Hematologic Malignancies

H. Joachim Deeg · David T. Bowen
Steven D. Gore · Torsten Haferlach
Michelle M. Le Beau · Charlotte Niemeyer

Myelodysplastic Syndromes

Second Edition

Springer

Hematologic Malignancies

H. Joachim Deeg • David T. Bowen
Steven D. Gore • Torsten Haferlach
Michelle M. Le Beau • Charlotte Niemeyer

Myelodysplastic Syndromes

Second Edition

Springer

H. Joachim Deeg, MD
Division of Clinical Research
Fred Hutchinson Cancer
Research Center
Seattle, WA
USA

University of Washington
School of Medicine
Seattle, WA
USA

David T. Bowen, MD
Department of Hematology
Leeds General Infirmary
Leeds
UK

Steven D. Gore, MD
Department of Pediatrics
The Sidney Kimmel Comprehensive
Cancer Center at Johns Hopkins
Division of Hematologic Malignancies
Johns Hopkins School of Medicine
Baltimore, MD
USA

Torsten Haferlach, MD
MLL Munich Leukemia Laboratory
Munich
Germany

Michelle M. Le Beau, PhD
Section of Hematology/Oncology
Cancer Research Center
University of Chicago
Chicago, IL
USA

Charlotte Niemeyer, MD
Division of Pediatric Hematology
and Oncology
Department of Pediatrics and
Adolescent Medicine
University Hospital of Freiburg
Mathildenstrasse 1
Freiburg
Germany

ISBN 978-3-642-36228-6 ISBN 978-3-642-36229-3 (eBook)
DOI 10.1007/978-3-642-36229-3
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2013943710

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Contents

Contributors

Ulrike Bacher, MD MLL Munchner Leukamie Laboratory, Munich, Germany

David T. Bowen, MD Department of Hematology, Leeds General Infirmary, Leeds, UK

Manojkumar Bupathi Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

Anneclaire J. De Roos Department of Environmental and Occupational Health, Drexel University School of Public Health, Philadelphia, PA, USA

H. Joachim Deeg, MD Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

University of Washington School of Medicine, Seattle, WA, USA

Guillermo Garcia-Manero, MD Department of Leukemia, University of Texas MD Anderson Cancer Center, Houston, TX, USA

Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Steven D. Gore, MD Department of Pediatrics, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Division of Hematologic Malignancies, Johns Hopkins School of Medicine, Baltimore, MD, USA

Sarah M. Greenblatt Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL, USA

Torsten Haferlach, MD MLL Munich Leukemia Laboratory, Munich, Germany

Martin Jadersten Karolinska University Hospital, Huddinge Hematology Center M54, Stockholm, Sweden

Aly Karsan, MD Department of Genome Sciences, British Columbia Cancer Research Centre, Vancouver, BC, Canada

Wolfgang Kern, MD MLL Munich Leukemia Laboratory, Munich, Germany

Michelle M. Le Beau, PhD Section of Hematology/Oncology, Cancer Research Center, University of Chicago, Chicago, IL, USA

Jaroslaw P. Maciejewski, MD, PhD Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

Department of Hematologic Oncology and Blood Disorders, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

Experimental Hematology and Hematopoiesis Section, Taussig Cancer Center/R40, Cleveland, OH, USA

Charlotte Niemeyer, MD Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, University Hospital of Freiburg, Mathildenstrasse 1, Freiburg, Germany

Stephen D. Nimer, MD Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL, USA

Harold J. Olney, MD, CM Department of Medicine and Hematology-Transfusion Medicine, Centre Hospitalier de L'universite De Montreal, Montreal, Canada

Gustavo Rivero, MD Department of Internal Medicine, The Section of Hematology/Oncology, Baylor College of Medicine, Houston, TX, USA

Bart L. Scott, MD Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

University of Washington School of Medicine, Seattle, WA, USA

Ramon V. Tiu Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

Department of Hematologic Oncology and Blood Disorders, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

Arjan A. van de Loosdrecht, MD, PhD Department of Hematology, Cancer Center Amsterdam, VU University Medical Center, Amsterdam, The Netherlands

Amer M. Zeidan Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD, USA

Part I

Epidemiology and Clinical Presentation

Clinical Presentation and Differential Diagnosis

1

Bart L. Scott

1.1 Clinical Presentation

The most frequent presenting symptom is fatigue, and the majority of patients have a macrocytic anemia at time of diagnosis (Sekeres et al. 2008). Large retrospective series have indicated that most patients do not have leukopenia or thrombocytopenia at time of presentation (Greenberg et al. 1997). However, there are some patients who do present with recurrent infections and easy bruising and bleeding events. Upon further questioning frequently, a prolonged history of symptomatic anemia can be elicited; however, there are a few patients who present with isolated thrombocytopenia or leukopenia. Few patients have circulating peripheral myeloblasts at time of presentation. Splenomegaly as a presenting sign in MDS is rare and should result in alternative diagnostic considerations such as myeloproliferative neoplasms (MPNs) or MDS/MPN overlap.

1.2 Diagnosis

The diagnosis of MDS is based upon the World Health Organization (WHO) criteria (Table 1.1) (Vardiman et al. 2009). The WHO classification is helpful for determining prognosis (Malcovati et al. 2005) and in selection of therapy (Howe et al. 2004). Despite advancements in classification schemata, there is often discordance among pathologists in diagnosing lesser degrees of dysplasia (Naqvi et al. 2011). In a patient survey, the diagnosis of MDS was delayed on average for 3 years after initial presentation with a hematologic abnormality (Sekeres et al. 2011). The diagnosis of MDS is one exclusion as there are other disorders such as acute myeloid leukemia and myeloproliferative neoplasms which can result in dysplastic changes within the bone marrow. The suggested diagnostic workup is summarized in Table 1.1 (Greenberg et al. 2011).

1.2.1 Differential Diagnosis

Vitamin deficiencies such as folate and vitamin B12 can cause a megaloblastoid anemia with evidence of bone marrow dysplasia; therefore, testing for these vitamin deficiencies is considered a standard part of the evaluation of patients with macrocytic anemia. In addition, copper deficiency

B.L. Scott, MD
Division of Clinical Research,
Fred Hutchinson Cancer Research Center,
1100 Fairview Avenue N, D1-100,
Seattle, WA 98109-1024, USA

University of Washington School of Medicine,
Seattle, WA, USA
e-mail: bscott@fhcrc.org

Supported in part by grants HL084054, HL036444 and HL082941 from the National Institutes of Health, Bethesda, MD.

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_1, © Springer-Verlag Berlin Heidelberg 2013

Table 1.1 Suggested evaluation of MDS

History and physical examination
Complete blood cell count with differential
Reticulocyte count
Bone marrow aspiration with iron stain and biopsy
Cytogenetic testing by karyotype analysis
Serum erythropoietin level
RBC folate, serum B12
Serum ferritin, iron, total iron-binding capacity
Transfusion history
Thyroid-stimulating hormone
Helpful in some clinical situations
Flow cytometry of bone marrow
HLA typing if stem cell transplant candidate
HLA-DR 15 typing if immunosuppressive therapy considered
Jak 2 mutation analysis in patients with thrombocytosis (RARS-T)
Copper level in patients with bone marrow myeloblasts <5 %

Adapted from NCCN Guidelines version 2.2013

has been recently noted to lead to peripheral cytopenias and dysplastic changes within the bone marrow (Gregg et al. 2002; Huff et al. 2007). Excessive alcohol use has also been associated with a macrocytic anemia and dysplastic changes within the marrow. Endocrine abnormalities such as hypothyroidism may result in a macrocytic anemia. There are certain genetic disorders that are associated with the development of MDS such as Fanconi Anemia and dyskeratosis congenita. Therefore, genetic screening may be warranted in certain clinical situations such as a positive family history or young age at diagnosis. Hypoplastic MDS can be difficult to distinguish from aplastic anemia as there are few cells present within the marrow to be analyzed for the presence of dysplasia; cytogenetic testing and measurement of CD34-positive cells by flow cytometry or immunohistochemistry is particularly useful in this situation (Orazi et al. 1997). Testing for paroxysmal nocturnal hemoglobinuria should be considered in patients with early stage MDS as these disorders may coexist (Dunn et al. 1999). Additionally, it is acknowledged that MDS patients with a PNH clone are more likely to respond to immunosuppressive therapy (Wang et al. 2002).

1.2.2 Laboratory Features

MDS are disorders of blood; therefore, assessment is focused on hematologic analyses (Table 1.2). A complete blood count with examination of peripheral blood smear and platelet count is standard if MDS is suspected, particularly when looking for enlarged erythrocytes (treating with replacement therapy to rule out folate or vitamin B12 deficiency) or peripheral blasts. Measures for serum iron, total iron-binding capacity, ferritin, and folic acid are also recommended to evaluate for other potential causes of anemia, and lactate dehydrogenase (LDH), haptoglobin, reticulocyte count, and Coombs' tests are needed to rule out an underlying hemolytic process. Serum copper levels should also be tested in any patient with a suspicion of MDS and less than 5 % myeloblasts with a normal karyotype. Copper deficiency has become an increasingly recognized cause of cytopenias with marrow dysplasia (Gregg et al. 2002; Huff et al. 2007). A baseline serum erythropoietin value should be determined prior to the initiation of any growth factor therapy and preferably prior to initiation of red blood cell transfusion support (Hellstrom-Lindberg et al. 2003). Examination of the peripheral blood smear is a central part of the diagnosis of MDS and usually shows a macrocytic or normocytic anemia. Additionally, hypochromic changes, poikilocytosis, and anisocytosis are frequently observed. Abnormalities may be observed within the granulocytic lineage such as the pseudo-Pelger-Huët anomaly and hypogranulation. Thrombocytopenia is present at diagnosis in a minority of patients with MDS (Garcia-Manero et al. 2008). Certain subtypes of MDS are associated with an increased platelet count (del 5q). Morphologic abnormalities observed include enlarged platelets with poor granulation.

