 (IPSS) and 5 (IPSS-R) risk groups with different outcomes in terms of acute myeloid leukemia (AML) evolution and survival. "Lower-risk" MDS traditionally includes patients with low and intermediate 1 IPSS (and "higher-risk" MDS, patients with intermediate 2 and high IPSS) [14]. The IPSS-R, established on larger patient numbers, is more precise than the IPSS [15], reclassifying a significant proportion of "lower-risk" MDS into higher-risk, and vice versa. Therefore, although currently available drugs are often licensed based on the classical IPSS ("higher" versus "lower" IPSS), we feel an IPSS-R-based definition of lower risk is preferable: the term "lower-risk" MDS generally applies to cases with IPSS-R up to 3.5, including very low and low IPSS-R patients, and part of intermediate IPSS-R patients [15]. "Higher-risk" MDS include patients with IPSS-R $\geq$4.0, i.e., IPSS-R high and very high-risk, and the remaining intermediate IPSS-R patients. There is

P. Fenaux (✉) · L. Adès
Service D'hématologie Séniors, Hôpital St Louis, Assistance Publique – Hôpitaux de Paris (APHP) and Université de Paris, Paris, France
e-mail: pierre.fenaux@aphp.fr; lionel.ades@aphp.fr

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3_8

Table 8.1 Revised International Prognostic Scoring System (IPSS-R) for myelodysplastic syndromes [15]

Prognostic characteristic	Points						
	0	0.5	1	1.5	2	3	4
Cytogenetic risk category[a]	Very good		Good		Intermediate	Poor	Very poor
Blasts in bone marrow, %	≤2		>2–5%		5–10%	>10%	
Hemoglobin, g/dL	≥10		8 to <10	<8			
Platelet count, $\times 10^9$/L	≥100	50 to <100	<50				
Absolute neutrophil count, $\times 10^9$/L	≥0.8	<0.8					

IPSS-R risk group	Score	Median overall survival, years	Median time to 25% AML evolution, years
Very low	≤1.5	8.8	NR
Low	>1.5–3	5.3	9.4
Intermediate	>3–4.5	3.0	2.5
High	>4.5–6	1.6	1.7
Very high	>6	0.8	0.7

[a]Very good: –Y and del(11q) as single abnormalities; Good: Normal, del(5q), del(12p), and del(20q) as single abnormalities, double abnormalities including del(5q); Intermediate: del(7q), +8, +19, i(17q), and any other single abnormalities, any other double abnormalities; Poor: −7 and inv(3)/t(3q)/del(3q) as single abnormalities, double abnormalities including −7/del(7q), complex (3 abnormalities); Very poor: >3 abnormalities

some uncertainty for intermediate IPSS-R, where the treatment approach (either relatively intensive or less intensive) will particularly take into account additional factors:

New prognostic factors have indeed emerged in MDS, including: the presence of grade 2 or higher myelofibrosis associated with poorer survival [8]; immunophenotypic characteristics of blood and marrow cells in experienced laboratories [28]; and importantly the presence of somatic mutations, most of which worsen prognosis. New molecular technologies indeed identify somatic mutations in almost every MDS patient [7, 16, 37]. Those mutations, involving in particular genes encoding for splicing factors or epigenetic factors, are of help in the diagnosis of MDS in difficult cases (to confirm clonal disease), considering that some of those mutations are also present in healthy elderly individuals at low variant allele frequency [49]. In MDS, most mutations (especially *RUNX1*, *ASXL1*, *TP53*) have a negative impact on prognosis, while *TET2* appears to have a neutral effect and *SF3B1* is associated with a better outcome in patients without excess blasts [5, 33, 34, 37]. In addition, prognosis worsens with the number of mutations. We therefore assess somatic mutations by next-generation sequencing (NGS) (studying genes most frequently

mutated in myeloid malignancies), particularly *TP53* mutation in all lower-risk MDS with del 5q (who have *TP53* mutation in 20% of the cases), SF3B1 in patients with MDS and ringed sideroblasts (MDS-RS), as those two mutations are associated with poor and favorable prognosis, respectively [20, 31], and IDH1 and IDH2 mutations, due to the recent development of specific IDH1 and IDH2 inhibitors, although those mutations are seen mainly in higher-risk rather than LR-MDS [37, 50]. The presence of somatic mutation(s) may be of importance especially in younger patients with intermediate prognosis, suggesting more intensive surveillance and intensification of the treatment strategy including allogeneic hematopoietic cell transplantation (allo-HCT), although this approach is not yet supported by prospective studies.

Finally, comorbidities, frequent in elderly patients, can influence treatment choice, with intensive versus less intensive therapies [9].

Proposed Algorithms for the Treatment of Lower-Risk (LR) MDS

In lower-risk MDS, therapy generally mainly aims at improving cytopenia(s) especially anemia (which is generally the predominating cytopenia in LR –MDS), thereby improving quality of life [1]. For all patients, the therapeutic benefits of drugs must be balanced with their potential side effects.

Watchful Observation

In many lower-risk MDS patients with mild and asymptomatic cytopenias, no treatment may be required. Anemia is generally asymptomatic for Hb levels >10 g/dl, although the threshold below which anemia becomes symptomatic is variable, depending on age, comorbidities, and patient activities. Thrombocytopenia is rarely associated with bleeding for platelet counts >50 g/dl, while infections are rarely seen in lower-risk patients with absolute neutrophil count (ANC) >0.5 G/l. Regular monitoring of disease evolution is however required, especially if more than one cytopenia is present or in case of unfavorable somatic mutations (ASXL1, RUNX1, SRSF2, RAS, etc.) especially if several of them are present. This observational strategy in case of moderate cytopenias may change in the future if new and well-tolerated treatments that are capable of modifying the natural history of lower-risk MDS become available.

In addition, even moderate neutropenia or thrombocytopenia, in LR-MDS, may occasionally cause repeated infections and bleeding complications, respectively, due to neutrophil or platelet functional defects, and consequently may require treatment.

Treatment of Anemia

Anemia is generally the main symptom in lower-risk MDS, associated with fatigue and poor quality of life (QoL), higher risk of cardiovascular complications, higher risk of falls, and poor quality of life mainly in elderly patients [30]. One option is to treat anemia with repeated red blood cell (RBC) transfusions, but this leads to only transient improvement of anemia, low median average Hb levels, associated with poorer QoL, requires substantial hospital resources (hospital beds…), induces for patients a "dependence" to the hospital system, and leads to iron overload. For those reasons, drugs increasing the Hb level and avoiding RBC transfusions are preferred whenever possible. Erythropoietic-stimulating agents (ESAs) are generally the first-line drugs used for the anemia of LR-MDS.

Erythropoiesis-Stimulating Agents (ESAs)

Treatment with ESAs (i.e., recombinant erythropoietin [EPO] or darbepoetin [DAR]) as a single agent is considered the standard for first-line treatment in most LR-MDS patients with anemia, at least those without del 5q [38]. A prospective clinical trial of EPO alfa was recently completed in non-del 5q LR-MDS patients with anemia leading to its formal approval for LR-MDS with serum EPO <200 U/l in the European Union (EU), but not in most other countries [10]. A low (less than 200–500 U/l) endogenous EPO level and a transfusion requirement of less than 4 units every 8 weeks are predictive of a better response to ESA [18, 47]. Weekly doses of 30,000–60,000 units of EPO or 150–300 μg of DAR yield erythroid response rates above 50% (with concomitant improvement of QoL) when the baseline EPO level is low (about 80% of LR-MDS patients have EPO levels below 200 IU/L and only 10% of patients above 500 IU/L) and transfusion requirement absent or limited, with a dose-dependent effect (60,000 units/week of EPO or 300 μg/week of darbepoetin being superior to lower doses) [38]. Side effects of ESAs are limited if one avoids a too rapid increase in Hb level (by close monitoring of Hb level and dose reduction if required), which may be associated with increased thromboembolic risk [38]. Most responses to ESAs occur within 3 months of treatment onset, and the median duration of response is 18–24 months [38]. Predictive factors of good response to ESAs (in addition to baseline EPO level and RBC transfusion requirement) include lower-risk IPSS-R, presence of no more than 2 somatic mutations, and possibly the immunophenotypic analysis of myeloid cells [25, 47]; however, patients with ring sideroblasts (RS) appear to have a shorter duration of response to ESAs compared to non-RS patients [39]. In patients with no or loss of response to single-agent ESA, the addition of G-CSF can rescue about 20% of the cases [40].

ESAs are increasingly used before RBC transfusion dependence, when the Hb level is between 8 and 10 g/dl. The initial dose is often reduced in elderly patients due to frequent concomitant renal failure (for which high ESA dose may lead to a

rapid increase in Hb level). Weekly blood counts are necessary during the first weeks of treatment to adjust ESA dose, and then regular blood counts to maintain the Hb level between 11 and 12 g/dl.

Treatment of Anemia of LR-MDS with del 5q

ESAs provide lower erythroid response rates and shorter responses in LR-MDS with isolated del 5q than in non-del 5q patients [24]. In RBC-TD patients, lenalidomide gives red blood cell transfusion independence (RBC-TI) rates of about 75% (with a complete or partial cytogenetic response in 65% of the responders), with a median duration of 2.3 years [26]. It is approved as first-line treatment of LR-MDS with isolated del 5q in various countries including the USA, and only after ESA failure in the EU. Severe neutropenia and/or thrombocytopenia may occur during the first weeks of treatment, requiring close blood count monitoring and, in case of severe neutropenia (ANC <0.5 G/l), we recommend the addition of G-CSF [13].

TP53 mutation is present in 20% of the patients [20] and associated with lower hematological but more importantly lower cytogenetic response rates, shorter response to lenalidomide, and more progressions to HR-MDS or AML [32]. Therefore, *TP53* mutations should be regularly assessed in LR-MDS with del 5q. Patients presenting with or developing a *TP53* mutation during treatment with lenalidomide require intensified disease surveillance. Except perhaps for small or transient TP53-mutated clones [27], it is recommended to proceed, if possible, to allo-HCT especially when no response to lenalidomide is seen. In non-*TP53*-mutated patients, however, primary or secondary failure to lenalidomide, even in the absence of obvious disease progression, is also associated with relatively poor prognosis and requires more intensive treatment with hypomethylating agents (HMAs), followed if possible by allo-HCT [44].

Second-line Treatment of Anemia in Non-del 5q MDS

The erythroid response is not obtained with ESAs in all LR-MDS, and most responses are transient, so that second-line treatment of patients with anemia or RBC-TD is generally needed at some point.

Lenalidomide

In a phase 3 placebo-controlled study in non-del 5q patients with RBC-TD refractory to ESAs, 27% of lenalidomide-treated patients achieved RBC-TI ≥8 weeks and the median duration of response was 8.2 months [46]. In a GFM randomized clinical trial, the combination of lenalidomide and ESA significantly improved the erythroid response rate over lenalidomide alone, to about 45%, with a median response duration of 16 months in ESA-resistant non-del 5q LR-MDS patients [53].

Lenalidomide is however not registered in this indication in most countries, and its use is difficult to recommend outside of clinical trials or registries. In non-del 5q LR-MDS, neutropenia and thrombocytopenia induced by lenalidomide are generally milder than in del 5q patients, but still require close monitoring and the possible addition of G-CSF, if needed [53].

Immunosuppressive Agents

Anti-thymocyte globulin (ATG), with or without the addition of cyclosporine (CSA), yields response, including trilineage responses, in 16–67% of the patients [42], and in 48% of patients in the largest series published so far [48]. Various predictors of better response have been described, including MDS with single lineage dysplasia, absence of ring sideroblasts, a hypoplastic bone marrow, DR15 HLA type, age less than 60 years, female gender, normal karyotype or trisomy 8, presence of a PNH clone, and short duration of transfusion dependence, although they are often disputed [48]. Higher response rates are seen with horse compared with rabbit ATG [48]. ATG/CSA, using horse ATG, can probably be recommended in the relatively rare non-sideroblastic MDS patients aged less than 65–70 years without excess of marrow blasts, normal karyotype, with at least 2 cytopenias and/or hypoplastic MDS, after the failure of first-line therapy with ESAs.

Hypomethylating Agents (HMAs)

HMAs are approved in lower-risk MDS in several countries including the USA, but not in the EU. Response rates of 35–45% have been reported in several US studies, but somewhat lower response rates (20–30%) in two European studies. This difference in the results of these studies is possibly because they included mainly purely anemic patients, treated after ESA failure in the European studies [51, 52]. In LR-MDS, reduced HMA schedules are often used, especially with 5 day cycles of azacitidine [29], and even in one study 3 day cycles of decitabine [19]. HMAs may be particularly interesting as second-line treatment in anemic patients who also have neutropenia and/or thrombocytopenia, in whom lenalidomide may be difficult to use.

It is currently unknown if second-line treatments after ESA failure including Lenalidomide, HMAs, and ATG can improve survival over supportive care in LR-MDS [39].

Iron Chelation

As second-line treatments after ESA failure often have moderate response rates and yield transient responses, most LR-MDS patients eventually require regular RBC transfusions containing iron. This leads to the saturation of transferrin and the occurrence of non-transferrin-bound iron (NTBI) and labile plasma iron (LPI)

which cause oxidative damage in the liver, pancreas, and heart. Liver and cardiac MRI can adequately detect this iron overload. The risk of cardiac failure, in particular, becomes elevated when MRI cardiac T2* decreases below 20 ms [41].

Retrospective studies suggest that iron chelation, in case of iron overload in MDS, may improve survival [45]. A recent placebo-controlled study with iron chelation in RBC-TD LR-MDS also suggests an improvement in event-free survival in chelated patients, though several "events" were considered that included heart and liver damage as well as disease progression which possibly made the conclusion of the study difficult [3].

The most frequently used iron-chelating drug is deferasirox, especially with its new coated form. Most guidelines recommend a ferritin-guided chelating approach (in addition to liver and cardiac MRI monitoring) for the treatment of iron overload in MDS patients. The threshold of iron overload triggering the onset of chelation in RBC-TD LR-MDS is however disputed. In patients who receive allo-HCT, however, even moderate iron overload appears to lead to an increased risk of transplant-related mortality [2, 4]. Patients scheduled for allo-HSCT, or who could be candidates to allo-SCT in the future, may therefore require early chelation to maintain the serum ferritin level below 1000 ng/ml [21].

Allogeneic Hematopoietic S tem Cell Transplantation (Allo-HCT)

Allo-SCT is generally mostly indicated in HR-MDS, but some indications also appear to exist in lower risk MDS, although their benefit has not so far been demonstrated in prospective studies. Allo-SCT may be considered in lower-risk patients with poor prognostic features (including intermediate R-IPSS, several somatic mutations, *TP53* mutation in MDS with del 5q, clinically significant cytopenias without response to other treatments), weighing in all cases the advantages and risks of the procedure based on age, comorbidities, donor matching, and also patient opinion.

By contrast, patients with MDS-RS and isolated *SF3B1* mutation may be at very low risk of progression to higher-risk MDS and AML and, unless disease progression occurs, probably do not require HCT even if anemia does not respond to drug treatments. A prospective trial of the Groupe Francophone des Myélodysplasies (GFM) currently tests the role of allo-SCT in lower-risk MDS with additional risk factors (especially intermediate IPSS-R or severe cytopenias).