1.3 Bone Marrow Examination

Bone marrow evaluation is crucial to establish the diagnosis of MDS. In fact, a final diagnosis must be confirmed based on morphologic criteria available only from marrow examination. Marrow features play a role in treatment planning as well.

Table 1.2 WHO diagnostic classification of Myelodysplastic Syndromes (Vardiman et al. 2009)

Disease	Blood findings	BM findings
Refractory cytopenia with unilineage dysplasia (RCUD): (refractory anemia [RA]; refractory neutropenia [RN]; refractory thrombocytopenia [RT])	Unicytopenia or bicytopenia[a] No or rare blasts (<1 %)[b]	Unilineage dysplasia: ≥10 % of the cells in one myeloid lineage <5 % blasts <15 % of erythroid precursors are ring sideroblasts
Refractory anemia with ring sideroblasts (RARS)	Anemia No blasts	≥15 % of erythroid precursors are ring sideroblasts Erythroid dysplasia only <% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s) No or rare blasts (<1 %)[b] No Auer rods <1 × 10⁹/L monocytes	Dysplasia in ≥10 % of the cells in ≥2 myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes) <5 % blasts in marrow No Auer rods ±15 % ring sideroblasts
Refractory anemia with excess blasts-1 (RAED-1)	Cytopenia(s) <5 % blasts[b] No Auer rods <1 × 10⁹/L monocytes	Unilineage or multilineage dysplasia 5–9 % blasts[b] No Auer rods
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenia(s) 5–19 % blasts[c] Auer rods ± [a] <1 × 10⁹/L monocytes	Unilineage or multilineage dysplasia 10–19 % blasts[c] Auer rods ± [a]
Myelodysplastic syndorme—unclassified (MDS-U)	Cytopenias <1 % blasts[b]	Unequivocal dysplasia in <10 % of cells in one or more myeloid lineages when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS (see Table 1.3) <5 % blasts
MDS associated with isolated del(5q)	Anemia Usually normal or increased platelet count No or rare blasts (<1 %)	Normal to increased megakaryocytes with hypolobated nuclei <5 % blasts Isolated del(5q) cytogenetic abnormality No Auer rods

[a]Bicytopenia may occasionally be observe. Cases with pancytopenia should be classified as MDS-U
[b]If the marrow myeloblast percentage is <5 % but there are 2–4 % myeloblasts in the blood, the diagnostic classification is RAEB-1. Cases of RCUD and RCMD with 1 % myeloblasts in the blood should be classified as MDS-U
[c]Cases with Auer rods and <5 % myeloblasts in the blood and less than 10 % in the marrow should be classified as RAEB-2. Although the finding of 5–19 % blasts in the blood is, in itself, diagnostic of RAEB-2, cases of RAEB-2 may have <5 % blasts in the blood if they have Auer rods or 10–19 % blasts in the marrow or both. Similarly, cases of RAEB-2 may have <10 % blasts in the marrow but may be diagnosed by the other two findings, Auer rod + and/or 5–19 % blasts in the blood

For instance, a bone marrow biopsy is the only means to measure cellularity, which can influence the selection of therapy. The majority of patients with MDS have a hypercellular marrow; however, normocellular and hypocellular marrows have been observed (Tuzuner et al. 1995). The presence of marrow fibrosis has a negative impact on prognosis (Buesche et al. 2008), and fibrosis can only be assessed by obtaining a bone marrow biopsy. A marrow aspirate can be examined for evidence of hematopoietic cell maturation abnormalities, excessive marrow blasts (>5 %), and the presence of iron suggestive of ring sideroblasts, and the sample can be used for testing in flow

cytometry, cytogenetics, and fluorescence in situ hybridization testing as well. The presence of at least 10 % of the cells of a specific myeloid lineage (erythroid, granulocytic, or megakaryocytic) should show evidence of dysplasia in order to confirm the diagnosis of MDS. A presumptive diagnosis of MDS may be made by the presence of recurrent cytogenetic abnormalities as discussed below.

1.4 Cytogenetic Analysis

The WHO diagnostic schema now includes the presence of recurrent cytogenetic abnormalities as presumptive evidence of MDS even in the absence of significant dysplasia (Table 1.3) (Vardiman et al. 2009). Cytogenetic studies are important in determining treatment expectations and can be helpful in determining the most appropriate therapy. For example, patients with deletion 5q are well known to have increased response rates when treated with lenalidomide (List et al. 2006). In addition, specific cytogenetic changes are suggestive of patient prognosis (Schanz et al. 2011). Some mutations have been shown to predict disease progression whereas other genetic derangements may suggest sensitivity to specific medications. The reliability and prognostic significance of cytogenetic analyses have been documented in a multicenter analysis (Haase et al. 2007). The investigators reported that among 2,124 patients in Austria and Germany on whom they carried out cytogenetic testing, 97.6 % were successfully analyzed. They also observed that about half of the subjects had normal genetic profiles, but cytogenetic profiles allowed for the separation of the rest of the subjects into good, intermediate, or poor prognostic categories. The WHO diagnostic system has been revised to include certain cytogenetic changes such as del 5q. The importance of cytogenetic changes in determining prognosis has been emphasized in newer prognostic models (Schanz et al. 2012). It should be noted that t(8;21), inv(16), t(16;16), and t(15;17) would classify a patient as having AML regardless of the myeloblast percentage and occurrence of dysplasia.

Table 1.3 Recurrent chromosomal abnormalities considered sufficient for a presumptive diagnosis of MDS even in the absence of significant dysplasia (Vardiman et al. 2009)

Unbalanced abnormalities	Balanced abnormalities
−7 or del(7q)	t(11;16)(q23;p13.3)
−5 or del(5q)	t(3;21)(q26.2;q22.1)
i(17q) or t(17p)	t(1;3)(p36.3;q21.1)
−13 or del(13q)	t(2;11)(p21;q23)
del(11q)	inv(3)(q21q26.2)
del(12p) or t(12p)	t(6;9)(p23;q34)
del(9q)	
indic(X)(q13)	

Complex karyotype (three or more chromosomal abnormalities) involving one or more of the above abnormalities

1.5 Flow Cytometry

Flow cytometry is emerging as a prominent diagnostic and prognostic test in MDS. Flow cytometry is particularly useful in patients with hypoplastic MDS as it provides an accurate measurement of CD34+ cells and myeloid dyspoiesis which can be helpful to distinguish hypoplastic MDS from aplastic anemia. In addition, flow cytometry of the peripheral blood is the preferred diagnostic tool for PNH. With flow cytometry, multiple myeloid and monocytic antigens can be measured to look for abnormalities in hematopoietic development (Wells et al. 2003). These antigenic aberrancies have been used to develop a flow cytometric scoring system which has been validated in the transplant (Scott et al. 2008) and non-transplant setting (van de Loosdrecht et al. 2008). Additionally, flow cytometry is helpful as a diagnostic tool (Stetler-Stevenson et al. 2001). This is particularly relevant in patients with hypoplastic MDS where there is a low cellularity within the marrow which precludes an accurate assessment of dysplasia by morphology. Another advantage of flow cytometry is the ability to detect small quantities of disease burden known as minimal residual disease (MRD). Patients with MDS who have evidence of MRD pre-transplant are known to be at a higher risk of relapse following stem cell transplantation (Scott et al. 2008). Ultimately, this technology may prove useful in monitoring response to therapy and subsequently

altering treatment choices at earlier time points leading to improved outcomes.

1.6 Summary

MDS is a collection of disorders resulting in low blood counts and a propensity to progress to AML. The most common presentation is fatigue with a macrocytic anemia. The differential diagnosis is broad and requires the collaborative efforts of an experienced hematologist and hematopathologist. A careful history and physical examination is necessary to exclude other potential causes of a macrocytic anemia. Presentations with isolated neutropenia or thrombocytopenia are unusual but have been reported. A comprehensive diagnostic workup includes examination of a peripheral blood smear, bone marrow aspirate, and bone marrow biopsy. Cytogenetic testing is useful from a diagnostic and prognostic perspective and should be performed in all patients who have marrow examinations done to evaluate cytopenias. Newer techniques such as flow cytometry are being incorporated into diagnostic and prognostic schemes. Although our diagnostic tools have improved, a clinical suspicion of MDS in general is necessary to avoid delays in appropriate diagnosis and institution of treatment.

References

Buesche G, Teoman H, Wilczak W, Ganser A, Hecker H, Wilkens L, Gohring G, Schlegelberger B, Bock O, Georgii A, Kreipe H (2008) Marrow fibrosis predicts early fatal marrow failure in patients with myelodysplastic syndromes. Leukemia 22:313–322

Dunn DE, Tanawattanacharoen P, Boccuni P, Nagakura S, Green SW, Kirby MR, Kumar MSA, Rosenfeld S, Young NS (1999) Paroxysmal nocturnal hemoglobinuria cells in patients with bone marrow failure syndromes. Ann Intern Med 131:401–408

Garcia-Manero G, Shan J, Faderl S, Cortes J, Ravandi F, Borthakur G, Wierda WG, Pierce S, Estey E, Liu J, Huang X, Kantarjian H (2008) A prognostic score for patients with lower risk myelodysplastic syndrome. Leukemia 22:538–543

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 (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89:2079–2088 [erratum appears in Blood 1998;91(3):1100]

Greenberg PL, Attar E, Bennett JM, Bloomfield CD, DeCastro CM, Deeg HJ, Foran JM, Gaensler K, Garcia-Manero G, Gore SD, Head D, Komrokji R, Maness LJ, Millenson M, Nimer SD, O'Donnell MR, Schroeder MA, Shami PJ, Stone RM, Thompson JE, Westervelt P (2011) NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines™): myelodysplastic syndromes version 1.2011. J Natl Compr Canc Netw 9:30–56

Gregg XT, Reddy V, Prchal JT (2002) Copper deficiency masquerading as myelodysplastic syndrome. Blood 100:1493–1495

Haase D, Germing U, Schanz J, Pfeilstöcker M, Nösslinger T, Hildebrandt B, Kundgen A, Lübbert M, Kunzmann R, Giagounidis AAN, Aul C, Trümper L, Krieger O, Stauder R, Müller TH, Wimazal F, Valent P, Fonatsch C, Steidl C (2007) New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. Blood 110:4385–4395

Hellstrom-Lindberg E, Gulbrandsen N, Lindberg G, Ahlgren T, Dahl IM, Dybedal I, Grimfors G, Hesse-Sundin E, Hjorth M, Kanter-Lewensohn L, Linder O, Luthman M, Lofvenberg E, Oberg G, Porwit-MacDonald A, Radlund A, Samuelsson J, Tangen JM, Winquist I, Wisloff F, Scandinavian MDS (2003) 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 120: 1037–1046

Howe RB, Porwit-MacDonald A, Wanat R, Tehranchi R, Hellstrom-Lindberg E (2004) The WHO classification of MDS does make a difference. Blood 103: 3265–3270

Huff JD, Keung YK, Thakuri M, Beaty MW, Hurd DD, Owen J, Molnar I (2007) Copper deficiency causes reversible myelodysplasia. Am J Hematol 82:625–630

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 (2006) Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 355:1456–1465

Malcovati L, Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, Passamonti F, Arcaini L, Maffioli M, Bernasconi P, Lazzarino M, Cazzola M (2005) Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol 23:7594–7603

Naqvi K, Jabbour E, Bueso-Ramos C, Pierce S, Borthakur G, Estrov Z, Ravandi F, Faderl S, Kantarjian H, Garcia-Manero G (2011) Implications of discrepancy in morphologic diagnosis of myelodysplastic syndrome between referral and tertiary care centers. Blood 118:4690–4693

Orazi A, Albitar M, Heerema NA, Haskins S, Neiman RS (1997) Hypoplastic myelodysplastic syndromes can be distinguished from acquired aplastic anemia by CD34 and PCNA immunostaining of bone marrow biopsy specimens. Am J Clin Pathol 107: 268–274

Schanz J, Steidl C, Fonatsch C, Pfeilstocker M, Nosslinger T, Tuechler H, Valent P, Hildebrandt B, Giagounidis A, Aul C, Lubbert M, Stauder R, Krieger O, Garcia-Manero G, Kantarjian H, Germing U, Haase D, Estey E (2011) Coalesced multicentric analysis of 2,351 patients with myelodysplastic syndromes indicates an underestimation of poor-risk cytogenetics of myelodysplastic syndromes in the international prognostic scoring system. J Clin Oncol 29:1963–1970

Schanz J, Tuchler H, Sole F, Mallo M, Luno E, Cervera J, Granada I, Hildebrandt B, Slovak ML, Ohyashiki K, Steidl C, Fonatsch C, Pfeilstocker M, Nosslinger T, Valent P, Giagounidis A, Aul C, Lubbert M, Stauder R, Krieger O, Garcia-Manero G, Faderl S, Pierce S, Le Beau MM, Bennett JM, Greenberg P, Germing U, Haase D (2012) 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 30:820–829

Scott BL, Wells DA, Loken MR, Myerson D, Leisenring WM, Deeg HJ (2008) Validation of a flow cytometric scoring system as a prognostic indicator for posttransplantation outcome in patients with myelodysplastic syndrome. Blood 112:2681–2686

Sekeres MA, Schoonen WM, Kantarjian H, List A, Fryzek J, Paquette R, Maciejewski JP (2008) Characteristics of US patients with myelodysplastic syndromes: results of six cross-sectional physician surveys. J Natl Cancer Inst 100:1542–1551

Sekeres MA, Maciejewski JP, List AF, Steensma DP, Artz A, Swern AS, Scribner P, Huber J, Stone R (2011) Perceptions of disease state, treatment outcomes, and prognosis among patients with myelodysplastic syndromes: results from an internet-based survey. Oncologist 16:904–911

Stetler-Stevenson M, Arthur DC, Jabbour N, Xie XY, Molldrem J, Barrett AJ, Venzon D, Rick ME (2001) Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. Blood 98:979–987

Tuzuner N, Cox C, Rowe JM, Watrous D, Bennett JM (1995) Hypocellular myelodysplastic syndromes (MDS): new proposals. Br J Haematol 91:612–617

van de Loosdrecht AA, Westers TM, Westra AH, Dräger AM, van der Velden VHJ, Ossenkoppele GJ (2008) Identification of distinct prognostic subgroups in low- and intermediate-1-risk myelodysplastic syndromes by flow cytometry. Blood 111:1067–1077

Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellström-Lindberg E, Tefferi A, Bloomfield CD (2009) The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 114:937–951

Wang H, Chuhjo T, Yasue S, Omine M, Nakao S (2002) Clinical significance of a minor population of paroxysmal nocturnal hemoglobinuria-type cells in bone marrow failure syndrome. Blood 100:3897–3902

Wells DA, Benesch M, Loken MR, Vallejo C, Myerson D, Leisenring WM, Deeg HJ (2003) Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. Blood 102:394–403

Epidemiology and Etiology

2

Anneclaire J. De Roos

2.1 Introduction

Epidemiology seeks to describe patterns of disease according to demographic factors and other exposures, thereby elucidating etiologic factors (causes of disease) and predictors of prognosis (such as survival). Epidemiologic research of MDS has been fairly limited in comparison to other hematopoietic cancers (such as acute myeloid leukemia (AML)), no doubt due to difficulty in case-finding from a historical lack of reporting of MDS in cancer registries. The *International Classification of Diseases for Oncology* listed MDS as malignant for the first time in its 3rd edition in 2000 (ICD-O-3) (Fritz et al. 2000), thereby spurring registration of MDS in cancer registries worldwide. As a result, population-based data have been more readily available in the past decade for identifying MDS cases and describing the epidemiology of MDS, and the amount and quality of published studies on MDS have since increased. Nevertheless, the field continues to encounter challenges due to changing case definitions and likely incomplete case identification.

A.J. De Roos
Department of Environmental and Occupational Health, Drexel University School of Public Health
1505 Race St., Philadelphia, PA 19102
e-mail: aderoos@drexel.edu

2.2 Descriptive Epidemiology

2.2.1 Incidence

MDS incidence rates have been described in several reports in the past decade, since the ICD-O-3 classification of MDS as malignant (Fritz et al. 2000), making MDS a reportable cancer in registries worldwide. Prior to that time, incidence rates were described within hospitals or regions that had historically close cancer surveillance. Estimated incidence rates of MDS in the USA from cases registered in the Surveillance, Epidemiology and End Results (SEER) and North American Association of Central Cancer Registries (NAACR) programs are reported as 3.3–3.4 per 100,000 person-years (PY) (Ma et al. 2007; Rollison et al. 2008). These estimates are quite similar to rates reported in Europe and other regions (Table 2.1, all per 100,000 PY) such as the UK (3.8) (McNally et al. 1997), England (3.5) (Phekoo et al. 2006), Germany (2.5) (Neukirchen et al. 2011), Sweden (3.6) (Radlund et al. 1995), New Zealand (3.7) (Rodger and Morison 2011), and Australia (3.2) (Rodger and Morison 2011). Rates are not always perfectly comparable across studies due to differing MDS case definitions (e.g., inclusion of different histologies). The US studies (Ma et al. 2007; Rollison et al. 2008) classified MDS according to the ICD-O-3 (Fritz et al. 2000), which includes refractory anemia (RA, ICD-O-3 9980); refractory anemia with sideroblasts (RARS, ICD-O-3 9982); refractory anemia with excess blasts (RAEB, ICD-O-3

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_2, © Springer-Verlag Berlin Heidelberg 2013

Table 2.1 Incidence rates of myelodysplastic syndromes (MDS) reported in peer-reviewed published studies

Reference	Region	Period	Population	Case-finding	Classification	Standardization	Incidence (per 100,000/year)
Radlund et al. (1995)	Sweden (Jönköping)	1978–1992	310,000	Local surveillance	FAB	European standard population	3.6
Maynadie et al. (2011)	France (Côte d'Or, Burgundy Region)	1980–2004	467,998–512,272	Specialized local registry of hematologic malignancies, established in 1980	WHO	World standard population	1.3
Williamson et al. (1994)	UK (Bournemouth)	1981–1990	203,000–226,000	Specialized registration of cases diagnosed in a local hospital, with "strenuous efforts to document new cases by adopting a low threshold for performing marrow examination in those with suggestive peripheral blood findings"	FAB	Unstandardized	12.6
McNally et al. (1997)	UK	1984–1993	11–16 million	Specialized registry of the Leukemia Research Fund	FAB	Uniform standard population	3.8
Bauduer et al. (1998)	France (French Basque Country)	1993–1996	290,000	Centralized review of bone marrow slides from one hospital serving the entire region	FAB	Unstandardized	7.7
Neukirchen et al. (2011)	Germany (Düsseldorf)	1996–2005	575,000	Centralized review of bone marrow slides from private physicians and hospitals serving the region	WHO and FAB	European standard population	2.5 (WHO) 3.2 (FAB)
Phekoo et al. (2006)	England (South Thames)	1999–2000	5,499,841	Local registration	FAB	European standard population	3.5
Sant et al. (2010)	Europe (Northern, Central, Southern, Eastern)	2000–2002	89 million	EUROCARE network cancer registries	WHO	Unstandardized	1.8
Ma et al. (2007)	USA	2001–2003	76 million	Surveillance, Epidemiology and End Results (SEER) registry	ICD-O-3	US standard population	3.4
Rollison et al. (2008)	USA	2001–2003	240 million	North American Association of Central Cancer Registries (NAACR)	ICD-O-3	US standard population	3.3

Irwin et al. (2011)	New Zealand (Wellington)	2002–2007	448,956	Review of all bone marrow biopsies performed at Wellington Hospital and a local private pathology laboratory	WHO	Unstandardized	2.7
De Roos et al. (2010a)	USA (Seattle, WA)	2005–2006	350,000	Members of a nonprofit healthcare system with MDS confirmed through SEER registry or chart review	ICD-O-3	US standard population	7.0
Rodger and Morison (2011)	New Zealand	2005–2007	4,200,000	New Zealand Cancer Registry	WHO	World standard population	3.7
Rodger and Morison (2011)	Australia	2005–2007	21 million	Australian Cancer Incidence and Mortality workbooks	WHO	World standard population	3.2