New Treatments for Anemia in Low-Risk MDS

In a phase 2 study in LR-MDS patients, luspatercept (ACE-536) [43], a specific activin receptor fusion protein acting as a ligand trap to neutralize negative regulators of late-stage erythropoiesis, yielded 63% erythroid responses, including 38% RBC transfusion independence. The presence of ring sideroblasts (RS) or *SF3B1* mutation was associated with better response (69% versus 43% in patients without RS). In a subsequent placebo-controlled randomized phase 3 study of luspatercept

in 229 RBC-TD MDS-RS or with *SF3B1* mutation, refractory to ESA, 37.8% of the patients treated with luspatercept achieved RBC-TI, with a median response duration of 30.8 weeks. Clinical benefit (i.e., RBC-TI and/or HI-E) was observed in 65% of the patients treated with luspatercept, and side effects were very limited, leading to the hope that this drug may soon be registered in that indication [11].

Other drugs are currently being tested in clinical trials. Drugs showing some efficacy include imetelstat, a telomerase inhibitor that leads to RBC-TI in 42% of ESA-resistant, lenalidomide, and HMA naïve LR-MDS patients in a recent report [12].

In conclusion Non-del 5q lower-risk MDS with anemia generally receive an ESA, except if they are both RBC-TD and have a serum EPO level >200 or 500 U/l, a relatively rare situation. After primary or secondary ESA failure, lenalidomide + ESAs, or hypomethylating agents or, in the case of MDS-RS, luspatercept (if approved) can be suggested but enrollment in a clinical trial or registry is always recommended. Selected patients may be candidates to receive horse ATG + cyclosporine or allo-HCT in case of poor risk features.

Patients with del 5q and RBC-TD anemia can receive lenalidomide, but can be treated with ESA before anemia becomes RBC-TD. In case of primary or secondary failure to lenalidomide, or if a significant and durable *TP53* clone appears, treating patients with HMA or if possible allo-HCT is strongly suggested. These recommendations are summarized in Fig. 8.1.

Treatment of Neutropenia and Thrombocytopenia

In lower-risk MDS, neutropenia and thrombocytopenia are less frequent than anemia, and are generally less profound and less symptomatic.

Neutropenia

Absolute neutrophil counts (ANC) are below 1.5 G/l in less than 10% of LR-MDS patients, and neutropenia is rarely associated with life-threatening infection unless it is related to therapy. Furthermore, infections may also be seen in the absence of significant neutropenia, if neutrophils dysfunction may be present. G-CSF can correct neutropenia in 60–75% of those cases, but its prolonged use has not shown any impact on survival, while a higher risk of progression to higher-risk MDS or AML has not been formally excluded. G-CSF may be used for transient periods, in patients who experience severe sepsis, although this rarely occurs in LR-MDS.

In case of fever or other signs of infection, however, neutropenic MDS patients should receive immediate broad-spectrum antibiotics. In the absence of previous infection episodes with resistant strains, amoxicilline-clavulanic acid and ciprofloxacine combinations are generally used in such situations.

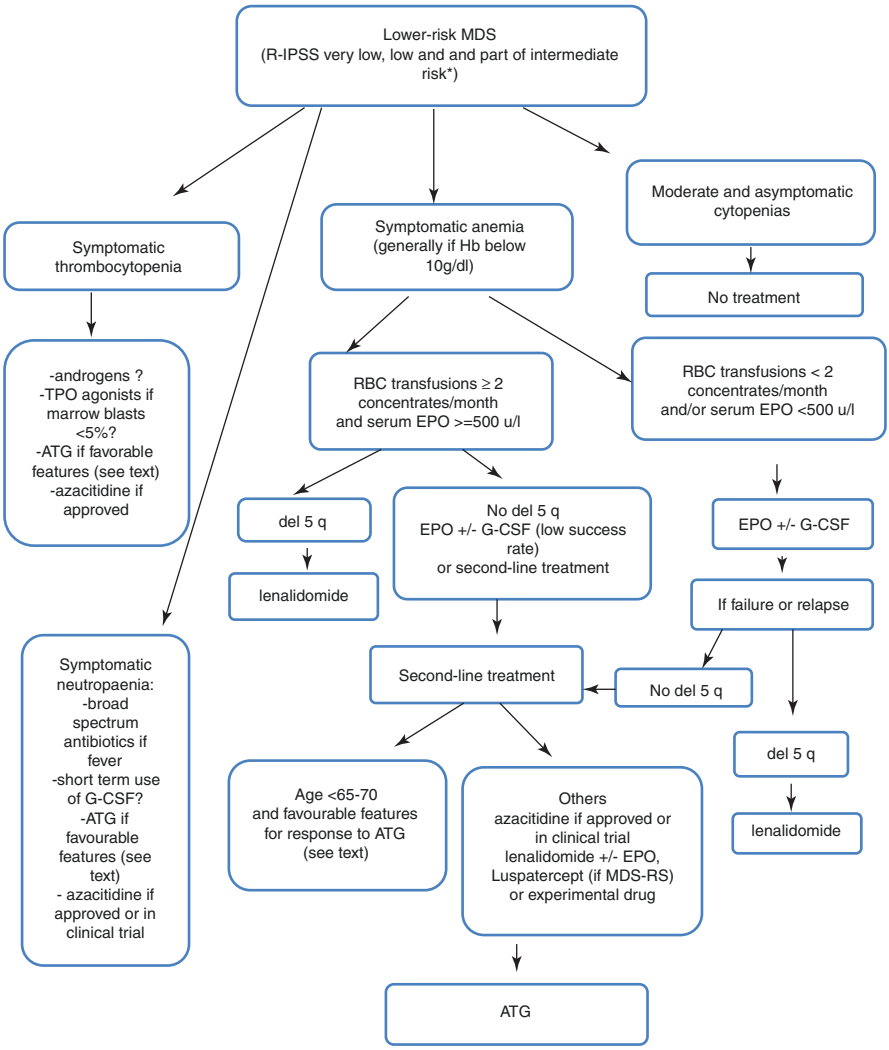

Fig. 8.1 Treatment algorithm for lower-risk MDS. *(for IPSS-R intermediate, whether they should initially receive treatment of lower-risk MDS or higher-risk MDS is also based on other factors including age, comorbidities, importance of cytopenias, somatic mutations, effect of first-line treatment, etc.)

Thrombocytopenia

Platelets below 50 G/l are seen in 30–35% of lower-risk MDS, but major bleeding is relatively rare unless therapy that interferes with hemostasis is used or platelet dysfunction is present. High-dose androgens can improve thrombocytopenia in about one-third of thrombocytopenic lower-risk MDS, but the response is generally transient [17].

In rare cases, a peripheral mechanism of platelet destruction may predominate in MDS, as shown by platelet lifespan studies, and ITP-like treatments can be tested, including splenectomy with some success [6].

The TPO receptor agonists romiplostim and eltrombopag are being investigated in MDS. In a phase 2 trial in lower-risk MDS with thrombocytopenia, high-dose romiplostim (500–1500 μg/week) yielded 55% platelet responses [22]. A reversible increase in marrow blasts was also seen in about 15% of the patients. In a subsequent randomized study versus placebo in LR-MDS with thrombocytopenia, romiplostim reduced the incidence of severe bleeding and platelet transfusions, but there was a suspected increase in the AML risk, however not confirmed with longer-term follow-up [23]. Eltrombopag also yields platelet response in about 50% of the patients, with no apparent increase in the risk of disease progression [35], including with longer-term follow-up [36]. As romiplostim and eltrombopag are not approved for MDS, they should be used with caution in clinical trials or specific programs, and in patients with marrow blasts <5%.

ATG and hypomethylating agents appear to give platelet response in 35–40% of the cases of lower-risk MDS, in addition to erythroid responses, and can be used in this situation.

In conclusion Thrombocytopenia is rarely the predominant cytopenia in low-risk MDS. Patients with platelets above 30–40 G/l rarely require treatment, unless bleeding (which can be due to concomitant platelet dysfunction) occurs. In patients with platelets below 30 G/l, and when thrombocytopenia is the only significant cytopenia, androgens can be tested (in the absence of underlying prostate carcinoma) followed in case of failure by TPO agonists if marrow blasts <5%. ITP-like treatments may be considered in a background of concomitant immune disorder or if very severe thrombocytopenia contrasts with the absence of any other cytopenia. If thrombocytopenia is associated with other cytopenias, HMAs or ATG may be considered, and a few of those patients (severe refractory thrombocytopenia, bleeding, etc.) may eventually require allo-SCT.

References

1. Adès L, Itzykson R, Fenaux P. Myelodysplastic syndromes. Lancet. 2014;383:2239–52.
2. Alessandrino EP, Della Porta MG, Bacigalupo A, Malcovati L, Angelucci E, Van Lint MT, Falda M, Onida F, Bernardi M, Guidi S, Lucarelli B, Rambaldi A, Cerretti R, Marenco P, Pioltelli P, Pascutto C, Oneto R, Pirolini L, Fanin R, Bosi A. Prognostic impact of pretransplantation transfusion history and secondary iron overload in patients with myelodysplastic syndrome undergoing allogeneic stem cell transplantation: a GITMO study. Haematologica. 2010;95:476–84.
3. Angelucci C et al. Safety and Efficacy, Including Event-Free Survival, of Deferasirox Versus Placebo in Iron-Overloaded Patients with Low- and Int-1-Risk Myelodysplastic Syndromes (MDS) (2018): Outcomes from the Randomized, Double-Blind Telesto Study, American Society of Hematology meeting, abstract no 234, 2018.

4. Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Alyea EP, Soiffer RJ, Antin JH. Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation. Blood. 2007;109(10):4586–8.
5. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, Kantarjian H, Raza A, Levine RL, Neuberg D, Ebert BL. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011;364:2496–506.
6. Bourgeois E, Caulier MT, Rose C, Dupriez B, Bauters F, Fenaux P. Role of splenectomy in the treatment of myelodysplastic syndromes with peripheral thrombocytopenia: a report on six cases. Leukemia. 2001;15(6):950–3.
7. Della Porta MG, Gallì A, Bacigalupo A, Zibellini S, Bernardi M, Rizzo E, Allione B, van Lint MT, Pioltelli P, Marenco P, Bosi A, Voso MT, Sica S, Cuzzola M, Angelucci E, Rossi M, Ubezio M, Malovini A, Limongelli I, Ferretti VV, Spinelli O, Tresoldi C, Pozzi S, Luchetti S, Pezzetti L, Catricalà S, Milanesi C, Riva A, Bruno B, Ciceri F, Bonifazi F, Bellazzi R, Papaemmanuil E, Santoro A, Alessandrino EP, Rambaldi A, Cazzola M. Clinical effects of driver somatic mutations on the outcomes of patients with myelodysplastic syndromes treated with allogeneic hematopoietic stem-cell transplantation. J Clin Oncol. 2016;67:3616–26.
8. Della Porta MG, Malcovati L. Myelodysplastic syndromes with bone marrow fibrosis. Haematologica. 2011;96(2):180–3.
9. Efficace F, Cottone F, Abel G, Niscola P, Gaidano G, Bonnetain F, Anota A, Caocci G, Cronin A, Fianchi L, Breccia M, Stauder R, Platzbecker U, Palumbo GA, Luppi M, Invernizzi R, Bergamaschi M, Borin L, Di Tucci AA, Zhang H, Sprangers M, Vignetti M, Mandelli F. Patient-reported outcomes enhance the survival prediction of traditional disease risk classifications: an international study in patients with myelodysplastic syndromes. Cancer. 2018;124(6):1251–9.
10. Fenaux P, Santini V, Spiriti MAA, Giagounidis A, Schlag R, Radinoff A, Gercheva-Kyuchukova L, Anagnostopoulos A, Oliva EN, Symeonidis A, Berger MH, Götze KS, Potamianou A, Haralampiev H, Wapenaar R, Milionis I, Platzbecker U. A phase 3 randomized, placebo-controlled study assessing the efficacy and safety of epoetin-α in anemic patients with low-risk MDS. Leukemia. 2018;32:2648–58.
11. Fenaux P, Platzbecker U, Mufti G, Garcia-Manero G, Buckstein R, Santini V, Diez Campelo M, Cavo M, Cazzola M, Ilhan O, Sekeres MA, González JFF, Arrizabalaga B, Salvi F, Vyas P, Bowen D, Selleslag D, DeZern AE, Jurcic J, Germing U, Götze K, Quesnel B, Beyne-Rauzy O, Cluzeau T, Voso MT, Mazure D, Vellenga E, Greenberg P, Hellström-Lindberg E, Zeidan A, Laadem A, Benzohra A, Zhang J, Rampersad A, Linde P, Sherman ML, Komrokji R, List AF. The MEDALIST trial: results of a phase 3, double-blind, multicenter, placebo-controlled, randomized study of luspatercept to treat Anemia in patients with very low-, low-, or intermediate-risk myelodysplastic syndromes (MDS) with ring sideroblasts who require red blood cell (RBC) transfusions. N Engl J Med. 2020;382(2):140–51.
12. Fenaux P, Steensma DP, Van Eygen K, Raza A, Santini V, Germing U, Font P, Díez-Campelo M, Thepo S, Vellenga E, Patnaik MM, Jang JH, Sherman L, Sun L, Varsos H, Rizo A, Wan Y, Huang F, Bussolari J, Rose E, Platzbecker U. Treatment with imetelstat provides durable transfusion Independence in heavily transfused non-del(5q) lower risk MDS relapsed/refractory to erythropoiesis-stimulating agents. European Hematology Association meeting 2019, abstract S 837, 2019.
13. Giagounidis A, Fenaux P, Mufti GJ, Muus P, Platzbecker U, Sanz G, Cripe L, Von Lilienfeld-Toal M, Wells RA. Practical recommendations on the use of lenalidomide in the management of myelodysplastic syndromes. Ann Hematol. 2008;87(5):345–52. Review.
14. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, Sanz M, Vallespi T, Hamblin T, Oscier D, Ohyashiki K, Toyama K, Aul C, Mufti G, Bennett J. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89:2079–88.
15. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, Bennett JM, Bowen D, Fenaux P, Dreyfus F, Kantarjian H, Kuendgen A, Levis A, Malcovati L, Cazzola M, Cermak J, Fonatsch C, Le Beau MM, Slovak ML, Krieger O, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120:2454–65.

16. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, Schnittger S, Sanada M, Kon A, Alpermann T, Yoshida K, Roller A, Nadarajah N, Shiraishi Y, Shiozawa Y, Chiba K, Tanaka H, Koeffler HP, Klein HU, Dugas M, Aburatani H, Kohlmann A, Miyano S, Haferlach C, Kern W, Ogawa S. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28:241–7.
17. Hebbar M, Kaplan C, Caulier MT, Wattel E, Morel P, Wetterwald M, Bauters F, Fenaux P. Low incidence of specific anti-platelet antibodies detected by the MAIPA assay in the serum of thrombocytopenic MDS patients and lack of correlation between platelet autoantibodies, platelet lifespan and response to danazol therapy. Br J Haematol. 1996;94(1):112–5.
18. Hellström-Lindberg E, Gulbrandsen N, Lindberg G, Ahlgren T, Dahl IMS, Dybedal I, Grimfors G, Hesse-Sundin E, Hjorth M, Kanter-Lewensohn L, Linder O, Luthman M, Löfvenberg E, Oberg G, Porwit-MacDonald A, Rådlund A, Samuelsson J, Tangen JM, Winquist I, Wisloff F, et al. A validated decision model for treating the anaemia of myelodysplastic syndromes with erythropoietin + granulocyte colony-stimulating factor: significant effects on quality of life. Br J Haematol. 2003;120:1037–46.
19. Jabbour E, Short NJ, Montalban-Bravo G, Huang X, Bueso-Ramos C, Qiao W, Yang H, Zhao C, Kadia T, Borthakur G, Pemmaraju N, Sasaki K, Estrov Z, Cortes J, Ravandi F, Alvarado Y, Komrokji R, Sekeres MA, Steensma DP, DeZern A, Roboz G, Kantarjian H, Garcia-Manero G. Randomized phase 2 study of low-dose decitabine vs low-dose **azacitidine** in lower-risk MDS and MDS/MPN. Blood. 2017;130(13):1514–22.
20. Jädersten M, Saft L, Smith A, Kulasekararaj A, Pomplun S, Göhring G, Hedlund A, Hast R, Schlegelberger B, Porwit A, Hellström-Lindberg E, Mufti GJ. TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. J Clin Oncol. 2011;29:1971–9.
21. Jaekel N, Lieder K, Albrecht S, Leismann O, Hubert K, Bug G, Kröger N, Platzbecker U, Stadler M, de Haas K, Altamura S, Muckenthaler MU, Niederwieser D, Al-Ali HK. Efficacy and safety of deferasirox in non-thalassemic patients with elevated ferritin levels after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2016;51(1): 89–95.
22. Kantarjian H, Fenaux P, Sekeres MA, Becker PS, Boruchov A, Bowen D, Hellstrom-Lindberg E, Larson RA, Lyons RM, Muus P, Shammo J, Siegel R, Hu K, Franklin J, Berger DP. Safety and efficacy of romiplostim in patients with lower-risk myelodysplastic syndrome and thrombocytopenia. J Clin Oncol. 2010;28(3):437–44.
23. Kantarjian HM, Fenaux P, Sekeres MA, Szer J, Platzbecker U, Kuendgen A, Gaidano G, Wiktor-Jedrzejczak W, Carpenter N, Mehta B, Franklin J, Giagounidis A. Long-term follow-up for up to 5 years on the risk of leukaemic progression in thrombocytopenic patients with lower-risk myelodysplastic syndromes treated with romiplostim or placebo in a randomised double-blind trial. Lancet Haematol. 2018;(3):e117–26.
24. Kelaidi C, Park S, Brechignac S, Mannone L, Vey N, Dombret H, Aljassem L, Stamatoullas A, Adès L, Giraudier S, de Botton S, Raynaud S, Lepelley P, Picard F, Leroux G, Daniel MT, Bouscary D, Dreyfus F, Fenaux P, Groupe Francophone des Myélodysplasies (GFM). Treatment of myelodysplastic syndromes with 5q deletion before the lenalidomide era; the GFM experience with EPO and thalidomide. Leuk Res. 2008;32:1049–53.
25. Kosmider O, Passet M, Santini V, Platzbecker U, Andrieu V, Zini G, Beyne-Rauzy O, Guerci A, Masala E, Balleari E, Bulycheva E, Dreyfus F, Fenaux P, Fontenay M, Park S, GFM, FISM and D-MDS. Are somatic mutations predictive of response to erythropoiesis stimulating agents in lower risk myelodysplastic syndromes? Haematologica. 2016;101(7):e280–3.
26. List A, Dewald G, Bennett J, Giagounidis A, Raza A, Feldman E, Powell B, Greenberg P, Thomas D, Stone R, Reeder C, Wride K, Patin J, Schmidt M, Zeldis J, Knight R, Myelodysplastic Syndrome-003 Study Investigators. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med. 2006;355:1456–65.
27. Lodé L, Ménard A, Flet L, Richebourg S, Loirat M, Eveillard M, Le Bris Y, Godon C, Theisen O, Gagez AL, Cartron G, Commes-Maerten T, Villemagne B, Luycx O, Godmer P, Pellat-

Deceunynck C, Soussi T, Béné MC, Delaunay J, Peterlin P. Emergence and evolution of *TP53* mutations are key features of disease progression in myelodysplastic patients with lower-risk del(5q) treated with lenalidomide. Haematologica. 2018;103(4):e143–6.
28. Van De Loosdrecht AA, Ireland R, Kern W, Della Porta MG, Alhan C, Balleisen JS, Bettelheim P, Bowen DT, Burbury K, Eidenschink L, Cazzola M, Chu SSC, Cullen M, Cutler JA, Dräger AM, Feuillard J, Fenaux P, Font P, Germing U, Haase D, et al. Rationale for the clinical application of flow cytometry in patients with myelodysplastic syndromes: position paper of an International Consortium and the European LeukemiaNet Working Group. Leuk Lymphoma. 2013;54:472–5.
29. Lyons RM, Cosgriff TM, Modi SS, Gersh RH, Hainsworth JD, Cohn AL, McIntyre HJ, Fernando IJ, Backstrom JT, Beach CL. Hematologic response to three alternative dosing schedules of azacitidine in patients with myelodysplastic syndromes. J Clin Oncol. 2009;27(11):1850–6.
30. Malcovati L, Porta MG, Pascutto C. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol. 2005;23:7594–603.
31. Malcovati L, Karimi M, Papaemmanuil E, Ambaglio I, Jädersten M, Jansson M, Elena C, Gallì A, Walldin G, Della Porta MG, Raaschou-Jensen K, Travaglino E, Kallenbach K, Pietra D, Ljungström V, Conte S, Boveri E, Invernizzi R, Rosenquist R, Campbell PJ, Cazzola M, Hellström Lindberg E. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015;126(2):233–41.
32. Mossner M, Jann J-C, Nowak D, Platzbecker U, Giagounidis A, Götze K, Letsch A, Haase D, Shirneshan K, Braulke F, Schlenk RF, Haferlach T, Schafhausen P, Bug G, Lübbert M, Ganser A, Büsche G, Schuler E, Nowak V, Pressler J, et al. Prevalence, clonal dynamics and clinical impact of TP53 mutations in patients with myelodysplastic syndrome with isolated deletion (5q) treated with lenalidomide: results from a prospective multicenter study of the german MDS study group (GMDS). Leukemia. 2016;30:1956–9.
33. Nazha A, Narkhede M, Radivoyevitch T, Seastone DJ, Patel BJ, Gerds AT, Mukherjee S, Kalaycio M, Advani A, Przychodzen B, Carraway HE, Maciejewski JP, Sekeres MA. Incorporation of molecular data into the Revised International Prognostic Scoring System in treated patients with myelodysplastic syndromes. Leukemia. 2016;30:2214–20.
34. Nazha A, Al-Issa K, Hamilton BK, Radivoyevitch T, Gerds AT, Mukherjee S, Adema V, Zarzour A, Abuhadra N, Patel BJ, Hirsch CM, Advani A, Przychodzen B, Carraway HE, Maciejewski JP, Sekeres MA. Adding molecular data to prognostic models can improve predictive power in treated patients with myelodysplastic syndromes. Leukemia. 2017;31(12):2848–50.
35. Oliva EN, Alati C, Santini V, Poloni A, Molteni A, Niscola P, Salvi F, Sanpaolo G, Balleari E, Germing U, Fenaux P, Stamatoullas A, Palumbo GA, Salutari P, Impera S, Avanzini P, Cortelezzi A, Liberati AM, Carluccio P, Buccisano F, Voso MT, Mancini S, Kulasekararaj A, Morabito F, Bocchia M, Cufari P, Spiriti MA, Santacaterina I, D'Errigo MG, Bova I, Zini G, Latagliata R. Eltrombopag versus placebo for low-risk myelodysplastic syndromes with thrombocytopenia (EQoL-MDS): phase 1 results of a single-blind, randomised, controlled, phase 2 superiority trial. Lancet Haematol. 2017;4(3):e127–36.
36. Oliva EN, Alati C, Santini V, Poloni A, Molteni A, Niscola P, Salvi F, Sanpaolo G, Balleari E, Germing U, Fenaux P, Stamatoullas A, Palumbo GA, Salutari P, Impera S, Avanzini P, Cortelezzi A, Liberati AM, Carluccio P, Buccisano F, Voso MT, Mancini S, Kulasekararaj A, Morabito F, Bocchia M, Cufari P, Spiriti MA, Santacaterina I, D'Errigo MG, Bova I, Zini G, Latagliata R. Long term effect of Eltrombopag versus placebo for low-risk myelodysplastic syndromes with thrombocytopenia (EQoL-MDS): interim results of a single-blind, randomised, controlled, phase 2 superiority trial. American Society for Hematology meeting 2019, submitted abstract, 2019.
37. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, Yoon CJ, Ellis P, Wedge DC, Pellagatti A, Shlien A, Groves MJ, Forbes SA, Raine K, Hinton J, Mudie LJ, McLaren S, Hardy C, Latimer C, Della Porta MG, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122(22):3616–27.

38. Park S, Grabar S, Kelaidi C, Beyne-Rauzy O, Picard F, Bardet V, Coiteux V, Leroux G, Lepelley P, Daniel M-T, Cheze S, Mahé B, Ferrant A, Ravoet C, Escoffre-Barbe M, Adès L, Vey N, Aljassem L, Stamatoullas A, Mannone L, et al. Predictive factors of response and survival in myelodysplastic syndrome treated with erythropoietin and G-CSF: the GFM experience. Blood. 2008;111:574–82.
39. Park S, Hamel JF, Toma A, Kelaidi C, Thépot S, Campelo MD, Santini V, Sekeres MA, Balleari E, Kaivers J, Sapena R, Götze K, Müller-Thomas C, Beyne-Rauzy O, Stamatoullas A, Kotsianidis I, Komrokji R, Steensma DP, Fensterl J, Roboz GJ, Bernal T, Ramos F, Calabuig M, Guerci-Bresler A, Bordessoule D, Cony-Makhoul P, Cheze S, Wattel E, Rose C, Vey N, Gioia D, Ferrero D, Gaidano G, Cametti G, Pane F, Sanna A, Germing U, Sanz GF, Dreyfus F, Fenaux P. Outcome of lower-risk patients with myelodysplastic syndromes without 5q deletion after failure of erythropoiesis-stimulating agents. J Clin Oncol. 2017;35(14):1591–7.
40. Park S, Greenberg P, Yucel A, Farmer C, O'Neill F, De Oliveira BC, Fenaux P. Clinical effectiveness and safety of erythropoietin-stimulating agents for the treatment of low- and intermediate-1-risk myelodysplastic syndrome: a systematic literature review. Br J Hematol. 2019;184(2):134–60. Review
41. Pascal L, Beyne-Rauzy O, Brechignac S, Marechaux S, Vassilieff D, Ernst O, Berthon C, Gyan E, Gourin MP, Dreyfus F, Fenaux P, Rose C. Cardiac iron overload assessed by T2* magnetic resonance imaging and cardiac function in regularly transfused myelodysplastic syndrome patients. Br J Haematol. 2013;162(3):413–5.
42. Passweg JR, Giagounidis AAN, Simcock M, Aul C, Dobbelstein C, Stadler M, Ossenkoppele G, Hofmann W-K, Schilling K, Tichelli A, Ganser A. Immunosuppressive therapy for patients with myelodysplastic syndrome: a prospective randomized multicenter phase III trial comparing antithymocyte globulin plus cyclosporine with best supportive care--SAKK 33/99. J Clin Oncol Off J Am Soc Clin Oncol. 2011;29:303–9.
43. Platzbecker U, Germing U, Götze KS, Kiewe P, Mayer K, Chromik J, Radsak M, Wolff T, Zhang X, Laadem A, Sherman ML, Attie KM, Giagounidis A. Luspatercept for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes (PACE-MDS): a multicentre, open-label phase 2 dose-finding study with long-term extension study. Lancet Oncol. 2017;18(10):1338–47.
44. Prebet T, Cluzeau T, Park S, Sekeres MA, Germing U, Ades L, Platzbecker U, Gotze K, Vey N, Oliva E, Sugrue MM, Bally C, Kelaidi C, Al Ali N, Fenaux P, Gore SD, Komrokji R. (2017). Outcome of patients treated for myelodysplastic syndromes with 5q deletion after failure of lenalidomide therapy. Oncotarget. 2017;8(47):81926–35.
45. Rose C, Brechignac S, Vassilief D, Pascal L, Stamatoullas A, Guerci A, Larbaa D, Dreyfus F, Beyne-Rauzy O, Chaury MP, Roy L, Cheze S, Morel P, Fenaux P, GFM (Groupe Francophone des Myélodysplasies). Does iron chelation therapy improve survival in regularly transfused lower risk MDS patients? A multicenter study by the GFM (Groupe Francophone des Myélodysplasies). Leuk Res. 2010;34(7):864–70.
46. Santini V, Almeida A, Giagounidis A, Gröpper S, Jonasova A, Vey N, Mufti GJ, Buckstein R, Mittelman M, Platzbecker U, Shpilberg O, Ram R, Del Cañizo C, Gattermann N, Ozawa K, Risueño A, MacBeth KJ, Zhong J, Séguy F, Hoenekopp A, et al. Randomized phase III study of lenalidomide versus placebo in RBC transfusion-dependent patients with lower-risk non-del(5q) myelodysplastic syndromes and ineligible for or refractory to erythropoiesis-stimulating agents. J Clin Oncol. 2016;34:2988–96.
47. Santini V, Schemenau J, Levis A, Balleari E, Sapena R, Adès L, Guerci A, Beyne-Rauzy O, Gourin MP, Cheze S, Stamatoullas A, Sanna A, Gioia D, Cametti G, Ferrero D, Raffoux E, Rose C, Poloni A, Prebet T, Legros L, Natarajan-Amé S, Fenaux P, Germing U, Dreyfus F, Park S. Can the revised IPSS predict response to erythropoietic-stimulating agents in patients with classical IPSS low or intermediate-1 MDS? Blood. 2013;122(13):2286–8.
48. Stahl M, DeVeaux M, de Witte T, Neukirchen J, Sekeres MA, Brunner AM, Roboz GJ, Steensma DP, Bhatt VR, Platzbecker U, Cluzeau T, Prata PH, Itzykson R, Fenaux P, Fathi AT, Smith A, Germing U, Ritchie EK, Verma V, Nazha A, Maciejewski JP, Podoltsev NA, Prebet

T, Santini V, Gore SD, Komrokji RS, Zeidan AM. The use of immunosuppressive therapy in MDS: clinical outcomes and their predictors in a large international patient cohort. Blood Adv. 2018;2(14):1765–72.
49. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, Ebert BL. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126:9–16.
50. Stein EM, Fathi AT, DiNardo CD, Pollyea DA, Swords RT, Roboz GJ, Collins R, Sekeres MA, Stone RM, Attar EC, Tosolini A, Xu Q, Amatangelo M, Gupta I, Knight RD, Botton SD, Tallman MS, Kantarjian HM. Enasidenib (AG-221), a potent Oral inhibitor of mutant isocitrate dehydrogenase 2 (IDH2) enzyme, induces hematologic responses in patients with myelodysplastic syndromes (MDS). Blood. 2016;128:343.
51. Thépot S, Ben Abdelali R, Chevret S, Renneville A, Beyne-Rauzy O, Prébet T, Park S, Stamatoullas A, Guerci-Bresler A, Cheze S, Tertian G, Choufi B, Legros L, Bastié JN, Delaunay J, Chaury MP, Sanhes L, Wattel E, Dreyfus F, Vey N, Chermat F, Preudhomme C, Fenaux P, Gardin C, Groupe Francophone des Myélodysplasies (GFM). A randomized phase II trial of azacitidine +/− epoetin-β in lower-risk myelodysplastic syndromesresistant to erythropoietic stimulating agents. Haematologica. 2016;101(8):918–25.
52. Tobiasson M, Dybedahl I, Holm MS, Karimi M, Brandefors L, Garelius H, Grövdal M, Högh-Dufva I, Grønbæk K, Jansson M, Marcher C, Nilsson L, Kittang AO, Porwit A, Saft L, Möllgård L, Hellström-Lindberg E. Limited clinical efficacy of azacitidine in transfusion-dependent, growth factor-resistant, low- and Int-1-risk MDS: results from the nordic NMDSG08A phase II trial. Blood Cancer J. 2014;4:e189.
53. Toma A, Kosmider O, Chevret S, Delaunay J, Stamatoullas A, Rose C, Beyne-Rauzy O, Banos A, Guerci-Bresler A, Wickenhauser S, Caillot D, Laribi K, De Renzis B, Bordessoule D, Gardin C, Slama B, Sanhes L, Gruson B, Makhoul PC, Chouffi B, et al. Lenalidomide with or without erythropoietin in transfusion dependent erythropoiesis-stimulating agent-refractory lower risk MDS without 5q deletion. Leukemia. 2016;30:897–905.