9983); refractory anemia with excess blasts in transformation (RAEB-t, ICD-O-3 9984); refractory cytopenia with multilineage dysplasia (RCMD, ICD-O-3 9985); MDS with 5q deletion (5q− syndrome, ICD-O-3 9986); therapy-related MDS, not otherwise specified (NOS) (t-MDS, ICD-O-3 9987); and MDS, NOS (MDS-U, ICD-O-3 9989). MDS case classification according to the World Health Organization (WHO) revision (adopted in 2001) does not include RAEB-t but rather classifies it as AML (Jaffe 2001). The WHO classification also excludes MDS patients who have had previous chemotherapy. Several of the studies using the WHO classification reported somewhat lower rates, including those from France (1.3 per 100,000 PY) (Neukirchen et al. 2011) and throughout Europe (1.8) (Sant et al. 2010). Several of the European incidence studies, particularly before the year 2000, included chronic myelomonocytic leukemia (CMML) (Bauduer et al. 1998; McNally et al. 1997; Phekoo et al. 2006; Williamson et al. 1994), as defined under the previously used French-American-British (FAB) cooperative group classification system (Bennett et al. 1982); CMML is classified in a myelodysplastic/myeloproliferative neoplasm overlap category by WHO (Jaffe 2001). Rates also differ importantly based on methods of standardization; several rates reported in Table 2.1 are crude (unstandardized) rates (Bauduer et al. 1998; Irwin et al. 2011; Williamson et al. 1994), which do not account for differing age distributions between different regions. Use of the world standard population versus the European or US standard population can also affect results, as the world standard population has a younger age distribution than the European or US alternatives.

Regardless of the methods of rate estimation, MDS incidence increases sharply with age, with a median age at diagnosis in the 70s in the US and European populations (Ma et al. 2007; Neukirchen et al. 2011; Phekoo et al. 2006; Sekeres et al. 2008). Younger median age at diagnosis, typically in the 50s, has been observed in several Eastern countries, such as Japan (Kuendgen et al. 2007), China (Chen et al. 2005), Central Africa (Mukiibi and Paul 1994), and Jordan (Awidi et al. 2009), suggesting different histologies or exposures in these regions compared to Western countries. MDS is more common in men than women, except for the subtype with 5q deletion (Maynadie et al. 2011; Neukirchen et al. 2011). The male excess is most prominent among ages 50 and older. A detailed analysis of the male-to-female MDS incidence ratio in the UK revealed a U-shaped pattern, with a male excess until age 15 (ages at which MDS is extremely rare), a female excess in ages 30–50, and a male excess increasing prominently after age 50 (Cartwright et al. 2002). A similar pattern was observed for most acute myeloid leukemia (AML) and myeloproliferative disease (MPD) subtypes, suggesting a shared etiology. MDS is more common among whites and non-Hispanics than among other racial/ethnic groups in the USA (Ma et al. 2007; Rollison et al. 2008), and American Indians/Alaska Natives have the lowest rates in the USA (Ma et al. 2007). In contrast, higher rates were reported in New Zealand Maoris (4.9 per 100,000) than non-Maori ethnicities (3.7 per 100,000) (Rodger and Morison 2011). A study conducted in Japan suggests lower MDS incidence than in Western countries (Shimizu et al. 1995), and similarly, Asian Americans/Pacific Islanders in the USA have lower rates than US whites (Ma et al. 2007). Any changes in MDS incidence over time have been difficult to establish and when observed have been generally attributed to changes in diagnostic practices and reporting (Germing et al. 2004). In studies that reported MDS incidence by subtype, RA has usually been the most frequently diagnosed type, followed by RARS and RAEB (Bauduer et al. 1998; Ma et al. 2007; McNally et al. 1997). However, reclassification of many previously defined RA as RCMD under the WHO classification has resulted in RCMD as the most common subtype in recent studies (Irwin et al. 2011; Neukirchen et al. 2011). Declarative subtype distributions have been impossible to establish in the US registry-based studies, as approximately half of all cases registered in SEER and NAACR are classified as MDS-U (Rollison et al. 2008). CMML is quite common in the studies that included it under the FAB classification, with frequency ranging from 11 % in the French Basque

region (Bauduer et al. 1998) to 31 % in Bournemouth, UK (Williamson et al. 1994).

It is now recognized that MDS incidence is probably underestimated due to a combination of factors including incomplete case registration and underdiagnosis. There are several lines of evidence suggesting incomplete case registration. Incidence rates vary widely between different regions – between US SEER registries, from 3.0 (per 100,000 PY) in metropolitan Atlanta to 6.6 in the Seattle-Puget Sound region from 2001 to 2008 (2011), and between EUROCARE network European cancer registries, from 0.27 in Eastern Europe to 2.1 in the UK and Ireland from 2000 to 2002 (Sant et al. 2010). Differential reporting practices likely play a role in the geographic discrepancies, although true differences in incidence between regions are also possible. Differential completeness in reporting by registries may occur because of differing case-finding and validation methods (e.g., passive vs. active case-finding). For example, patients who are diagnosed outside of a hospital setting are likely to be missed. This may be illustrated by the fact that only 4 % of cases registered in NAACR (encompassing 82 % of the US population) were reported by physicians' offices, as opposed to hospitals or laboratories (Rollison et al. 2008). A recent US study identified incident MDS patients from Medicare records using an algorithm requiring two claims with an MDS-relevant diagnosis code in addition to ordering of typical diagnostic tests for MDS, specifically blood counts and bone marrow biopsy or aspiration (Cogle et al. 2011). The algorithm had high specificity (99.8 %) and moderate sensitivity (78.1 %) when compared to SEER-identified cases as the gold standard. MDS incidence was much higher using the Medicare algorithm than it was based on SEER-reported cases, with rates among persons 65 years and older of 75 (per 100,000 PY) versus 20, respectively (Cogle et al. 2011). These results suggest that patients diagnosed in the outpatient setting are frequently not reported to SEER.

Underdiagnosis likely also contributes to underestimation of MDS incidence. Definitive diagnosis of MDS requires a bone marrow biopsy, and the fact that potential MDS patients may not undergo detailed work-up likely leads to underdiagnosis of the disease. The third National Health and Nutrition Examination Survey (NHANES) cross-sectional study in the USA identified 11.0 % of men and 10.2 % of women as anemic, with 5.8 % of the anemic population having unexplained anemia and peripheral blood features suggestive of MDS (macrocytosis, thrombocytopenia, or neutropenia) (Guralnik et al. 2004). A similar survey conducted in Italy found unexplained anemia with blood features of MDS in 8.1 % of the anemic elderly (Tettamanti et al. 2010). Underdiagnosis of MDS was also suggested by a study of patients enrolled in a health plan in Western Washington State, which found that half of all patients with new MDS diagnoses (definite/probable or possible cases) were not reported to SEER, and inclusion of all cases led to an overall incidence rate of 10.2 per 100,000 PY (De Roos et al. 2010a). The "possible" cases, identified by diagnosis code and corroborated by chart review, did not receive bone marrow biopsy. There was evidence that definitive diagnosis was less likely to be pursued in less severe cases, as "possible" cases had higher average hemoglobin levels, platelet counts, and white blood cells upon presentation than did SEER cases (De Roos et al. 2010a). A higher than typically reported rate of MDS was also found in a UK study that aimed for complete identification of MDS cases through periodic health examinations followed by pursuit of bone marrow biopsy in patients with suggestive blood findings (Williamson et al. 1994). The estimated (crude) incidence rate in the UK study was 12.6 per 100,000 person-years (including CMML but not including patients with previous chemotherapy or radiotherapy). These studies suggest that underdiagnosis contributes to underestimation of MDS incidence, probably due to less severe cases that typically do not receive diagnostic work-up.

2.2.2 Prevalence and Survival

There are an estimated 12,000 new MDS cases diagnosed per year in the USA and 20,000 in Europe, based on reported incidence rates

(Germing et al. 2008). MDS prevalence, or the number of persons living with the disease, was estimated as 7.2 per 100,000 persons in Germany, using the WHO classification in 2003 (Neukirchen et al. 2011). However, with such a wide range in incidence estimates (Table 2.1), prevalence is uncertain. Based on the NHANES study finding that 5.8 % of the anemic population had "unexplained anemia" with peripheral blood features suggestive of MDS (Guralnik et al. 2004), Sekeres estimated that this finding would translate to 170,000 persons living with MDS in the USA while acknowledging that this figure is likely an overestimate (Sekeres 2011). Nevertheless, current prevalence figures based only on registry-reported incidence rates are probably underestimates due to the issues of incomplete reporting and underdiagnosis described above (Sect. 2.2.1). Furthermore, MDS prevalence is expected to increase as the population in developed countries ages.

The number of prevalent cases is also dependent on survival following MDS diagnosis, which is generally poor. Median survival has been reported as 23–34 months (Irwin et al. 2011; Maynadie et al. 2011; Phekoo et al. 2006). Relative survival, which accounts for competing causes of death by age group, was reported as 47 % 2 years from diagnosis among US cases with MDS as their first primary cancer (Ma et al. 2007). Superior survival has been observed among women compared to men (Ma et al. 2007; Maynadie et al. 2011; Phekoo et al. 2006) and younger versus older patients (Ma et al. 2007; Phekoo et al. 2006). Several studies indicate longer survival among patients in Asian countries than in Western countries (Kuendgen et al. 2007). However, survival can vary widely by MDS subtype (Germing et al. 2008; Ma et al. 2007; Phekoo et al. 2006), in addition to cytogenetic abnormalities, blast counts, number of dysplastic lineages, and blood cell counts (Belli et al. 2002; Bowles et al. 2006; Germing et al. 2008; Greenberg et al. 1997; Haus et al. 2006), and the concept of overall survival has limited utility for individual patients. MDS prevalence will increase as therapies resulting in improved survival are developed and disseminated.

2.3 Disease Etiology

2.3.1 Therapy-Related MDS

Among the few known risk factors for development of MDS is prior cytotoxic therapy. MDS is sometimes termed "secondary" (vs. "primary" or de novo) if its diagnosis follows treatment with chemotherapy or radiation for any of a variety of diseases (but most frequently for cancer) or if following accidental exposure to ionizing radiation or benzene (discussed in Sect. 2.3.2.3). We will use the terms "therapy-related" and "de novo" MDS (instead of "secondary" and "primary," respectively), as use of the word "secondary" differs in the context of cancer registration (2000). Therapy-related myeloid neoplasms (t-MN) are defined by the WHO classification as one, heterogeneous entity that contains a composite of MDS, AML, and MDS/MPN (Vardiman et al. 2009). Sekeres et al. reported from a survey of 101 US physicians that 10 % of recently diagnosed MDS patients were considered to be therapy related based on recent chemotherapy, radiation therapy, or other chemical exposure (Sekeres et al. 2008). However, based on the fact that 26 % of newly diagnosed MDS patients reported in SEER from 2001 to 2006 had previous cancers (De Roos et al. 2007), previous cancer treatments may contribute to a greater proportion of newly diagnosed MDS than clinically recognized. The risk of developing t-MN differs greatly according to the type of the initial cancer. For example, up to 10 % of patients treated for lymphoproliferative neoplasm developed t-MN within 10 years, whereas approximately 0.55 % of breast cancer patients developed t-AML within 8 years (Leone et al. 2010). These differing risks are certainly due to varying cytotoxicities of treatment regimens as well as the underlying susceptibility for myeloid neoplasm of the patient group with the initial cancer (i.e., the same genetic profile may increase susceptibility to both lymphoproliferative and myeloid neoplasms).

Clinical observations suggest a worse prognosis for therapy-related MDS than for de novo MDS (Finch 2004; Levine and Bloomfield 1992;

Singh et al. 2007). Therapy-related MDS cases have been observed to be less responsive to treatment and evolve more frequently into AML (Finch 2004; Levine and Bloomfield 1992). Comparisons of histopathologic features of therapy-related and de novo MDS indicate biologic differences that may account for differences in clinical outcomes. Clonal chromosomal abnormalities are found in 40–50 % of patients with de novo MDS compared to up to 95 % of therapy-related MDS (Catenacci and Schiller 2005) (although it is notable that newer, more sensitive technologies detect such abnormalities in a larger proportion of de novo MDS (Tiu et al. 2011)). Additionally, the proportion of "high-risk" cytogenetics (e.g., deletions of chromosome 7 or complex karyotypes) is higher in therapy-related than de novo MDS (Bloomfield 1986; Rubin et al. 1990). Monosomy of chromosome 5 or deletion of 5q (−5/5q−) and/or monosomy of chromosome 7 or deletion of 7q (−7/7q−) is frequently associated with prior chemotherapy, in particular with alkylating agents (Leone et al. 2010). In contrast, no prototypical chromosomal patterns have been found for radiation-related myeloid neoplasms (Leone et al. 2010). Similar to clinical observations, an analysis of SEER data observed shorter survival for MDS patients who had a previous cancer diagnosis than for de novo cases and found that the increased risk was fairly constant throughout a 47-month period of follow-up after MDS diagnosis (De Roos et al. 2007). Shortened survival associated with previous cancer was most pronounced for MDS cases diagnosed within 5 years of the previous cancer diagnosis, although previous lymphoproliferative neoplasm was associated with shorter MDS survival even when MDS was diagnosed up to 20 years after the lymphoproliferative neoplasm diagnosis. Previous radiation treatment for cancer was an independent predictor of death in MDS patients, significantly so for MDS cases diagnosed between 5 and 10 years after irradiation (De Roos et al. 2007). These results suggest that previous cancer therapies may contribute to MDS etiology up to a decade or longer after treatment.

2.3.2 Lifestyle and Environmental Risk Factors for MDS

Few risk factors are known for MDS, aside from therapies for previous cancers and other conditions. Epidemiologic research to date has largely focused on smoking, alcohol consumption, and occupational exposures to solvents and alcohol. Epidemiologic studies of "lifestyle" (e.g., smoking, alcohol, obesity) and "environmental" (e.g., occupation, hobbies) risk factors for MDS are summarized in Table 2.2. Most studies have relied on convenience samples of MDS cases and controls, such as hospital patients. Because approximately one-third of MDS patients develop AML (Steensma and Bennett 2006), MDS was sometimes considered in the past as "preleukemia" or "aleukemia" in epidemiologic studies or was grouped with AML. Indeed, MDS may share risk factors with AML, as demonstrated by similar magnitude risks observed in the cohort of atomic bomb survivors in Hiroshima and Nagasaki, Japan, with significant excess risks of AML and MDS of 4.3 and 5.3 per 1 Gy dose of ionizing radiation, respectively (Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation NRC 2006; Preston et al. 1994; Richardson et al. 2009). Nevertheless, aside from direct genotoxicity to the bone marrow, potential mechanisms of development of these myeloid cell neoplasms are not well understood, and there are also likely to be risk factors that are not shared between AML and MDS.

2.3.2.1 Lifestyle

Several lifestyle-related factors including smoking and alcohol consumption have been investigated as potential causes of MDS in multiple studies. However, the risks associated with these factors are as yet not well described in terms of etiologically relevant timing of exposure and histologic subtype-specific effects. Smoking has been significantly or nonsignificantly associated with increased risk in multiple studies (Bjork et al. 2009; Dalamaga et al. 2002; Ido et al. 1996; Ma et al. 2009; Mele et al. 1994; Nisse et al.

Table 2.2 Epidemiologic studies of lifestyle and environmental factors in relation to myelodysplastic syndromes (MDS)

Reference	Study design, region, and time period of case diagnosis	Study population	Data collection	Results[a]
Goldberg et al. (1990)	Hospital-based case–control study	52 de novo MDS cases recruited from hospital over undefined time period	Questionnaire with items about smoking, lifetime occupational history, and exposure to solvents, insecticides, and other chemicals through occupation and hobbies	*Solvents*
	USA (Philadelphia, PA)	52 controls from the same hospital (primary care and cardiology) and one of its affiliates, excluding those with previous cancer or blood dyscrasia, matched by age, sex, and socioeconomic group	"Significant" exposure defined as five or more contacts with these agents per lifetime	"Significantly" exposed: 20 % cases vs. 42 % controls ($p=0.9$); note that cases with prior benzene exposure were excluded from the study
	1976–not specified			*Pesticides* "Significantly" exposed: 71 % cases vs. 29 % controls ($p=0.002$)
Albin et al. (2003)	Population-based case–control study	330 MDS cases cytogenetically analyzed at the Department of Clinical Genetics, Lund	Telephone interview conducted in 1995–1997 with the study subject or next of kin (next-of-kin interviews were conducted for 88 % of cases and 26 % of controls), containing questions on smoking, lifelong occupation, specific job tasks, hobbies, and hair dye	*Solvents*
	Southern Sweden 1976–1993	337 population controls, matched by age, gender, and county of residence	Assessment of occupational and hobby exposures by an occupational hygienist	Exposed: OR = 1.4 (95 % CI = 0.9–2.2) Moderate or high exposure: OR = 0.8 (95 % CI = 0.3–1.9) Duration 15–20 years: OR = 1.2 (95 % CI = 0.7–2.0) Benzene: OR = 1.0 (95 % CI = 0.5–1.7) Chlorinated organic solvents: OR = 0.8 (95 % CI = 0.3–1.9) *Pesticides* Exposed: OR = 0.8 (95 % CI = 0.5–1.4) *Other significant associations* EMF occupational exposure, high: OR = 2.6 (95 % CI = 1.0–6.2)

Brown et al. (1990)	Population-based case–control study	63 cases of "myelodysplasia" from IA registry and from a special surveillance network of MN hospitals and pathology laboratories	In-person interview with questions on smoking, alcohol consumption, residential history, lifetime occupational history, and farming history	*Alcohol consumption*
Brown et al. (1992)	USA (IA and MN)	818 controls from random-digit dialing (ages <65) and Medicare (ages ≥65)	Assignment of occupational exposures by a job-exposure matrix	Any: OR = 1.6 (95 % CI = 0.9–2.7)
Blair et al. (2001)	1981–1984			Consumption >23 drinks/week: OR = 2.1 (95 % CI = 1.0–4.6) Beer or wine only: OR = 1.2 (95 % CI = 0.5–2.5) Liquor only: OR = 0.9 (95 % CI = 0.3–2.4) Other combinations: OR = 2.4 (95 % CI = 1.3–4.6) *Solvents* Benzene, high exposure: OR = 2.6 (95 % CI = 0.7–9.7) *Pesticides* Ever farmed: OR = 0.8 (95 % CI = 0.5–1.4) Farmed ≥45 years: OR = 0.5 (95 % CI = 0.2–1.4) Insecticides: OR = 0.6 (95 % CI = 0.3–1.1) Herbicides: OR = 0.7 (95 % CI = 0.3–1.5) Fungicides: OR = 0.7 (95 % CI = 0.2–3.2) *Other significant associations* Industrial groups Nonmetallic minerals, except fuels: OR = 5.9 (95 % CI = 1.2–30) Plumbing, heating, and air conditioning: OR = 4.0 (95 % CI = 1.1–15) Miscellaneous nondurable goods: OR = 4.7 (95 % CI = 1.1–20)

(continued)

Table 2.2 (continued)