Chapter 9
Treatment Algorithms for Higher-Risk Disease

Bryan C. Hambley and Amy E. DeZern

Introduction

Myelodysplastic syndromes (MDS) encompass a wide spectrum of pathophysiologic processes, with broad variation in the natural history of the disease [1]. Morbidity and mortality from MDS are related to cytopenias (particularly transfusion refractory anemia and thrombocytopenia), opportunistic infections, leukemic transformation, among other sequelae of the disease and its treatment [2, 3]. For clinicians managing patients with higher-risk MDS, choosing the appropriate timing and therapeutic approach (Fig. 9.1) is critical to achieving optimal outcomes for an individual patient.

This chapter will focus on therapeutic management for higher-risk patients, as classified by the Revised International Prognostic Scoring System for Myelodysplastic Syndromes (IPSS-R) [1] with a score of ≥4.5 as well as those with intermediate-risk disease who may require treatment more akin to higher-risk patients. These patients have a median survival of 0.8, 1.6, and 3.0 years for very high risk, high, and intermediate categories, respectively [1]. Their risk for transformation to acute myeloid leukemia (AML) is markedly higher than those with lower-risk disease [1]. Emerging data indicate the incorporation of mutational profiling may supplement the prognostic utility of the IPSS-R score (which includes cytogenetic, but not molecular, characterization) [4, 5]. Once a patient has been identified as having higher-risk MDS, treatment considerations range from supportive transfusions and palliative care in the less robust or frail

B. C. Hambley · A. E. DeZern (✉)
Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA

Division of Hematology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
e-mail: adezern1@jhmi.edu

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3_9

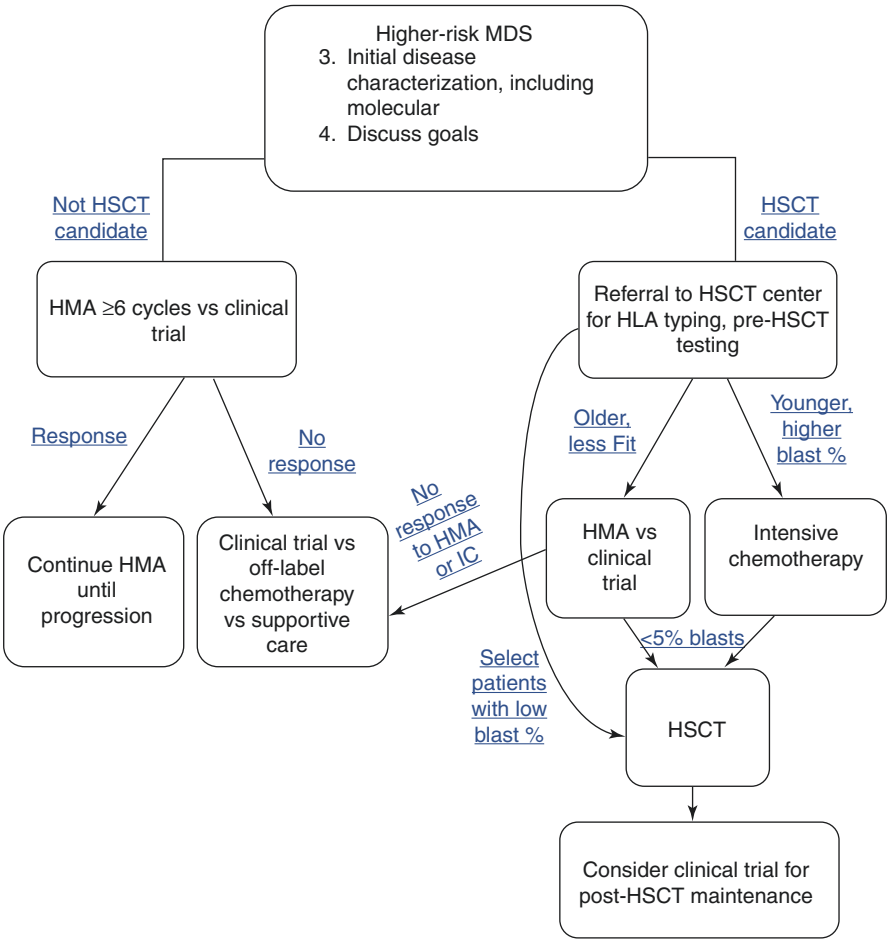

Fig. 9.1 Higher-risk MDS 2019 treatment algorithm

patient to HMAs for disease control to allogeneic stem cell transplant with curative intent in younger or more fit patients.

Initial Steps

Bone marrow aspirate and biopsy, including flow cytometry (where available), conventional karyotyping, and molecular profiling for somatic mutations from aspirate are important initial steps in the characterization of MDS [6–9].

All patients with higher-risk MDS should be assessed for fitness, both to evaluate ability to tolerate various treatment paradigms as well as guide patient expectations in terms of disease and treatment toxicity. In-depth assessment of activity, fatigue,

and ability to take a long walk appears to enhance predictive capacity compared with more general assessments such as the Eastern Cooperative Group (ECOG) performance status and the Charlson Comorbidity Index [10–12].

Hypomethylating Agent Selection

Currently, only three agents are approved for the treatment of MDS by the U.S. Food and Drug Administration (FDA): azacitidine, decitabine, and lenalidomide [13]. The vast majority of higher-risk patients will receive one of the two approved hypomethylating agents at diagnosis. Azacitidine was approved by the FDA in 2004 after the CALGB-9221 study showed delay to leukemic transformation or death, improved quality of life, and decreased transfusion burden [14]. Decitabine was approved by the FDA in 2006, after the D-0007 trial showed improved response rates, decreased transfusion requirements, and a trend toward improved progression-free survival [15]. Each was approved based on data showing efficacy in limiting the clinical impact of MDS through better outcomes compared to supportive care. All are currently used as monotherapies with modest results.

Several multicenter randomized studies have compared treatment with HMAs to supportive care in MDS patients, including the CALGB-9221 (azacitidine), D-0007 (decitabine), AZA-001 (azacitidine), and EORTC-06011 (decitabine) trials [14–17]. These studies have demonstrated delayed progression to AML following HMA therapy, with an improvement in median progression-free survival over supportive care ranging from 3 to 8 months. Only the AZA-001 trial showed statistically significant superior overall survival with an HMA [16]. The interpretation of these trials has varied. Some construe these outcomes as decidedly positive; others view these data as dramatically demonstrating the need for more effective agents. Regardless, optimal outcomes with HMA therapy demand close attention to the details of treatment. Therapy must be started expeditiously, used with the correct dose and schedule, and continued until progression occurs. Typically, this requires at least 6 cycles comprised of azacitidine for 7 days at 75 mg/m^2 or decitabine for 5 days at 20 mg/m^2, both administered every 28 days [18].

No randomized studies have compared decitabine and azacitidine in patients with higher-risk MDS. One retrospective study reported overall survival and leukemic transformation were not significantly different between those receiving azacitidine and decitabine, though a subgroup of patients 65 years and older experienced superior survival with azacitidine [19]. A retrospective evaluation of 642 patients with higher-risk MDS reported significantly longer survival in females treated with decitabine versus azacitidine (median overall survival [OS] 21 versus 13 months), with no survival difference between agents in males [20]. While the clinical significance of these retrospective studies remains unclear, much interest has focused on the potential benefit of decitabine in patients with a *TP53* mutation (see below "Applying Molecular Data to the Therapeutic Paradigm").

Hypomethylating Agent Dosing Schedule

The trials proving the superiority of azacitidine over supportive/conventional care used seven consecutive days of 75 mg/m^2 given subcutaneously [14, 16]. Some infusion clinics are not structured to accommodate weekend dosing of chemotherapy, and alternative dosing schedules have been investigated as listed in Table 9.1. The 2006 study by Kantarjian and colleagues of decitabine versus supportive care used a dose of 15 mg/m^2 every 8 hours for 3 days, virtually requiring inpatient hospital admission [15]. The alternative daily dosing of decitabine has been investigated to minimize hospital admission needs while maintaining efficacy.

Combination Therapy with Hypomethylating Agents

While HMAs provide improved outcomes compared with supportive care alone, they are not a curative treatment [21]. In hopes of improving both response rates and durability of response, combination therapy has been explored in-depth as both first-line therapy and in the setting of HMA failure. Combination therapy is based on the theory that efficacy is increased by combining drugs with different mechanisms of action. Unfortunately, the bulk of available evidence seems to oppose any such synergy, and it appears that the addition of other agents to HMAs does not provide marked benefit over single HMA therapy. The most promising combination has been of the two approved

Table 9.1 Hypomethylating agent dosing schedules

Agent	Dosing schedule	Comments
Azacitidine	75 mg/m^2 SC or IV daily for 7 days each cycle	Standard treatment in most trials Requires weekend infusion clinic availability
Azacitidine	75 mg/m^2 SC or IV daily for 5 days, off for 2 days, and on for 2 days	No weekend infusion needs Retrospective data indicate similar outcomes to 7 consecutive day dosing [72]
Azacitidine	50 mg/m^2 SC or IV for 5 days, off 2 days, on 5 days	Requires 10 total infusion clinic visits Equivalent response rate to 7 day schedule with weekend holiday in prospective trial (not compared to 7 consecutive days) [73]
Azacitidine	75 mg/m^2 SC or IV for 5 days	Retrospective data indicate lower response that regimens with 7 treatment days [72]
Decitabine	15 mg/m^2 IV Q8 hours for 3 days	Standard dose in initial trials leading to FDA approval Q8 hour dosing virtually requires inpatient admission
Decitabine	20 mg/m^2 IV daily for 5 days	In trial, superior to SC dosing and 10 days of infusions each cycle [74]
Decitabine	20 mg/m^2 IV daily for 10 days	Notable efficacy in trial of *TP53*-mutated AML/MDS [31]

agents azacitidine and lenalidomide [22]. Among 36 patients enrolled (18 phase 1, 18 phase 2), the overall response rate was 72%: 16 patients achieved a complete response (CR), and 10 had hematologic improvement. Median CR duration was 17 months (range, 3–39). In reviewing the patient population, it was noted that *TET2/DNMT3A/IDH1/2* mutational status was associated with response in some patients [22], arguing that modest response rates in trials could be increased within select populations. Other combinations have shown less promise and increased treatment toxicities have been seen. For example, azacitidine has been combined with various histone deacetylase [HDAC] inhibitors without a significant increase in efficacy but with increased toxicity [23–27]. The most recent cooperative group effort, the S1117 trial, compared azacitidine monotherapy to either azacitidine and vorinostat or azacitidine and lenalidomide [25]. This study was stopped early due to the lack of improved outcomes of either combination arm beyond single-agent azacitidine [25]. Enrollment criteria were broad in this upfront trial, and patients with CMML and treatment-related MDS were enrolled. Future reviews of these data may suggest populations (defined molecularly or otherwise) that could benefit from further investigation of combination regimens.

Initial Intensive Chemotherapy

In patients with the physical and psychosocial fitness to receive intensive chemotherapy and blasts counts nearing the arbitrary 20% threshold for AML, dismal long-term outcomes with HMA-based approaches may lead to consideration of intensive induction chemotherapy. Younger patients, those presenting with the rapidly progressing disease, and those whose treatment goals include allogeneic stem cell transplant may be considered for this approach. In these patients, the initial strategy is to achieve the deepest remission possible with initial treatment—minimizing the amount of active disease at the time of HSCT.

Intensive chemotherapy with cytarabine+anthracycline combinations can frequently achieve complete or partial remissions, with occasional long-term progression-free survival [28, 29]. Despite increased remission rates with intensive chemotherapy, prospective and retrospective reports have indicated decreased survival with intensive upfront chemotherapy [16, 29]. While the AZA-001 prospectively compared 42 patients randomized to azacitidine or intensive chemotherapy, the trial excluded patients planned for allogeneic transplant [16]. The treatment paradigm of intensive chemotherapy followed by HSCT compared to HMA-based treatment followed by HSCT is currently being evaluated in NCT01812252 [30].

Applying Molecular Data to the Therapeutic Paradigm

With a retrospective review of larger cohorts of patients treated and examination of the molecular phenotypes, it may become possible to identify subsets of patients with specific somatic mutations that have higher or lower response rates to individual

therapies. This is the first step toward using novel molecular data to guide therapy and improve outcomes for MDS patients and emphasizes why full characterization at diagnosis is so important. While a deviation from current standard therapeutic strategies may not be guided at this time by molecular data, this information will be increasingly important in disease classification, prognosis, and therapeutic selection as our biologic knowledge of these diseases increases.

Two recent studies suggest that high-risk entity of *TP53*-mutant MDS/AML might be best treated with decitabine. In a study of 116 patients with MDS/AML, outcomes were explored with a 10-day course of decitabine every 28 days [31]. Patients with a *TP53* mutation had a significantly higher overall response rate compared with wild type (21 [100%] of 21 patients vs. 32 [41%] of 78 patients; $p < 0.001$) and higher rate of complete remission/incomplete marrow recovery (CR/CRi; 13 [62%] of 21 patients vs. 26 [33%] of 78 patients; $p = 0.04$) [31]. In another retrospective study evaluating 109 patients with MDS treated with decitabine, *TP53* mutations were identified in 13.8% of patients [32]. *TP53* was the only somatic gene mutation predictive for complete response (CR), with 10 of 15 patients with *TP53* mutations (66.7%) achieving CR versus 20 (21%) of 94 with wild type ($p = 0.001$) [32]. Of those with monosomies, 80% achieved CR. The median overall survival remained disappointing at 14 months. These favorable response rates have not been uniformly seen in *TP53*-mutant disease treated with decitabine in all studies. Additionally, *TP53* mutant clones probably do not display exclusive sensitivity to decitabine compared with azacitidine so the use of either HMA remains reasonable [33]. Survival in *TP53*-mutated MDS remains poor, and therapies with more durable responses are needed [34, 35].