Reference	Study design, region, and time period of case diagnosis	Study population	Data collection	Results[a]
				Finance, taxation, and monetary policy: OR = 3.2 (95 % CI = 1.1–9.1) Occupational groups Miscellaneous professionals: OR = 3.5 (95 % CI = 1.1–11) Sales occupations, consumable: OR = 2.8 (95 % CI = 1.0–7.8) Metals fabricating: OR = 3.0 (95 % CI = 1.1–7.6) Truck drivers, light: OR = 9.4 (95 % CI = 2.1–42)
Farrow et al. (1989)	Hospital-based case–control study UK (Wales) 1985–1986	63 MDS cases from the hematology department of the University Hospital of Wales 63 controls from outpatient clinics in the same hospital (surgery and ear, nose, and throat), matched by day of hospital visit, age, and sex	In-person interview including demographics, lifetime residence, smoking, alcohol consumption, hobbies, lifetime occupational history, and exposure to 70 specific agents	*Significant associations (no risk estimates provided)*: Single (vs. married, men only) No children (vs. children) Petrol diesel fumes and liquids exposure Ammonia exposure
Mele et al. (1994)	Hospital-based case–control study Italy (Rome, Bologna, and Pavia) 1986–1990	111 RAEB cases from hematology departments of three hospitals 1161 controls from outpatients in same hospitals (hematology and other departments) with nonneoplastic conditions, excluding platelet disorders, leukocytosis, leukopenia, and MGUS	In-person interview with questions about smoking, occupational exposures, and hair dye use	*Smoking* Ever: OR = 1.7 (95 % CI = 1.0–3.0) Former: OR = 2.7 (95 % CI = 1.2–6.3) Current: OR = 1.2 (95 % CI = 0.4–3.3) Pack-years ≥20: OR = 2.4 (95 % CI = 1.0–5.8); *p*-trend = 0.03

				Solvents Painter occupation: OR = 5.4 (95 % CI = 0.5–61.0) Shoemaker occupation: OR = 4.3 (95 % CI = 0.9–21.1) *Pesticides* Pesticide use: OR = 0.8 (95 % CI = 0.5–1.4) Herbicide use: OR = 0.7 (95 % CI = 0.2–2.8) Agricultural occupation: OR = 1.1 (95 % CI = 0.6–2.0)
West et al. (1995)	Hospital-based case–control study	400 MDS cases newly diagnosed at participating hospitals	In-person interview with detailed questions on occupations, hobbies, and exposure to 70 specific agents	*Smoking*
	UK (South East Wales, Wessex, and West Yorkshire)	400 controls identified from outpatient and inpatient clinics of the same hospitals (medicine, ear, nose, and throat, orthopedics, and geriatrics), excluding patients with previous cancer, matched by hospital, area of residence, age, sex, and year of diagnosis	An exposure index was calculated for each agent using frequency and duration of exposure; exposure thresholds were set as follows: low exposure (≥10 h), moderate exposure (≥50 h), and high exposure (≥2500 h); some exposures grouped	Ever: OR = 1.2 (95 % CI = 0.8–1.6)
	Years not specified			Smoked ≥25 cigarettes/day: OR = 1.0 *Solvents* Paints/Solvents/Glues, high exposure: OR = 0.8 Halogenated organics, high exposure: OR = 2.2 (95 % CI = 1.1–4.5) Solvents, high exposure: OR = 1.2 Degreasing agents, high exposure: OR = 2.0 (95 % CI = 0.8–5.5) Vinyl chloride, high exposure: OR = 1.7 Dry-cleaning fumes, high exposure: OR = 2.0 Paints/varnishes, high exposure: OR = 0.7

(continued)

Table 2.2 (continued)

Reference	Study design, region, and time period of case diagnosis	Study population	Data collection	Results[a]
				Glues, high exposure: OR = 0.8 *Pesticides* Agrochemicals, high exposure: OR = 0.3 *Other significant associations* No children (vs. children): OR = 1.5 (95 % CI = 1.0–2.1) Dental X-rays: OR = 1.9 (95 % CI = 1.3–2.8) Occupation in education, welfare and health: OR = 1.9 (95 % CI = 1.1–3.4) Metals, high exposure: OR = 1.8 (95 % CI = 1.2–2.7) Radiation, high exposure: OR = 2.3 (95 % CI = 1.1–4.7) Copper compounds, high exposure: OR = 2.4 (95 % CI = 1.2–4.6)
Rigolin et al. (1998)	Hospital-based case–control study Italy (Ferrara) 1990–1996	178 de novo MDS cases referred to the Institute of Haematology of Ferrera 178 controls identified from outpatient files of the same hospital, excluding patients admitted for cancer or work-related diseases or accidents, matched by geographical area, sex, and age	In-person interview with detailed questions on occupations and hobbies, with special reference to pesticides, solvents, and petroleum products An exposure index was calculated for each agent using frequency and duration of exposure; subjects with an exposure index >2400 h were considered exposed	*Solvents* Exposed: OR = 7.1 (95 % CI = 2.4–20.9) Occupation as painters, printers, shoe makers, chemical industry: OR = 0.8 (95 % CI = 0.3–2.0) *Pesticides* Exposed: OR = 2.1 (95 % CI = 1.3–3.6) Occupation as farmer or gardener: OR = 1.1 (95 % CI = 0.7–1.1)

Nisse et al. (2001)	Population-based case–control study	204 de novo MDS diagnosed in the hematology department of the University Hospital of Lille	In-person interview with questions about demographics, lifetime residence, radiation, smoking, lifetime occupational history, and exposure to 70 specific agents	*Smoking*
	France (Lille)	204 controls identified from the electoral register, matched by age and sex	Lifetime cumulative exposure to each potentially hazardous agent was estimated using frequency and duration of exposure	Current: OR = 3.4 (95 % CI = 1.7–7.3)
	1991–1996			Pack-years ≥20: OR = 2.6 (95 % CI = 1.1–6.8) *Solvents* Exposed: OR = 2.6 (95 % CI = 1.6–5.4) *Pesticides* Exposed: OR = 3.2 (95 % CI = 1.1–11.2) Agricultural worker: OR = 2.8 (95 % CI = 1.4–6.2) *Other significant associations* Gardening >1 time/week: OR = 2.1 (95 % CI = 1.3–3.2) Living near an industrial plant ≥6 months: OR = 3.1 (95 % CI = 1.8–5.7) Occupations Health professionals: OR = 8.0 (95 % CI = 1.1–355) Technical and commercial sales representatives: OR = 3.2 (95 % CI = 1.0–13.7) Machine operators: OR = 2.8 (95 % CI = 1.3–6.4) Textile workers: OR = 2.8 (95 % CI = 1.3–6.5) Occupational exposures Oil: OR = 4.2 (95 % CI = 2.0–9.9)

(continued)

Table 2.2 (continued)

Reference	Study design, region, and time period of case diagnosis	Study population	Data collection	Results[a]
				Ammonia: OR = 4.0 (95 % CI = 1.1–22.1) Fertilizers: OR = 2.9 (95 % CI = 1.2–8.0) Cereal dust: OR = 2.6 (95 % CI = 1.2–6.0) Poultry: OR = 15.0 (95 % CI = 2.3–631) Livestock: OR = 3.6 (95 % CI = 1.6–9.2) Infectious agents: OR = 2.9 (95 % CI = 1.7–5.4) Cotton and flax dusts: OR = 3.4 (95 % CI = 1.6–8.2)
Ido et al. (1996)	Hospital-based case–control study Japan 1992–1993	116 de novo MDS cases from 32 hospitals 116 controls from outpatients, excluding blood disorders and liver disease, matched by hospital, age, and sex	Self-administered questionnaire with items on smoking, alcohol consumption, pet keeping, hair dye use, and a list of occupations with probable/possible exposure to organic solvents or lead	*Smoking* Ever: OR = 1.8 (95 % CI = 0.8–3.9) *Alcohol consumption* Any: OR = 2.2 (95 % CI = 1.1–4.2) Ethanol consumption ≥300 ml/week: OR = 2.4 (95 % CI = 0.9–6.2); *p*-trend = 0.02 *Solvents* Exposed: OR = 1.5 (95 % CI = 0.9–2.6)

Nagata et al. (1999)	Population-based case–control study Japan 1995–1996	111 de novo MDS from 28 hospitals 830 controls from telephone directories, matched by prefecture and sex	Self-administered questionnaire with items on smoking, alcohol consumption, hair dye use, and occupational exposure to organic solvents	*Smoking* Former: OR = 1.0 (95 % CI = 0.5–2.4) Current: OR = 1.1 (95 % CI = 0.5–2.3) Cigarettes per day ≥20: OR = 1.2 (95 % CI = 0.6–2.5); *p*-trend = 0.51 Duration >20 years: OR = 1.1 (95 % CI = 0.5–2.4); *p*-trend = 0.89 *Alcohol consumption* Former: OR = 0.9 (95 % CI = 0.3–3.0) Current: OR = 1.0 (95 % CI = 0.6–1.9) Ethanol intake ≥25 g/day: OR = 1.3 (95 % CI = 0.7–2.5); *p*-trend = 0.45 *Solvents* Exposed: OR = 1.7 (95 % CI = 0.7–3.7) *Other significant associations* Hair dye use: OR = 2.0 (95 % CI = 1.2–3.4) Hair dye use duration ≥10 years: OR = 3.0 (95 % CI = 1.4–6.2); *p*-trend = 0.003 Hair dye use total frequency ≥70: OR = 2.1 (95 % CI = 1.1–4.1); *p*-trend = 0.01
Dalamaga et al. (2002)	Hospital-based case–control study Greece (Athens) 1995–2000	84 de novo MDS cases hospitalized in the Veterans' General Hospital 84 controls admitted to the same hospital (orthopedics) for acute nonneoplastic and noninfectious conditions, matched by age and sex	In-person interview with questions on demographics, smoking, and alcohol consumption	*Smoking* Ever (but no alcohol): OR = 1.7 (95 % CI = 0.6–5.2) *Alcohol consumption* Drinks ≥1 glasses/day (but no smoking): OR = 3.3 (95 % CI = 0.5–21.1) Both alcohol and smoking: OR = 9.5 (95 % CI = 3.5–25.8) *Other significant associations* Never married (vs. ever): OR = 4.0 (95 % CI = 1.0–15.1)

(continued)

Table 2.2 (continued)

Reference	Study design, region, and time period of case diagnosis	Study population	Data collection	Results[a]
Strom et al. (2005)	Hospital-based case–control study	354 de novo MDS cases from MD Anderson Cancer Center	Self-administered questionnaire with items about smoking, alcohol consumption, and lifetime occupational history	*Smoking*
	USA (Houston, TX) 1999–2003	452 controls from friends, family members, and visitors at outpatient clinics	Assignment of solvent and pesticide exposures using a job-exposure matrix	Ever: OR = 1.7 (95 % CI = 1.2–2.3) Pack-year (per-unit increase): OR = 1.01 (95 % CI = 1.005–1.02) *Alcohol consumption* Wine: OR = 0.5 (95 % CI = 0.4–0.8) Other alcoholic beverages: OR = 0.8 (95 % CI = 0.5–1.1) *Solvents* High exposure: OR = 2.1 (95 % CI = 1.2–3.5); *p*-trend = 0.01 *Pesticides* High exposure: OR = 4.6 (95 % CI = 1.6–12.7); *p*-trend = 0.003
Pekmezovic et al. (2006)	Hospital-based case–control study	80 de novo MDS from the Institute of Hematology, Clinical Centre of Serbia, Belgrade	In-person interview with items on demographics, lifetime residence, smoking, alcohol consumption, diet, coffee consumption, and occupational exposure to radiation and chemicals	*Smoking*
	Serbia (Belgrade)	160 controls from outpatient departments (cardiology, gastroenterology, ophthalmology) for nonmalignant and noninfectious diseases; also had blood work to confirm no blood disorders or liver disease		Ever: OR = 1.1 (95 % CI = 0.6–1.9)
	2000–2003			Duration >25 years: OR = 1.0 (95 % CI = 0.4–2.3)

				Frequency >20 cigarettes/day: OR = 1.3 (95 % CI = 0.4–3.6) *Alcohol consumption* Any: OR = 3.0 (95 % CI = 1.7–5.3) Consumption weekly (vs. rarely): OR = 1.7 (95 % CI = 0.6–4.3) Spirits (vs. wine or beer): OR = 11.8 (95 % CI = 3.8–36.4) *Pesticides* Exposed: OR = 7.3 (95 % CI = 2.9–18.1) *Other significant associations* Rural residence (vs. urban): OR = 2.3 (95 % CI = 1.1–4.7) Coffee consumption: OR = 4.8 (95 % CI = 2.2–10.8) Coffee consumption >4 cups/day: OR = 7.5 (95 % CI = 1.9–34.6) Egg consumption ≥7 times/week: OR = 2.8 (95 % CI = 1.2–6.3) Radiation exposure: OR = 10.6 (95 % CI = 1.2–92.3)
Bjork et al. (2009)	Population-based case–control study Southern Sweden 2001–2004	75 de novo MDS cases from the regional cancer registry 132 population controls and 146 hospital-based controls, frequency matched by age, sex, and county of residence	In-person interview with detailed questions on smoking history	*Smoking* Ever: OR = 1.3 (95 % CI = 0.7–2.2) Recent: OR = 1.8 (95 % CI = 1.0–3.3) Duration (years, per-unit increase): OR = 1.009 (95 % CI = 0.996–1.023) Duration ≥20 years: OR = 1.4 (95 % CI = 0.8–2.5) Pack-years (per-unit increase): OR = 1.013 (95 % CI = 1.001–1.026) Pack-years ≥20 OR = 1.6 (95 % CI = 0.9–3.1)

(continued)

Table 2.2 (continued)

Reference	Study design, region, and time period of case diagnosis	Study population	Data collection	Results[a]
Ma et al. (2009)	Prospective cohort study	NIH-AARP Diet and Health Study (National Institutes of Health-American Association of Retired Persons); established in 1995–1996; included over 560,000 people throughout the US	Self-administered questionnaire with items on demographics, smoking, alcohol consumption, diet, and physical activity	*Smoking*
	USA 2001–2003	193 MDS cases identified through linkage to the SEER registry	BMI calculated from self-reported weight and height	Ever: HR = 1.9 (95 % CI = 1.4–2.7) Former: HR = 1.7 (95 % CI = 1.2–2.4) Former, >1 pack/day: HR = 1.9 (95 % CI = 1.2–2.8) Current: HR = 3.2 (95 % CI = 2.0–5.0) Current, >1 pack/day: HR = 4.7 (95 % CI = 2.7–8.2) *Alcohol consumption* Drinks per day, third tertile: HR = 0.8 (95 % CI = 0.6–1.2); *p*-trend = 0.57 *Other significant associations* Body mass index (per-unit increase): HR = 1.07 (95 % CI = 1.03–1.10) Obese vs. normal weight: HR = 2.2 (95 % CI = 1.5–3.2)

Kokouva et al. (2011)	Hospital-based case–control study	78 prevalent MDS cases from medical records of two hospitals	In-person interview with questions on demographics, residence, smoking, alcohol consumption, and occupational history; subjects with at least 1 year of work in farming were asked detailed questions on pesticide exposure	*Pesticides*
	Greece (Thessaly)	455 controls admitted to the same hospitals (for acute conditions or to the internal medicine or blood donor departments) who were self-declared cancer-free	Phytopathologist review of questionnaires with grouping of pesticide exposures by target pest and active ingredients	Medium/high exposure (vs. low/none): OR = 1.9 (95 % CI = 1.0–3.5)
	2003–2006		Quantitative total pesticide exposure was calculated from number of treatments per year, years of use, and area cultivated	High exposure (vs. low/none): OR = 2.1 (95 % CI = 1.0–4.3) Agricultural occupation: OR = 1.7 (95 % CI = 1.1–2.8) Pesticide application by spraying: OR = 0.3 (95 % CI = 0.1–0.8) Pesticide application during planting: OR = 1.5 (95 % CI = 0.4–5.2) Pesticide application to seed: OR = 5.1 (95 % CI = 1.5–16.8) Smoking during pesticide application: OR = 3.7 (95 % CI = 1.2–12.1)

(continued)

Table 2.2 (continued)

Reference	Study design, region, and time period of case diagnosis	Study population	Data collection	Results[a]
Lv et al. (2011)	Hospital-based case–control study	403 de novo MDS cases diagnosed at 27 major hospitals	In-person interview with questions on demographics, smoking, alcohol consumption, hair dye use, residential environment, lifetime occupational history, and specific occupational exposures	*Smoking*
	China (Shanghai)	806 controls hospitalized for conditions not related to hematologic diseases, excluding patients with a history of any malignant or nonmalignant hematologic disease, matched by hospital, age, and sex	Additional expert assessment of occupational exposures	Ever: OR = 1.4 (95 % CI = 1.0–2.0)
	2003–2006			Former: OR = 1.5 (95 % CI = 0.9–2.3) Current: OR = 1.4 (95 % CI = 0.9–2.1) Duration ≥20 years: OR = 1.2 (95 % CI = 0.9–1.6); *p*-trend = 0.44 Cigarettes per day ≥20: OR = 1.1 (95 % CI = 0.8–1.5); *p*-trend = 0.52 Pack-years ≥20: OR = 1.1 (95 % CI = 0.7–1.6); *p*-trend = 0.20 *Alcohol consumption* Ever: OR = 1.3 (95 % CI = 0.9–1.8) Former: OR = 1.2 (95 % CI = 0.8–2.0) Current: OR = 1.4 (95 % CI = 0.8–2.4) Consumption per week ≥70 g: OR = 1.7 (95 % CI = 0.9–3.2); *p*-trend = 0.28 Duration ≥20 years: OR = 1.1 (95 % CI = 0.7–1.6); *p*-trend = 0.34 *Solvents* Benzene exposure, ever: OR = 4.3 (95 % CI = 1.9–10.0)

				Benzene >10 years: OR = 5.0 (95 % CI = 1.6–15.9); *p*-trend = 0.001 Benzene, high intensity: OR = 12.5 (95 % CI = 2.8–55.7); *p*-trend = 0.001 Daily benzene exposure: OR = 4.9 (95 % CI = 1.3–18.6); *p*-trend < 0.001 Glues exposure, ever: OR = 2.8 (95 % CI = 0.9–8.8) *Pesticides* Pesticide exposure, ever: OR = 2.2 (95 % CI = 1.2–3.8) Herbicide exposure, ever: OR = 5.3 (95 % CI = 1.4–20.1) *Other significant associations* No education (vs. 6–9 years): OR = 20.7 (95 % CI = 2.7–156); *p*-trend = 0.001 Hair dye use ≥2 times/year: OR = 1.5 (95 % CI = 1.0–2.1); *p*-trend = 0.02 Live in a new or renovated building: OR = 1.7 (95 % CI = 1.1–2.0)

OR odds ratio, *CI* confidence interval, *HR* hazard ratio, *RAEB* refractory anemia with excess blasts
[a]Unless otherwise noted, reference group is no exposure

2001; Strom et al. 2005). A meta-analysis of ten studies found a significant increased risk of MDS associated with smoking (RR = 1.5, 95 % CI = 1.2–1.7), with evidence of heterogeneity of the effect between studies but no evidence of publication bias (Du et al. 2010). Ma et al. found in a prospective cohort of American Association of Retired Persons (AARP) members that smoking-related risks were higher for current smokers (HR = 3.2, 95 % CI = 2.0–5.0) than past smokers (HR = 1.7, 95 % CI = 1.2–2.4) (compared to never smokers) and increased by the number of packs per day (Ma et al. 2009). Bjork et al. also found a higher risk associated with "recent" smoking than ever smoking and estimated 1.3 % increased risk of MDS per pack-year of exposure, amounting to an excess risk of 71 % (95 % CI = 3–180) associated with 40 pack-years of smoking (Bjork et al. 2009). Several studies evaluated risk factors for subgroups of MDS cases classified by histologic subtype or chromosomal abnormalities. One study found that RAEB/RAEB-t patients were more likely to be current smokers than RA/RARS patients (18 % vs. 10 %) (Strom et al. 2005) and that MDS patients with chromosomal abnormalities were more likely to be ever smokers than those with normal karyotype (73 % vs. 59 %) (Strom et al. 2005). A study conducted in China similarly found significant associations with smoking only for the RAEB subtype (Lv et al. 2011). Another study found that smoking was particularly associated with MDS characterized by any of several specific chromosomal abnormalities (−5/5q−,−7/7q−, +8), estimating a 2.8-fold increased risk for this MDS subgroup associated with ever smoking (95 % CI = 1.0–7.6) (Bjork et al. 2009). Studies of AML have also found particularly strong smoking associations for AML cases with specific chromosomal abnormalities, including −7 or 7q− (Sandler et al. 1993) and those with t(8;21) abnormalities (Moorman et al. 2002). Further evidence for cytogenetic differences in smoking-related MDS is supported by an observation of increased mortality among cases who ever smoked (HR = 1.2, 95 % CI = 1.0–1.3), with even shorter survival associated with higher smoking frequency (Ma et al. 2011).