Assessing Response to Initial Therapy in Higher-Risk Patients

Assessment of higher-risk MDS after initial therapy is important to monitor for treatment response and disease progression. This information may be of particular use in patients requiring a change of therapy for progressive disease, clinical trial eligibility, as well as those requiring improved disease status prior to proceeding to allogeneic transplant. Peripheral blood counts are important markers of disease, though cytopenias often worsen in the initial cycles of therapy [36]. There are no prospective management studies comparing the timing of bone marrow assessment, though a common time point in clinical trials of azacitidine was to obtain a bone marrow biopsy after 4–6 cycles of treatment [14, 16, 25]. Further bone marrow biopsies outside of a clinical trial are largely at the discretion of the treating physicians and are particularly important in the setting of suspected disease progression [36]. Morphologic, cytogenetic, and molecular evaluations provide important data for treatment and prognosis (Table 9.2). There is a growing clinical interest in the assessment of minimal residual disease in MDS [37].

Table 9.2 Treatment response testing

Test	Timing	Rationale
Complete blood count	At least biweekly with initial 2 cycles, monitoring for clinical cytopenias to assess transfusion needs	Guide transfusion support Assess for improvement in cytopenias
Bone marrow aspirate and surgical pathology	After 4–6 cycles of HMA (optimally just prior to next cycle) With suspected progression Guide timing of allogeneic transplant	Assess for disease response and plan alternative therapy as needed Evaluate for leukemic transformation Confirm desired response prior to proceeding to allogeneic transplant
Flow cytometry (from BM aspirate, where available)	As above	Further characterize disease response [6]
Conventional karyotype (from BM aspirate)	As above	Assess for clonal evolution, may alter risk stratification/prognosis [7, 8]. If poor growth, add on MDS FISH (FISH not required in all patients)
Molecular profiling for somatic mutations (from BM aspirate)	At diagnosis at a minimum; reassess if changing therapy	The emergence of certain mutations may play a role in prognostication and clinical trial eligibility [7, 9].

Therapy After Hypomethylating Agent Failure

In spite of demonstrable improvements in MDS treatment with HMAs and the fact that HMAs remain the only effective therapy for higher-risk MDS, the disease is still incurable outside of HSCT. Furthermore, in cases of absence or loss of response to either azacitidine or decitabine, outcomes are extremely poor [38, 39]. Primary HMA failure is defined as either no response to or progression during HMA therapy; median OS in this situation is about 6 months [38, 39]. Secondary HMA failure is defined as relapse after an initial response, and has a median OS of 7.4 months [39]. Therapeutic options for either primary or secondary HMA failure are limited (Table 9.3). HMA failure is usually a class effect; there are few data to suggest a benefit from switching between decitabine and azacitidine when one or the other has been ineffective. Intolerance to one HMA remains a viable reason to switch to the alternative agent. Various other treatment approaches which have been studied are listed in Table 9.3, characterized by low response rates, poor durability, and increased toxicity compared with supportive care. Clinical trials remain the emphasis in this patient group where possible.

The development of HMA failure, and the associated grim prognosis, often leads to revisiting discussions of HSCT, even in patients who have previously declined this option. However, HSCT has limited applicability in this population, especially in the setting of physical deconditioning.

Table 9.3 Treatment after HMA failure

Salvage therapy	Trial Results	Therapeutic Utility
Cytarabine +Anthracycline	Retrospective analysis, ORR 39% for 7+3 subgroup 37% of responders proceeding to allogeneic transplant [75]	Salvage option in patients with a plan to proceed to HSCT after HMA failure
Clofarabine +Cytarabine	44% response rate, frequent infectious complications, 9/70 proceeded to allogeneic transplant [76]	Potential alternative cytarabine+anthracycline in patients planned for HSCT
Low dose cytarabine	Low response rates and short OS in both upfront and HMA failure settings [39, 77]	Minimal role as a single agent
Lenalidomide	Low response rates with high toxicity related to infection and cytopenias [78, 79]	Minimal utility outside of del5q in patients with HMA refractory MDS
Alternating HMA	Response rates of 19–40%, poor durability [80–82]	Limited, can consider switching from decitabine to azacitidine (or azacitidine to decitabine) if poor tolerance
Supportive care and hospice		Appropriate option to discuss in those who are not clinical trial or allogeneic transplant candidates

[a]Above agents, with the exception of HMAs and lenalidomide, are investigational or off-label for MDS

While various relapse regimens described in Table 9.3 have the possibility of response, the clinical reality is that many patients experience HMA failure and simultaneous deterioration in their fitness to tolerate further chemotherapy. Moreover, a national database study revealed that 28% of patients with MDS were admitted to the ICU in their last month of life, and 7% received chemotherapy in their last 2 weeks of life [40]. Supportive care at the time of HMA failure may be the only reasonable option for elderly or deconditioned patients or those who are otherwise ineligible (due to geographic, donor, social, or financial reasons) for either HSCT or enrollment in a clinical trial. To prevent this dire situation, more effective interventions from the onset and along the entire continuum of the disease are needed. More effective first-line agents are urgently needed for MDS, but almost as important is the identification of optimum treatment strategies in the setting of HMA failure. Novel therapies, especially in higher-risk disease, are at the center of ongoing clinical trials to improve treatment after HMA failure.

Targeted Therapy in Higher Risk Disease

A wide spectrum of somatic mutations, with no single dominant mutation, have been implicated in MDS [41]. Various genetic and cellular pathways have been implicated, with many genes expressing their own characteristic pathophysiology

and some suggested higher-risk phenotypes [41, 42]. Compared with AML and myeloproliferative neoplasms, many MDS mutations are loss-of-function mutations which have been more difficult to target therapeutically [43]. Nevertheless, the identification of somatic mutations with characteristic disease manifestations along with limited standard-of-care options for MDS has led to great interest in developing therapies targeted at a patient's specific disease. These therapies may be investigated earlier in higher-risk disease when combined in trials with AML populations. APR-246 has shown promising responses in a phase Ib/II clinical trial of patients with *TP53*-mutant MDS/AML, and a phase III trial (NCT03745716) is currently enrolling [44]. Ivosidenib achieved responses in 11/12 patients with *IDH1*-mutated MDS included in a phase I trial [45]. Given these results and the effectiveness of *IDH* inhibition in AML, multiple phase II and III trials are currently investigating ivosidenib and enasidenib for *IDH1*- and *IDH2*-mutated MDS. In vitro and mouse models have shown vitamin C can activate *TET2*-mutant cells, and clinical trials to investigate this concept are ongoing [46, 47]. Select targeted therapies under active investigation are listed in Table 9.4.

Table 9.4 Select targeted therapy trials enrolling higher-risk MDS patients in 2019

Mutation targeted	Therapy	Inclusion	Study name and status
SF3B1, SRSF2, U2AF1, ZRSR2	*H3B-8800*	MDS: HMA refractory higher-risk and transfusion-dependent lower-risk AML not fit for intensive induction CMML refractory to therapy	NCT02841540 Phase 1, currently enrolling
TET2	*Vitamin C* (can be combined with HMA)	MDS with <20% blasts and platelets>20,000/uL	NCT03433781 Phase 1b/2a, currently enrolling
TET2	*Ascorbic acid* + HMA	MDS, MDS/MPN, or AML not fit for intensive induction	NCT03397173 Phase 2, currently enrolling
IDH1 R132	*FT-2102* +/− HMA or Cytarabine	AML or MDS (IPSS-R INT, HIGH, or VERY HIGH) either not candidate for or relapsed/refractory to standard therapy	NCT02719574 Phase 1/2, currently enrolling
IDH1 R132	*Ivosidenib*	Relapsed/refractory hematologic malignancies	NCT02074839 Phase 1 results reported June 2018, including 12 patients with MDS (with 11/12 ORR, with 5/12 CR) [45]

(continued)

Table 9.4 (continued)

Mutation targeted	Therapy	Inclusion	Study name and status
IDH1 R132	*Ivosidenib* + Venetoclax +/− Azacitidine	AML relapsed/refractory or not candidate for standard induction MDS and MPN with >10% bone marrow blasts	NCT03471260 Phase 1b/2, currently enrolling
IDH2	*Enasidenib* + Azacitidine	MDS, CMML, and AML with <30% blasts Arms for HMA naïve and HMA relapsed/refractory	NCT03383575 Phase 2, currently enrolling
IDH2	*Enasidenib*	MDS, CMML, and AML with <30% blasts	NCT03383575 Phase 2, currently enrolling
IDH1 or *IDH2*	*Ivosidenib* (IDH1) or *Enasidenib* (IDH2) + standard chemotherapy	Newly diagnosed AML or MDS with 10–19% marrow blasts (MDS-EB2)	NCT03839771 Phase 3, currently enrolling
IDH1 or *IDH2*	*AG-881*	Hematologic malignancies	NCT02492737 Phase 1, enrollment completed and results pending
TP53	*APR-246* (with AZA compared to AZA alone)	MDS and AML with <30% blasts	NCT03745716 For Phase 2 of combination arm (9/11 [82%]) CR rate in Phase 1b reported in abstract December 2018 [44] Phase 3, currently enrolling

Upfront Treatment Considerations in Patients Planned for HSCT

Currently, the only treatment modality capable of curing MDS is HSCT. Unfortunately, the applicability of HSCT has historically been limited by the inherent risks of the procedure and the older age and attendant comorbidities of typical MDS patients. Though the recent advent of reduced-intensity preparative regimens and more available alternative donors has begun to expand the use of HSCT, outcomes remain mixed [48].

There are three key issues applicable to all treating physicians regarding the management of higher-risk MDS patients planned for HSCT: identifying patients who may proceed directly to HSCT, determining the optimal disease response and timing of HSCT, and selecting initial therapy.

Retrospective data indicate some patients with MDS, particularly with a low blast percentage, have up to 57% 5-year OS proceeding to HSCT without

chemotherapy prior to a conditioning regimen; however, the applicability of this approach to reduced intensity, nonmyeloablative, and unrelated donors is unclear [30, 49, 50]. A clinical rationale for upfront chemotherapy prior to HSCT is the recognition that patients with >5% bone marrow blasts at the time of transplant have increased relapse post-HSCT [51, 52]. In patients selected for pre-HSCT chemotherapy, cytoreduction with either an HMA or intensive chemotherapy is reasonable based upon retrospective reports and a prospective randomized trial (NCT01812252) to answer this question is actively enrolling [30, 53, 54].

Supportive Care

Regardless of treatment intensity planned, all patients with higher-risk MDS require multifaceted supportive care.

Infections and Prophylactic Antimicrobials

Infectious complications are a common cause of morbidity and mortality in MDS [55, 56]. Infectious complications are associated with cytopenias and higher-risk disease [55, 56]. While antimicrobial prophylaxis decreases febrile episodes in MDS patients undergoing treatment with HMAs, the impact of prophylactic antibiotics and G-CSF on serious infections is unclear [56, 57]. In the absence of high-quality prospective studies, the National Comprehensive Cancer Network guidelines do not recommend routine prophylactic antibiotics or G-CSF except in patients with recurrent infections [58].

Growth Factors in Combination with Chemotherapy

Prior chapters have discussed the use of growth factors as single agents for lower-risk MDS. A common clinical scenario in higher-risk MDS is whether to combine growth factors with HMAs.

Combining an erythropoietin-stimulating agent (ESA) with azacitidine may improve transfusion independence [59].

Randomized phase 2 studies have demonstrated improved platelet levels and decreased transfusion needs with romiplostim when combined with HMAs in low- and intermediate-risk MDS only [60, 61]. Similarly, the ASPIRE trial randomized patients with AML or MDS undergoing supportive care to either eltrombopag or placebo, showing increased platelet counts and lower "clinically relevant thrombocytopenic events" [62].

Transfusions

Anemia and thrombocytopenia occur commonly in high-risk MDS. Severe anemia can lead to fatigue, dyspnea, and negatively impact the quality of life. Hemoglobin less than 9 g/dL in males and less than 8 g/dL in females has been identified as an independent predictor of mortality in MDS [63]. Severe thrombocytopenia can increase the risk of potentially serious bleeding complications. While the deleterious impact of cytopenias are clear, understanding the threshold at which the benefits of transfusion outweigh the harms is not straightforward and based on clinical judgment. RBC transfusions in MDS may be used to improve quality of life and mitigate end-organ injury; while no universal threshold exists, patients with hemoglobin <8 g/dL often see symptomatic benefits [64]. An ongoing study for both lower- and higher-risk MDS, NCT02099669, is evaluating the impact of an RBC transfusion goal of 11–12 g/dL versus 8.5–10 g/dL on quality of life in patients with MDS [65].

Prospective evidence for platelet transfusion in MDS is limited, and the practice of transfusion to maintain platelet counts over 10–20 × 10^9/L is largely extrapolated from the leukemia literature [64, 66].

To reduce the risk of alloimmunization to HLA antigens, leukoreduction of red blood cell and platelet products has become nearly universally adopted in the United States and Europe [67]. While alloimmunization to minor red blood cell antigens may be relatively common in the MDS population, these antibodies only rarely complicate bone marrow transplant, and to date minor antigen RBC matching has not routinely been implemented in MDS to prevent alloimmunization [68, 69].

Irradiation is particularly important in patients proceeding to allogeneic stem cell transplant (beginning either during pre-transplant chemotherapy or at the initiation of pre-transplant conditioning chemotherapy) [67, 70].

Moreover, iron overload is associated with increased mortality in patients proceeding to allogeneic transplant, and should be a consideration when developing an approach to red blood cell transfusion in patients with MDS [71].

Summary

Achieving optimal outcomes in patients with MDS requires complete initial assessment, shared decision making with patients to set goals, and close clinical management to guide therapy. While our understanding of the pathophysiology and molecular underpinnings of MDS has continued to improve over recent years, this has not translated yet into new approved therapies. Beyond HMAs and HSCT, clinical trials will remain an important consideration in patients with higher-risk MDS.

References

1. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Sole F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454–65.
2. Montoro J, Vallespi T, Sancho E, Salamero O, Lopez-Andreoni L, Sanchez-Morata C, et al. Study of causes of death in patients with myelodysplastic syndrome: a single institution experience. Blood. 2011;118(21):5026.
3. Nachtkamp K, Stark R, Strupp C, Kundgen A, Giagounidis A, Aul C, et al. Causes of death in 2877 patients with myelodysplastic syndromes. Ann Hematol. 2016;95(6):937–44.
4. Nazha A, Narkhede M, Radivoyevitch T, Seastone DJ, Patel BJ, Gerds AT, et al. Incorporation of molecular data into the Revised International Prognostic Scoring System in treated patients with myelodysplastic syndromes. Leukemia. 2016;30(11):2214–20.
5. Hou H-A, Tsai C-H, Lin C-C, Chou W-C, Kuo Y-Y, Liu C-Y, et al. Incorporation of mutations in five genes in the revised International Prognostic Scoring System can improve risk stratification in the patients with myelodysplastic syndrome. Blood Cancer J. 2018;8(4):39.
6. van de Loosdrecht AA, Ireland R, Kern W, Della Porta MG, Alhan C, Balleisen JS, et al. Rationale for the clinical application of flow cytometry in patients with myelodysplastic syndromes: position paper of an International Consortium and the European LeukemiaNet Working Group. Leuk Lymphoma. 2013;54(3):472–5.
7. Lode L, Menard A, Flet L, Richebourg S, Loirat M, Eveillard M, et al. Emergence and evolution of TP53 mutations are key features of disease progression in myelodysplastic patients with lower-risk del(5q) treated with lenalidomide. Haematologica. 2018;103(4):e143–6.
8. Neukirchen J, Lauseker M, Hildebrandt B, Nolting A-C, Kaivers J, Kobbe G, et al. Cytogenetic clonal evolution in myelodysplastic syndromes is associated with inferior prognosis. Cancer. 2017;123(23):4608–16.
9. da Silva-Coelho P, Kroeze LI, Yoshida K, Koorenhof-Scheele TN, Knops R, van de Locht LT, et al. Clonal evolution in myelodysplastic syndromes. Nat Commun. 2017;8:15099.
10. Deschler B, Ihorst G, Platzbecker U, Germing U, Marz E, de Figuerido M, et al. Parameters detected by geriatric and quality of life assessment in 195 older patients with myelodysplastic syndromes and acute myeloid leukemia are highly predictive for outcome. Haematologica. 2013;98(2):208–16.
11. Fega KR, Abel GA, Motyckova G, Sherman AE, DeAngelo DJ, Steensma DP, et al. Non-hematologic predictors of mortality improve the prognostic value of the international prognostic scoring system for MDS in older adults. J Geriatr Oncol. 2015;6(4):288–98.
12. Abel GA, Klepin HD. Frailty and the management of hematologic malignancies. Blood. 2018;131(5):515–24.
13. DeZern AE. Nine years without a new FDA-approved therapy for MDS: how can we break through the impasse? Hematology Am Soc Hematol Educ Program. 2015;2015:308–16.
14. Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol. 2002;20(10):2429–40.
15. Kantarjian H, Issa J-PJ, Rosenfeld CS, Bennett JM, Albitar M, DiPersio J, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer. 2006;106(8):1794–803.
16. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009;10(3):223–32.
17. Lubbert M, Suciu S, Baila L, Ruter BH, Platzbecker U, Giagounidis A, et al. Low-dose decitabine versus best supportive care in elderly patients with intermediate- or high-risk myelo-

dysplastic syndrome (MDS) ineligible for intensive chemotherapy: final results of the randomized phase III study of the European Organisation for Research and Treatment of Cancer Leukemia Group and the German MDS Study Group. J Clin Oncol. 2011;29(15):1987–96.
18. Fenaux P, Bowen D, Gattermann N, Hellstrom-Lindberg E, Hofmann W-K, Pfeilstocker M, et al. Practical use of azacitidine in higher-risk myelodysplastic syndromes: an expert panel opinion. Leuk Res. 2010;34(11):1410–6.
19. Lee Y-G, Kim I, Yoon S-S, Park S, Cheong JW, Min YH, et al. Comparative analysis between azacitidine and decitabine for the treatment of myelodysplastic syndromes. Br J Haematol. 2013;161(3):339–47.
20. DeZern AE, Zeidan AM, Barnard J, Hand W, Al Ali N, Brown F, et al. Differential response to hypomethylating agents based on sex: a report on behalf of the MDS Clinical Research Consortium (MDS CRC). Leuk Lymphoma. 2017;58(6):1325–31.
21. Sekeres MA, Cutler C. How we treat higher-risk myelodysplastic syndromes. Blood. 2014;123(6):829.
22. Sekeres MA, Tiu RV, Komrokji R, Lancet J, Advani AS, Afable M, et al. Phase 2 study of the lenalidomide and azacitidine combination in patients with higher-risk myelodysplastic syndromes. Blood. 2012;120(25):4945–51.
23. Prebet T, Sun Z, Figueroa ME, Ketterling R, Melnick A, Greenberg PL, et al. Prolonged administration of azacitidine with or without entinostat for myelodysplastic syndrome and acute myeloid leukemia with myelodysplasia-related changes: results of the US Leukemia Intergroup trial E1905. J Clin Oncol. 2014;32(12):1242–8.
24. Issa J-P, Garcia-Manero G, Huang X, Cortes J, Ravandi F, Jabbour E, et al. Results of phase 2 randomized study of low-dose decitabine with or without valproic acid in patients with myelodysplastic syndrome and acute myelogenous leukemia. Cancer. 2015;121(4):556–61.
25. Sekeres MA, Othus M, List AF, Odenike O, Stone RM, Gore SD, et al. Randomized phase II study of azacitidine alone or in combination with lenalidomide or with vorinostat in higher-risk myelodysplastic syndromes and chronic myelomonocytic leukemia: North American Intergroup Study SWOG S1117. J Clin Oncol. 2017;35(24):2745–53.
26. Garcia-Manero G, Sekeres MA, Egyed M, Alimena G, Graux C, Cavenagh JD, et al. Panobinostat plus azacitidine in adult patients with MDS, CMML, or AML: results of a phase 2b study. Blood. 2015;126(23):2861.
27. Garcia-Manero G, Berdeja JG, Komrokji RS, Essell J, Lyons RM, Maris M, et al. A randomized, placebo-controlled, phase II study of Pracinostat in combination with azacitidine (AZA) in patients with previously untreated myelodysplastic syndrome (MDS). Blood. 2015;126(23):911.
28. Hofmann WK, Heil G, Zander C, Wiebe S, Ottmann OG, Bergmann L, et al. Intensive chemotherapy with idarubicin, cytarabine, etoposide, and G-CSF priming in patients with advanced myelodysplastic syndrome and high-risk acute myeloid leukemia. Ann Hematol. 2004 Aug;83(8):498–503.
29. Kantarjian HM, Jabbour E, DiNardo CD, Borthakur G, Daver NG, Faderl SH, et al. Outcome of patients with myelodysplastic syndrome (MDS) with bone marrow blasts between 10–30% treated with hypomethylating agents versus intensive chemotherapy. Nazha A, editor. Blood. 2013;122(21):2788.
30. de Witte T, Bowen D, Robin M, Malcovati L, Niederwieser D, Yakoub-Agha I, et al. Allogeneic hematopoietic stem cell transplantation for MDS and CMML: recommendations from an international expert panel. Blood. 2017;129(13):1753–62.
31. Welch JS, Petti AA, Miller CA, Fronick CC, O'Laughlin M, Fulton RS, et al. TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. N Engl J Med. 2016;375(21):2023–36.
32. Chang C-K, Zhao Y-S, Xu F, Guo J, Zhang Z, He Q, et al. TP53 mutations predict decitabine-induced complete responses in patients with myelodysplastic syndromes. Br J Haematol. 2017;176(4):600–8.

33. Müller-Thomas C, Rudelius M, Rondak I-C, Haferlach T, Schanz J, Huberle C, et al. Response to azacitidine is independent of p53 expression in higher-risk myelodysplastic syndromes and secondary acute myeloid leukemia. Haematologica. 2014;99(10):e179–81.
34. Bally C, Adès L, Renneville A, Sebert M, Eclache V, Preudhomme C, et al. Prognostic value of TP53 gene mutations in myelodysplastic syndromes and acute myeloid leukemia treated with azacitidine. Leuk Res. 2014;38(7):751–5.
35. Hunter AM, Sallman DA. Current status and new treatment approaches in TP53 mutated AML. Best Pract Res Clin Haematol. 2019;32(2):134–44.
36. Wells RA, Leber B, Zhu NY, Storring JM. Optimizing outcomes with azacitidine: recommendations from Canadian centres of excellence. Curr Oncol. 2014;21(1):44–50.
37. Platzbecker U, Middeke JM, Sockel K, Herbst R, Wolf D, Baldus CD, et al. Measurable residual disease-guided treatment with azacitidine to prevent haematological relapse in patients with myelodysplastic syndrome and acute myeloid leukaemia (RELAZA2): an open-label, multicentre, phase 2 trial. Lancet Oncol. 2018;19(12):1668–79.
38. Jabbour EJ, Garcia-Manero G, Strati P, Mishra A, Al Ali NH, Padron E, et al. Outcome of patients with low-risk and intermediate-1-risk myelodysplastic syndrome after hypomethylating agent failure: a report on behalf of the MDS Clinical Research Consortium. Cancer. 2015;121(6):876–82.
39. Prebet T, Gore SD, Esterni B, Gardin C, Itzykson R, Thepot S, et al. Outcome of high-risk myelodysplastic syndrome after azacitidine treatment failure. J Clin Oncol. 2011;29(24):3322–7.
40. Fletcher SA, Cronin AM, Zeidan AM, Odejide OO, Gore SD, Davidoff AJ, et al. Intensity of end-of-life care for patients with myelodysplastic syndromes: findings from a large national database. Cancer. 2016;122(8):1209–15.
41. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241–7.
42. Malcovati L, Karimi M, Papaemmanuil E, Ambaglio I, Jadersten M, Jansson M, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015;126(2):233–41.
43. Kennedy JA, Ebert BL. Clinical implications of genetic mutations in myelodysplastic syndrome. J Clin Oncol. 2017;35(9):968–74.
44. Sallman DA, DeZern AE, Steensma DP, Sweet KL, Cluzeau T, Sekeres MA, et al. Phase 1b/2 combination study of APR-246 and azacitidine (AZA) in patients with TP53 mutant myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Blood. 2018;132(Suppl 1):3091.
45. DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, et al. Durable remissions with ivosidenib in IDH1-mutated relapsed or refractory AML. N Engl J Med. 2018;378(25):2386–98.
46. Blaschke K, Ebata KT, Karimi MM, Zepeda-Martínez JA, Goyal P, Mahapatra S, et al. Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells. Nature. 2013;500(7461):222–6.
47. Cimmino L, Dolgalev I, Wang Y, Yoshimi A, Martin GH, Wang J, et al. Restoration of TET2 function blocks aberrant self-renewal and leukemia progression. Cell. 2017;170(6):1079–1095.e20.
48. Della Porta MG, Jackson CH, Alessandrino EP, Rossi M, Bacigalupo A, van Lint MT, et al. Decision analysis of allogeneic hematopoietic stem cell transplantation for patients with myelodysplastic syndrome stratified according to the revised International Prognostic Scoring System. Leukemia. 2017;31(11):2449–57.
49. Runde V, de Witte T, Arnold R, Gratwohl A, Hermans J, van Biezen A, et al. Bone marrow transplantation from HLA-identical siblings as first-line treatment in patients with myelodysplastic syndromes: early transplantation is associated with improved outcome. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant. 1998;21(3):255–61.

50. Nakai K, Kanda Y, Fukuhara S, Sakamaki H, Okamoto S, Kodera Y, et al. Value of chemotherapy before allogeneic hematopoietic stem cell transplantation from an HLA-identical sibling donor for myelodysplastic syndrome. Leukemia. 2005;19(3):396–401.
51. Sierra J, Perez WS, Rozman C, Carreras E, Klein JP, Rizzo JD, et al. Bone marrow transplantation from HLA-identical siblings as treatment for myelodysplasia. Blood. 2002;100(6):1997–2004.
52. Onida F, Brand R, van Biezen A, Schaap M, von dem Borne PA, Maertens J, et al. Impact of the International Prognostic Scoring System cytogenetic risk groups on the outcome of patients with primary myelodysplastic syndromes undergoing allogeneic stem cell transplantation from human leukocyte antigen-identical siblings: a retrospective analysis of the European Society for Blood and Marrow Transplantation-Chronic Malignancies Working Party. Haematologica. 2014;99(10):1582–90.
53. Damaj G, Duhamel A, Robin M, Beguin Y, Michallet M, Mohty M, et al. Impact of azacitidine before allogeneic stem-cell transplantation for myelodysplastic syndromes: a study by the Societe Francaise de Greffe de Moelle et de Therapie-Cellulaire and the Groupe-Francophone des Myelodysplasies. J Clin Oncol. 2012;30(36):4533–40.
54. Gerds AT, Gooley TA, Estey EH, Appelbaum FR, Deeg HJ, Scott BL. Pretransplantation therapy with azacitidine vs induction chemotherapy and posttransplantation outcome in patients with MDS. Biol Blood Marrow Transplant. 2012;18(8):1211–8.
55. Merkel D, Filanovsky K, Gafter-Gvili A, Vidal L, Aviv A, Gatt ME, et al. Predicting infections in high-risk patients with myelodysplastic syndrome/acute myeloid leukemia treated with azacitidine: a retrospective multicenter study. Am J Hematol. 2013;88(2):130–4.
56. Quinto AM, De March E, Castelli M, Piazza F, Adami F, Semenzato G, et al. Infections in patients with myelodysplastic syndrome/acute myeloid leukemia treated with azacitidine: report from a single center. Blood. 2014;124(21):5622.
57. Lee J-H, Lee K-H, Lee J-H, Kim D-Y, Kim S-H, Lim S-N, et al. Decreased incidence of febrile episodes with antibiotic prophylaxis in the treatment of decitabine for myelodysplastic syndrome. Leuk Res. 2011;35(4):499–503.
58. National Comprehensive Cancer Network. Myelodysplastic sydromes (Version 2.2019). [Internet]. 2018 [cited 2019 Jul 3]. Available from: https://www.nccn.org/professionals/physician_gls/PDF/mds.pdf.
59. Itzykson R, Thepot S, Beyne-Rauzy O, Ame S, Isnard F, Dreyfus F, et al. Does addition of erythropoiesis stimulating agents improve the outcome of higher-risk myelodysplastic syndromes treated with azacitidine? Leuk Res. 2012;36(4):397–400.
60. Kantarjian HM, Giles FJ, Greenberg PL, Paquette RL, Wang ES, Gabrilove JL, et al. Phase 2 study of romiplostim in patients with low- or intermediate-risk myelodysplastic syndrome receiving azacitidine therapy. Blood. 2010;116(17):3163–70.
61. Greenberg PL, Garcia-Manero G, Moore M, Damon L, Roboz G, Hu K, et al. A randomized controlled trial of romiplostim in patients with low- or intermediate-risk myelodysplastic syndrome receiving decitabine. Leuk Lymphoma. 2013;54(2):321–8.
62. Mittelman M, Platzbecker U, Afanasyev B, Grosicki S, Wong RSM, Anagnostopoulos A, et al. Eltrombopag for advanced myelodysplastic syndromes or acute myeloid leukaemia and severe thrombocytopenia (ASPIRE): a randomised, placebo-controlled, phase 2 trial. Lancet Haematol. 2018;5(1):e34–43.
63. Malcovati L, Della Porta MG, Strupp C, Ambaglio I, Kuendgen A, Nachtkamp K, et al. Impact of the degree of anemia on the outcome of patients with myelodysplastic syndrome and its integration into the WHO classification-based Prognostic Scoring System (WPSS). Haematologica. 2011;96(10):1433–40.
64. Malcovati L, Hellstrom-Lindberg E, Bowen D, Ades L, Cermak J, Del Canizo C, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood. 2013;122(17):2943–64.
65. Gu Y, Estcourt LJ, Doree C, Hopewell S, Vyas P. Comparison of a restrictive versus liberal red cell transfusion policy for patients with myelodysplasia, aplastic anaemia, and other congenital bone marrow failure disorders. Cochrane Database Syst Rev. 2015;10(10):CD011577.

66. Rebulla P, Finazzi G, Marangoni F, Avvisati G, Gugliotta L, Tognoni G, et al. The threshold for prophylactic platelet transfusions in adults with acute myeloid leukemia. Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto. N Engl J Med. 1997;337(26):1870–5.
67. Gajewski JL, Johnson VV, Sandler SG, Sayegh A, Klumpp TR. A review of transfusion practice before, during, and after hematopoietic progenitor cell transplantation. Blood. 2008;112(8):3036–47.
68. Novaretti MC, Sopelete CR, Velloso ER, Rosa MF, Dorlhiac-Llacer PE, Chamone DA. Immunohematological findings in myelodysplastic syndrome. Acta Haematol. 2001; 105(1):1–6.
69. Booth GS, Gehrie EA, Savani BN. Minor RBC Ab and allo-SCT. Bone Marrow Transplant. 2014;49(3):456–7.
70. Treleaven J, Gennery A, Marsh J, Norfolk D, Page L, Parker A, et al. Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force. Br J Haematol. 2011;152(1):35–51.
71. Koreth J, Antin JH. Iron overload in hematologic malignancies and outcome of allogeneic hematopoietic stem cell transplantation. Haematologica. 2010;95(3):364–6.
72. Garcia-Delgado R, de Miguel D, Bailen A, Gonzalez JR, Bargay J, Falantes JF, et al. Effectiveness and safety of different azacitidine dosage regimens in patients with myelodysplastic syndromes or acute myeloid leukemia. Leuk Res. 2014;38(7):744–50.
73. Lyons RM, Cosgriff TM, Modi SS, Gersh RH, Hainsworth JD, Cohn AL, et al. Hematologic response to three alternative dosing schedules of azacitidine in patients with myelodysplastic syndromes. J Clin Oncol. 2009;27(11):1850–6.
74. Kantarjian H, Oki Y, Garcia-Manero G, Huang X, O'Brien S, Cortes J, et al. Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. Blood. 2007;109(1):52–7.
75. Ball B, Komrokji RS, Adès L, Sekeres MA, DeZern AE, Pleyer L, et al. Evaluation of induction chemotherapies after hypomethylating agent failure in myelodysplastic syndromes and acute myeloid leukemia. Blood Adv. 2018;2(16):2063–71.
76. Jabbour E, Faderl S, Sasaki K, Kadia T, Daver N, Pemmaraju N, et al. Phase 2 study of low-dose clofarabine plus cytarabine for patients with higher-risk myelodysplastic syndrome who have relapsed or are refractory to hypomethylating agents. Cancer. 2017;123(4): 629–37.
77. Miller KB, Kim K, Morrison FS, Winter JN, Bennett JM, Neiman RS, et al. The evaluation of low-dose cytarabine in the treatment of myelodysplastic syndromes: a phase-III intergroup study. Ann Hematol. 1992;65(4):162–8.
78. Cherian MA, Tibes R, Gao F, Fletcher T, Fiala M, Uy GL, et al. A study of high-dose lenalidomide induction and low-dose lenalidomide maintenance therapy for patients with hypomethylating agent refractory myelodysplastic syndrome. Leuk Lymphoma. 2016;57(11): 2535–40.
79. Zeidan AM, Smith BD, Carraway HE, Gojo I, DeZern A, Gore SD. A phase 2 trial of high dose lenalidomide in patients with relapsed/refractory higher-risk myelodysplastic syndromes and acute myeloid leukaemia with trilineage dysplasia. Br J Haematol. 2017;176(2): 241–7.
80. Borthakur G, Ahdab SE, Ravandi F, Faderl S, Ferrajoli A, Newman B, et al. Activity of decitabine in patients with myelodysplastic syndrome previously treated with azacitidine. Leuk Lymphoma. 2008;49(4):690–5.
81. Harel S, Cherait A, Berthon C, Willekens C, Park S, Rigal M, et al. Outcome of patients with high risk Myelodysplastic Syndrome (MDS) and advanced Chronic Myelomonocytic Leukemia (CMML) treated with decitabine after azacitidine failure. Leuk Res. 2015;39(5): 501–4.
82. Apuri S, Al Ali N, Padron E, Lancet JE, List AF, Komrokji RS. Evidence for selective benefit of sequential treatment with hypomethylating agents in patients with myelodysplastic syndrome. Clin Lymphoma Myeloma Leuk. 2017;17(4):211–4.

Chapter 10
Hematopoietic Cell Transplantation for MDS Patients

Anne Sophie Kubasch and Uwe Platzbecker

Introduction

Allogeneic hematopoietic cell transplantation (HCT) remains the only potential curative therapy for a small subset of fit patients with myelodysplastic syndromes (MDS) [1], because currently available targeted therapeutic agents may lead to prolongation of overall survival but no cure of MDS. Individual stratification based on age, comorbidities, and MDS risk scores [2] is important to select patients for HCT, because overall only 10% of patients are potential candidates. In general, the earlier the transplantation takes place during the disease course, the better the chances of long-term cure [3]. Contrarily, patients with less advanced disease and without high-risk cytogenetic and molecular features should not be exposed to the risk associated with this procedure, because within the first year after HCT there is an approximately 20% risk of treatment-related mortality (TRM) [4]. Thus, the selection of the right patient population, the appropriate timing of HCT, and the optimal conditioning regimen are key questions that must be addressed. The introduction of reduce-intensity conditioning (RIC) regimens have substantially extended the use of HCT also to older patients with reduced fitness or present comorbidities [2]. Nevertheless, careful consideration should be given to who will optimally benefit from an HCT approach. In addition, relapse remains the main cause of failure for HCT and novel conditioning regimens and post-HCT prophylactic approaches are demanded [4].

A. S. Kubasch · U. Platzbecker (✉)
Medical Clinic and Policlinic, Department of Hematology, Cellular Therapy and Hemostaseology, Leipzig University Hospital, Leipzig, Germany

German MDS Study Group (D-MDS), Leipzig, Germany

The European Myelodysplastic Syndromes Cooperative Group (EMSCO), Leipzig, Germany
e-mail: uwe.platzbecker@medizin.uni-leipzig.de

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3_10

Indication and Timing for Allogeneic Hematopoietic Stem Cell Transplantation

Because the clinical course of MDS is highly variable, an accurate assessment of the prognosis by IPSS/IPSS-R is essential before deciding about HCT [2]. Cutler et al. made an attempt to facilitate the decision process and carried out an analysis to determine which approach offers the longest life expectancy [5]. Results showed that in transplant-eligible patients with lower-risk disease, HCT may be best carried out when progression occurs to IPSS intermediate-2 risk [5]. Patients with less advanced disease and good quality of life should not be exposed to the substantial risk of mortality of this procedure due to the favorable prognosis with standard treatment alone [1]. Nevertheless, the earlier the HCT is performed during the disease course, the better are the long-term results [6]. A prior study by the European Society for Blood and Marrow Transplantation (EBMT) in 246 IPSS low/intermediate-1 patients demonstrated a 3-year survival rate of 58% and a 30% overall non-relapse mortality rate [7]. Thus, clinically fit patients with lower-risk MDS failing first-line standard of care treatment options and harboring poor-risk features including frequent RBC transfusions (≥2 units per month), life-threatening cytopenias (neutrophil counts, <0.5 × 109/L, or platelet counts, <30 × 109/L), very poor prognostic cytogenetic or molecular markers like RUNX1, EZH2, NRAS, TP53, or ASXL1 should be considered for HCT as appropriate candidates if no clinical trial is available [6].This may be especially important for the large group of lower-risk patients with a high transfusion burden and lack of response to erythropoietin-stimulating agent (ESA) [1, 6].

For higher-risk (IPSS intermediate-2 and high risk) transplant-eligible patients, HCT should be performed as early as possible in the disease course, since any delay appears to be associated with a loss in life years [1, 5]. On the other hand, considering the potential treatment-related complications associated with HCT, that is, GvHD and infections, the stringent selection of patients by identifying the patient- and disease-related factors is unavoidable and an important predictor of outcome after HCT [6, 8]. HCT should be considered in patients up to the age of 70–75 years, in MDS patients with intermediate-2/high or IPSS-R high/very high in good clinical condition, and without severe comorbidities if an HLA-matched donor is available [9].

Moreover, patients that do not respond or lose response to established non-transplant therapies like hypomethylating agents (HMA) including azacitidine and decitabine are also potential candidates [1, 2]. In fact, higher-risk MDS patients who fail HMA treatment typically have very poor prognosis with a median survival of 5–6 months with best supportive care only [10, 11]. Given the dismal prognosis of HMA failure and the current lack of available other treatment options, HCT should be considered for those patients. Overall, choosing the optimal candidate and timing for transplant and integrating HCT into the therapeutic algorithm remains a challenge in many cases and the pros and cons of this procedure should be discussed in detail with the patients [1].

Risk Factors Influencing Outcome After Allogeneic Hematopoietic Cell Transplantation

Comorbidities, frailty, performance status (e.g., Karnofsky score), and age are relevant patient-related factors that determine outcome after HCT [12, 13]. In addition to conventional prognostic scoring systems like IPSS and IPSS-R, tools like the hematopoietic cell transplantation-specific comorbidity index (HCT-CI) demonstrated a strong prognostic impact on outcome after HCT [3, 12, 14]. The HCT-CI was developed to enable HCT-related risk assessment and to identify relevant comorbidities in the HCT population [3]. The comorbidity index comprises 17 different categories of organ dysfunction influencing non-relapse mortality and overall survival (OS) in the HCT setting. Positive findings are summated into a total score that enables the classification of patients into three risk groups: low risk (non-relapse mortality 14% at 2 years), intermediate risk (non-relapse mortality 21% at 2 years), and high risk (non-relapse mortality 41% at 2 years) [3].

Regarding pre-transplant blast count, patients with less than 5% bone marrow blasts showed a better outcome after HCT in prior studies [15]. For the rest of the primarily HCT-treated MDS patients, the overall survival was not significantly influenced by the percentage of bone marrow blasts [6], but fit, higher-risk patients with bone marrow blasts of more than 10% should be considered for early HCT after prior HMA or intensive chemotherapy treatment [6]. Concerning cytogenetic risk classification, complex karyotype abnormalities and monosomal karyotype predict for increased mortality, higher rates of relapse, and inferior survival after HCT [16]. When considering the mutational profile of the transplant-eligible patients, high risk somatic mutations like *TP53*, *RUNX1*, and *ASXL1* are independently associated with adverse outcome and shorter survival after allogeneic HCT [17]. In a prior small study including 87 transplant eligible patients, Bejar et al. demonstrated that mutations in *TP53*, *TET2*, or *DNMT3A* identify patients with shorter OS after HCT [18]. Subsequent larger studies could not confirm these results and showed that *TET2* and *DNMT3A* mutations had no impact on transplant outcomes [19]. Moreover, Della Porta et al. showed that in patients with MDS/AML, somatic mutation like *ASXL1*, *RUNX1*, or *TP53* are independently associated with unfavorable outcomes and shorter survival after allogeneic HCT [17]. Lindsley et al. also evaluated the association of mutations with transplantation outcomes like overall survival, relapse, and death without relapse in 1514 patients with MDS. Again, *TP53* mutations were associated with shorter survival and a shorter time to relapse compared to TP53 wild-type patients. Moreover, the emergence of *TP53* mutation in combination with a complex karyotype resulted in an unfavorable outcome and early relapse after HCT in prior studies [18]. In patients without *TP53* mutation, the presence of RAS pathway mutations was associated with shorter survival and a high risk of relapse. *JAK2* mutations were also associated with shorter survival and a high risk of death without relapse [20]. Thus, alternative conventional therapies (e.g., APR236) or disease-specific post-transplant strategies to prevent relapse are demanded for this patient population carrying high-risk somatic mutations.

Cytoreductive Treatment Prior to Allogeneic Hematopoietic Stem Cell Transplantation

Upfront HCT in higher-risk MDS patients is currently recommended in patients with less than 10% bone marrow blasts [6]. In the absence of randomized trials, the value of prior induction chemotherapy to reduce the percentage of bone marrow blasts prior HCT remains unclear [1]. A few retrospective studies have addressed the question, which cytoreductive approach prior to HCT conditioning is associated with superior outcome [21, 22]. Comparing intensive chemotherapy versus HMA therapy prior to the HCT, the relapse rates post HCT were similar for both cohorts after adjustment for several prognostic factors including cytogenetic risk [22]. Thus, a reduced toxicity approach using HMA treatment in order to "bridge" the time up to the identification of a compatible donor [1, 2] prior to conditioning for HCT is currently the preferred treatment in many centers. Nevertheless, there remains a substantial number of patients who display disease progression or severe infectious complications during the first 4 months of pre-transplant therapy and therefore cannot undergo subsequent transplantation.

In patients with an anticipated short-term benefit of HMAs (e.g., due to the presence of a complex karyotypes), HCT should be planned as early as possible because of the dismal prognosis of patients failing HMA therapy with a median survival time of less than 6 months [10]. In these cases, exposition to HMAs should be limited with the goal to achieve the highest potential reduction in disease burden prior to transplantation [1, 6]. The VidazaALLO study compared the 3-year overall survival after single agent azacitidine treatment with azacitidine followed by HCT according to donor availability in elderly patients with newly diagnosed untreated high-risk MDS aged 55–70 years (NCT01404741). Within the first 3 years, patients treated with azacitidine followed by HCT had an overall survival of 49% (95% CI: 36–61%) compared to 22% (95% CI: 6–44%) with azacitidine monotherapy. Thus, the VidazaALLO study demonstrated an improved event-free survival and overall survival in favor of HCT [23].

When considering remission-induction using intensive chemotherapy regimens, prior studies demonstrated considerable toxicity leading to treatment-related mortality (TRM) in up to 16% of transplant-eligible patients [24]. The higher response rates and better tolerability of the liposomal cytarabine-daunorubicin formulation (CPX-351) compared to conventional chemotherapy makes it an attractive treatment opportunity prior to transplant. Within the German MDS study group, the randomized PALOMA study is currently comparing CPX-351 versus azacitidine versus intensive chemotherapy treatment prior to HCT in patients with higher-risk MDS and oligoblastic AML (NCT04061239).

Moreover, it is widely accepted that systemic iron overload directly contributes to outcome after HCT in MDS [25, 26]. Available data showed that patients with either higher ferritin or a pre-transplant liver iron content greater than or equal to 125 μmol/g had an increased incidence of non-relapse mortality after HCT [27]. The results of the ALLIVE study demonstrated that elevated labile plasma iron (LPI) levels before or during HCT predict an increased incidence of

treatment-related non-relapse mortality (33% vs 7%) and a decreased overall survival in patients with AML or MDS [27]. Therefore, eligible patients should receive appropriate iron chelation prior to HCT.

Conditioning Intensity Prior to Allogeneic Hematopoietic Stem Cell Transplantation

As the intensity of transplant conditioning is linked to mortality, the development of reduced intensity conditioning (RIC) regimens has allowed the successful application of HCT in older patients with MDS [1, 28]. Recent retrospective analyses have suggested that HCT in older higher-risk MDS patients undergoing RIC regimens is superior compared to treatment with HMA, although the observed benefit occurred later following HCT [29, 30]. Many retrospective studies have assessed the value of RIC regimens compared with conventional myeloablative conditioning (MAC) regimens in patients with MDS. Kröger et al. demonstrated that RIC resulted in at least a 2-year relapse-free survival and overall survival similar to MAC in patients with MDS or secondary AML and a median age of 50 years [29]. In contrast to these results, Scott et al. showed a non-significant higher overall survival following MAC compared to RIC [30]. Moreover, RIC was associated with a lower treatment-related mortality but higher relapse rates compared with MAC [30]. These results support that higher-risk patients with good performance status and no comorbidities are candidates for MA regimens, but less fit and comorbid patients should be considered for RIC schedules [6].

Post-transplantation Strategies

Since HCT represents an intensive and possible curative treatment for eligible MDS patients, relapse after HCT remains one of the most important causes of treatment failure and mortality with very limited salvage therapies. While many patients have a high early mortality from relapse, some respond to salvage treatment and achieve sustained remissions. In fact, the risk of relapse is mainly determined by the disease stage at the time of transplantation and the relapse rate of patients is significantly influenced by the cytogenetic risk, exceeding 50% in patients with very poor-risk karyotype according to the IPSS-R [1, 2, 8]. Declining donor chimerism or mixed chimerism early after HCT are usually considered signs of imminent relapse. Measurement of chimerism in sorted CD34 cells has been used as minimal residual disease (MRD) monitoring after HCT in MDS [6]. Therapeutic options for MDS relapse after HCT consist of treatment with HMA or intensive chemotherapy, donor lymphocyte infusions (DLIs), second HSCT, or palliative care [1, 6]. DLIs can be administered prophylactically at the time of persisting or declining mixed donor chimerism or therapeutically in cases of confirmed relapse.

Oral azacitidine is currently under investigation as maintenance therapy following HCT in higher-risk MDS or AML patients [1]. Recently, the phase 3, randomized, placebo-controlled QUAZAR AML-001 study demonstrated that maintenance treatment with oral azacitidine (CC-486) [31] results in a significant improvement in overall survival compared to placebo in newly diagnosed AML patients after achieving the first complete response (CR) or complete response with incomplete blood count recovery (CRi) with prior induction chemotherapy [31].

Alternative approaches include pre-emptive MRD-triggered azacitidine treatments as shown by the recently published results of the multicenter prospective RELAZA2 trial [32]. Patients who had achieved a CR after conventional chemotherapy or HSCT were prospectively screened for MRD by either quantitative PCR for mutant *NPM1*, leukemia-specific fusion genes (DEK-NUP214, RUNX1-RUNX1T1, CBFb-MYH11), or analysis of donor-chimerism in flow cytometry-sorted CD34-positive cells. MRD-positive patients in confirmed CR received azacytidine treatment. After the first six cycles, MRD status was reassessed and patients with major responses (MRD negativity) were eligible for a treatment de-escalation. Six months after initiation of azacitidine, 58% patients were relapse-free and alive ($p < 0.0001$). Thus, MRD-guided pre-emptive therapy with azacitidine was able to prevent or delay hematological relapse in these MRD-positive patients with MDS or AML who are at a high risk of relapse [32]. Further studies may incorporate novel strategies to prevent relapse as either pre-emptive or maintenance therapy into their concepts.

Conclusion

HCT remains the only potential curative therapy for patients with MDS. Choosing the right candidates and the optimal moment for transplant remains a challenge in many cases. Fit patients with IPSS intermediate 2 or high-risk MDS should be transplanted early in their disease course, if a suitable HLA-matched related or unrelated donor is available [1, 6]. In MDS patients with lower-risk IPSS and without poor-risk features, HCT can be postponed until disease progression to higher-risk disease. Older patients (>60 or 65 years of age) and patients with clinically relevant comorbidities can still be candidates for lower-intensity conditioning regimens [1, 6]. Clinical trials, which investigate less toxic but intensive regimens prior HCT and further prophylactic strategies to prevent relapse are currently recruiting and results are eagerly awaited.

References

1. Platzbecker U. Treatment of MDS. Blood [Internet]. 2019;133(10):1096–107. Available from: http://www.bloodjournal.org/lookup/doi/10.1182/blood-2018-10-844696.
2. Platzbecker U, Giagounidis A. Allogeneic HSCT in patients with MDS. TumorDiagnostik Ther [Internet]. 2014;35(07). Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0034-1389177.

3. Sorror ML. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood [Internet]. 2005;106(8):2912–9. Available from: http://www.bloodjournal.org/cgi/doi/10.1182/blood-2005-05-2004.
4. Schetelig J, de Wreede LC, van Gelder M, Koster L, Finke J, Niederwieser D, et al. Late treatment-related mortality versus competing causes of death after allogeneic transplantation for myelodysplastic syndromes and secondary acute myeloid leukemia. Leukemia [Internet]. 2019;33(3):686–95. Available from: http://www.nature.com/articles/s41375-018-0302-y.
5. Cutler CS. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. Blood [Internet]. 2004;104(2):579–85. Available from: http://www.bloodjournal.org/cgi/doi/10.1182/blood-2004-01-0338.
6. de Witte T, Bowen D, Robin M, Malcovati L, Niederwieser D, Yakoub-Agha I, et al. Allogeneic hematopoietic stem cell transplantation for MDS and CMML: recommendations from an international expert panel. Blood [Internet]. 2017;129(13):1753–62. Available from: http://www.bloodjournal.org/lookup/doi/10.1182/blood-2016-06-724500.
7. Robin M, Porcher R, Zinke-Cerwenka W, van Biezen A, Volin L, Mufti G, et al. Allogeneic haematopoietic stem cell transplant in patients with lower risk myelodysplastic syndrome: a retrospective analysis on behalf of the Chronic Malignancy Working Party of the EBMT. Bone Marrow Transplant [Internet]. 2017;52(2):209–15. Available from: http://www.nature.com/articles/bmt2016266.
8. Platzbecker U, Mufti G. Allogeneic stem cell transplantation in MDS: How? When? Best Pract Res Clin Haematol [Internet]. 2013;26(4):421–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S152169261300056X.
9. Platzbecker U, Schetelig J, Finke J, Trenschel R, Scott BL, Kobbe G, et al. Allogeneic hematopoietic cell transplantation in patients age 60-70 years with de novo high-risk myelodysplastic syndrome or secondary acute myelogenous leukemia: comparison with patients lacking donors who received azacitidine. Biol Blood Marrow Transplant [Internet]. 2012;18(9):1415–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1083879112001826.
10. Prébet T, Gore SD, Esterni B, Gardin C, Itzykson R, Thepot S, et al. Outcome of high-risk myelodysplastic syndrome after azacitidine treatment failure. J Clin Oncol [Internet]. 2011;29(24):3322–7. Available from: http://ascopubs.org/doi/10.1200/JCO.2011.35.8135.
11. Jabbour E, Garcia-Manero G, Batty N, Shan J, O'Brien S, Cortes J, et al. Outcome of patients with myelodysplastic syndrome after failure of decitabine therapy. Cancer [Internet]. 2010;116(16):3830–4.
12. Sorror ML, Sandmaier BM, Storer BE, Maris MB, Baron F, Maloney DG, et al. Comorbidity and disease status–based risk stratification of outcomes among patients with acute myeloid leukemia or myelodysplasia receiving allogeneic hematopoietic cell transplantation. J Clin Oncol [Internet]. 2007;25(27):4246–54. Available from: http://ascopubs.org/doi/10.1200/JCO.2006.09.7865.
13. Lim Z, Brand R, Martino R, van Biezen A, Finke J, Bacigalupo A, et al. Allogeneic hematopoietic stem-cell transplantation for patients 50 years or older with myelodysplastic syndromes or secondary acute myeloid leukemia. J Clin Oncol [Internet]. 2010;28(3):405–11. Available from: http://ascopubs.org/doi/10.1200/JCO.2009.21.8073.
14. Sorror ML, Storb RF, Sandmaier BM, Maziarz RT, Pulsipher MA, Maris MB, et al. Comorbidity-age index: a clinical measure of biologic age before allogeneic hematopoietic cell transplantation. J Clin Oncol [Internet]. 2014;32(29):3249–56. Available from: http://ascopubs.org/doi/10.1200/JCO.2013.53.8157.
15. Runde V, de Witte T, Arnold R, Gratwohl A, Hermans J, van Biezen A, et al. Bone marrow transplantation from HLA-identical siblings as first-line treatment in patients with myelodysplastic syndromes: early transplantation is associated with improved outcome. Bone Marrow Transplant [Internet]. 1998;21(3):255–61. Available from: http://www.nature.com/articles/1701084.
16. van Gelder M, de Wreede LC, Schetelig J, van Biezen A, Volin L, Maertens J, et al. Monosomal karyotype predicts poor survival after allogeneic stem cell transplantation in chromosome 7

abnormal myelodysplastic syndrome and secondary acute myeloid leukemia. Leukemia [Internet]. 2013;27(4):879–88. Available from: http://www.nature.com/articles/leu2012297.
17. Della Porta MG, Gallì A, Bacigalupo A, Zibellini S, Bernardi M, Rizzo E, et al. Clinical effects of driver somatic mutations on the outcomes of patients with myelodysplastic syndromes treated with allogeneic hematopoietic stem-cell transplantation. J Clin Oncol [Internet]. 2016;34(30):3627–37. Available from: http://ascopubs.org/doi/10.1200/JCO.2016.67.3616.
18. Bejar R, Stevenson KE, Caughey B, Lindsley RC, Mar BG, Stojanov P, et al. Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. J Clin Oncol [Internet]. 2014;32(25):2691–8. Available from: http://ascopubs.org/doi/10.1200/JCO.2013.52.3381.
19. Grimm J, Bill M, Jentzsch M, Beinicke S, Häntschel J, Goldmann K, et al. Clinical impact of clonal hematopoiesis in acute myeloid leukemia patients receiving allogeneic transplantation. Bone Marrow Transplant [Internet]. 2019;54:1189–97.
20. Lindsley RC, Saber W, Mar BG, Redd R, Wang T, Haagenson MD, et al. Prognostic mutations in myelodysplastic syndrome after stem-cell transplantation. N Engl J Med [Internet]. 2017;376(6):536–47. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1611604.
21. Damaj G, Duhamel A, Robin M, Beguin Y, Michallet M, Mohty M, et al. Impact of azacitidine before allogeneic stem-cell transplantation for myelodysplastic syndromes: a study by the Société Française de Greffe de Moelle et de Thérapie-Cellulaire and the Groupe-Francophone des Myélodysplasies. J Clin Oncol [Internet]. 2012;30(36):4533–40. Available from: http://ascopubs.org/doi/10.1200/JCO.2012.44.3499.
22. Gerds AT, Gooley TA, Estey EH, Appelbaum FR, Deeg HJ, Scott BL. Pretransplantation therapy with azacitidine vs induction chemotherapy and posttransplantation outcome in patients with MDS. Biol Blood Marrow Transplant [Internet]. 2012;18(8):1211–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1083879112000353.
23. Platzbecker U, Kröger N, et al. 5-azacytidine (5-Aza) induction followed by allogeneic stem cell transplantation versus continuous 5-Aza in elderly MDs patients (55-70 years). A prospective randomized study (VidazaAllo Study). 45th Annual Meeting of the European Society for Blood and Marrow Transplantation (EBMT). Frankfurt, Germany; 2019.
24. Nakai K, Kanda Y, Fukuhara S, Sakamaki H, Okamoto S, Kodera Y, et al. Value of chemotherapy before allogeneic hematopoietic stem cell transplantation from an HLA-identical sibling donor for myelodysplastic syndrome. Leukemia [Internet]. 2005;19(3):396–401. Available from: http://www.nature.com/articles/2403640.
25. Platzbecker U, Bornhäuser M, Germing U, Stumpf J, Scott BL, Kröger N, et al. Red blood cell transfusion dependence and outcome after allogeneic peripheral blood stem cell transplantation in patients with de novo myelodysplastic syndrome (MDS). Biol Blood Marrow Transplant [Internet]. 2008;14(11):1217–25. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1083879108003558.
26. Wermke M, Schmidt A, Middeke JM, Sockel K, von Bonin M, Schonefeldt C, et al. MRI-based liver iron content predicts for nonrelapse mortality in MDS and AML patients undergoing allogeneic stem cell transplantation. Clin Cancer Res [Internet]. 2012;18(23):6460–8. Available from: http://clincancerres.aacrjournals.org/cgi/doi/10.1158/1078-0432.CCR-12-1683.
27. Wermke M, Eckoldt J, Götze KS, Klein SA, Bug G, de Wreede LC, et al. Enhanced labile plasma iron and outcome in acute myeloid leukaemia and myelodysplastic syndrome after allogeneic haemopoietic cell transplantation (ALLIVE): a prospective, multicentre, observational trial. Lancet Haematol [Internet]. 2018;5(5):e201–10. Available from: https://linkinghub.elsevier.com/retrieve/pii/S235230261830036X.
28. Koreth J, Pidala J, Perez WS, Deeg HJ, Garcia-Manero G, Malcovati L, et al. Role of reduced-intensity conditioning allogeneic hematopoietic stem-cell transplantation in older patients with de novo myelodysplastic syndromes: an international collaborative decision analysis. J Clin Oncol [Internet]. 2013;31(21):2662–70. Available from: http://ascopubs.org/doi/10.1200/JCO.2012.46.8652.

29. Kröger N, Iacobelli S, Franke G-N, Platzbecker U, Uddin R, Hübel K, et al. Dose-reduced versus standard conditioning followed by allogeneic stem-cell transplantation for patients with myelodysplastic syndrome: a prospective randomized phase III study of the EBMT (RICMAC trial). J Clin Oncol [Internet]. 2017;35(19):2157–64. Available from: http://ascopubs.org/doi/10.1200/JCO.2016.70.7349.
30. Scott BL, Pasquini MC, Logan BR, Wu J, Devine SM, Porter DL, et al. Myeloablative versus reduced-intensity hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes. J Clin Oncol [Internet]. 2017;35(11):1154–61. Available from: http://ascopubs.org/doi/10.1200/JCO.2016.70.7091.
31. Roboz GJ, Montesinos P, Selleslag D, Wei A, Jang J-H, Falantes J, et al. Design of the randomized, Phase III, QUAZAR AML Maintenance trial of CC-486 (oral azacitidine) maintenance therapy in acute myeloid leukemia. Future Oncol [Internet]. 2016;12(3):293–302. Available from: https://www.futuremedicine.com/doi/10.2217/fon.15.326.
32. Platzbecker U, Middeke JM, Sockel K, Herbst R, Wolf D, Baldus CD, et al. Measurable residual disease-guided treatment with azacitidine to prevent haematological relapse in patients with myelodysplastic syndrome and acute myeloid leukaemia (RELAZA2): an open-label, multicentre, phase 2 trial. Lancet Oncol [Internet]. 2018;19(12):1668–79. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1470204518305801.

Index

A. Nazha (ed.), *Diagnosis and Management of Myelodysplastic Syndromes*,
https://doi.org/10.1007/978-3-030-51878-3