Alcohol consumption has been significantly or nonsignificantly associated with increased risk of MDS in several studies (Brown et al. 1992; Dalamaga et al. 2002; Ido et al. 1996; Pekmezovic et al. 2006), with dose–response by total ethanol consumption (ml/week) in a study conducted in Japan (Ido et al. 1996). However, several studies have found no consistent association with alcohol consumption (Lv et al. 2011; Ma et al. 2009; Nagata et al. 1999), and a meta-analysis of five studies found only a nonsignificant increase in MDS risk with alcohol consumption (RR = 1.3, 95 % CI = 0.8–2.2) and significant heterogeneity between studies (Du et al. 2010). Several studies found differing risks according to the type of alcohol consumed, with a higher risk reported for hard liquor (spirits) versus wine or beer (OR = 11.8, 95 % CI = 3.8–36.4) (Pekmezovic et al. 2006) and significantly decreased MDS risk associated with wine consumption (OR = 0.5, 95 % CI = 0.4–0.8) (Strom et al. 2005). One study conducted in Greece found an interaction between smoking and alcohol consumption, with a 9.5-fold increased risk associated with both exposures versus neither (95 % CI = 3.5–25.8) (Dalamaga et al. 2002).

Ma et al. found in a prospective cohort of American Association of Retired Persons (AARP) members that obesity was significantly associated with risk of MDS and there was an increasing risk by increasing categories of body mass index (kg/m^2). Highly obese participants (BMI ≥ 35) had 2.5-fold increased risk (95 % CI = 1.5–4.4) of developing MDS during the study follow-up, compared to participants with normal weight (BMI of 18.5 to <25) (Ma et al. 2009). Higher weight was associated with MDS in another study, although the association with BMI was not significant (Dalamaga et al. 2007). The plausibility of these findings is strengthened by several studies describing increased risk of myeloid leukemia with overweight and obesity (De Roos et al. 2010b; Lichtman 2010; MacInnis et al. 2005; Ross et al. 2004).

Several other lifestyle factors have been evaluated in only a few studies, and in general, the detail of the questionnaires used was limited. Indicators of socioeconomic status have not been

associated with risk of MDS in most of the few studies that assessed such factors, including education (Dalamaga et al. 2002; Pekmezovic et al. 2006; Rigolin et al. 1998; Strom et al. 2005), professional versus nonprofessional occupation (Pekmezovic et al. 2006), and race/ethnicity (Strom et al. 2005). One exception is a study conducted in Shanghai, China, which found an increased risk of MDS, albeit imprecise, associated with no education (vs. 6–9 years education; OR = 20.7, 95 % CI = 22.7–156) (Lv et al. 2011). Several studies found that risk of MDS was higher among those who were never married (Dalamaga et al. 2002; Farrow et al. 1989) or had no children (Farrow et al. 1989; West et al. 1995); however, a potential biological mechanism for these associations has not been investigated. Rural versus urban residence was associated with increased risk in a study conducted in Serbia (Pekmezovic et al. 2006). The one prospective study of potential lifestyle risk factors for MDS found no consistent associations with vigorous physical activity, fruit and vegetable intake, or total meat intake (Ma et al. 2009). The Serbian study also found no associations with most dietary factors, except increased risks associated with egg intake (≥7 times/week vs. none, OR = 2.8, 95 % CI = 1.2–6.3) and coffee consumption (any vs. none, OR = 4.8, 95 % CI = 2.2–10.8) (Pekmezovic et al. 2006). Use of hair dyes was significantly associated with increased risk of MDS (OR = 2.0, 95 % CI = 1.2–3.4) in a study conducted in Japan (Nagata et al. 1999), with a clear trend of increasing risk by duration and cumulative frequency of use. Several other studies found less consistent increases associated with hair dye use, in other words, nonsignificant associations or significant associations with no evidence of dose–response (Ido et al. 1996; Lv et al. 2011; Mele et al. 1994).

2.3.2.2 Farming and Pesticide Exposure

Farming, as an occupation, has been associated with increased risks of many types of hematopoietic and lymphatic cancers, including myeloid and lymphoid leukemias, non-Hodgkin lymphoma (NHL), Hodgkin lymphoma, and multiple myeloma (Blair and Freeman 2009; Keller-Byrne et al. 1995), and pesticides are suspected etiologic agents. With regards to MDS, specifically, multiple studies have found associations of increased risk with pesticide exposure (Goldberg et al. 1990; Kokouva et al. 2011; Lv et al. 2011; Nisse et al. 2001; Pekmezovic et al. 2006; Rigolin et al. 1998; Strom et al. 2005). Other studies found no association between MDS and pesticide exposure (Albin et al. 2003; Brown et al. 1990; Mele et al. 1994; West et al. 1995) or farming occupation (Brown et al. 1990; Mele et al. 1994; Rigolin et al. 1998). The studies of MDS have relied either on self-reported pesticide exposure, which is subject to recall bias in retrospective studies, or exposure assigned according to occupational title, which offers little information on the level of exposure or types of pesticides used. A study conducted in an agricultural region of Greece asked comparatively detailed questions about pesticide use history (Kokouva et al. 2011). They reported a 2.1-fold increased risk of MDS associated with high versus low/no pesticide exposure (95 % CI = 1.0–4.3) and a 1.7-fold increased risk associated with working in an agricultural occupation (95 % CI = 1.1–2.8). Particularly high risks were found for pesticide application to seed (OR = 5.1, 95 % CI = 1.5–16.8) and smoking during pesticide application (OR = 3.7, 95 % CI = 1.2–12.1) (Kokouva et al. 2011). Additional information supporting pesticides as potentially causal for MDS are epidemiologic associations with other myeloid cell malignancies. A focused review of pesticide-exposed cohorts found a significant increased risk of AML among five studies (RR = 1.6, 95 % CI = 1.0–2.3). The relative risk of any myeloid leukemia was highest among pesticide manufacturing workers (three risk estimates from two studies of phenoxy herbicide, chlorophenol, and alachlor pesticide manufacturing workers, RR = 6.3, 95 % CI = 1.9–21), followed by pesticide applicators (five studies of applicators of a variety of pesticides, RR = 2.1, 95 % CI = 1.4–3.3), and was not significantly increased for farmers and agricultural workers (nine studies with pesticide exposure mostly assumed based on agricultural occupation, RR = 1.03), implying dose–response in the contribution of pesticides to

the etiology of myeloid neoplasms, given more frequent and intense exposures in manufacturing and applicator jobs than in typical farming occupations (Van Maele-Fabry et al. 2007). In summary, there is a substantial amount of preliminary data suggesting an association between pesticide exposure and MDS incidence; however, the existing studies leave room for improvement in terms of researching specific farming- or pesticide-related agents as potential causal factors.

2.3.2.3 Solvents

Solvents have been a suspected and scrutinized group of exposures for risks of myeloid cell neoplasms. Plausible mechanisms by which various solvents may induce myeloid malignancies include DNA mutations (McHale et al. 2008; Motohashi et al. 1999; Zhang et al. 2007), DNA hypomethylation (Tao et al. 2004), and immune dysregulation (Andrys et al. 1997; Blossom and Gilbert 2006; Griffin et al. 2000). "Solvents" is a very broad category that includes many distinct chemicals in various formulations, primarily used for cleaning and degreasing and for blending other products (e.g., paints). The most evidence for a risk of MDS exists for benzene, an aromatic hydrocarbon solvent which is a known cause of AML based on sufficient data from human studies and clear evidence of the bone marrow as a site of benzene toxicity (Golding and Watson 1999). Cohort studies of benzene-exposed workers are individually small and underpowered, but a meta-analysis of nine studies found that the risk of AML was 3.2-fold greater (95 % CI = 1.1–9.5) with high benzene exposure (>100 ppm-years) than for unexposed (Khalade et al. 2010). Few of these cohort studies included MDS or reported on it separately, but a couple noted significantly higher numbers of MDS cases than expected (grouped with myelofibrosis in one study) (Cowles et al. 1991; Honda et al. 1995). The timing of exposure may be important with regard to MDS etiology. Hayes et al. found in his study of workers in China exposed to an average benzene intensity of less than 10 ppm that increased risk of AML/MDS was significantly associated with exposure within the past 10 years (RR = 5.3, 95 % CI = 1.8–15.6; p-trend = 0.003), but was not clearly associated with exposure in the more distant past (p-trend = 0.51) (Hayes et al. 1997).

Several case–control studies have investigated solvents in relation to MDS (Table 2.2) and have found evidence supporting increased risk associated with benzene exposure (Blair et al. 2001; Lv et al. 2007) or solvent exposure in general (Goldberg et al. 1990; Ido et al. 1996; Nagata et al. 1999; Nisse et al. 2001; Rigolin et al. 1998; Strom et al. 2005; West et al. 1995). These studies are subject to the same limitations as the studies on pesticides, in terms of the possibility of recall bias and limited exposure information. One study conducted in Houston, Texas, USA, found that the association with solvents was stronger for RAEB/RAEB-t patients than for RA/RARS; a significant trend for increasing risk across levels of solvent exposure was observed only for RAEB/RAEB-t (p-trend = 0.001 vs. p-trend = 0.34 for RA/RARS), relating to a 2.7-fold increased risk of RAEB/RAEB-t for high-solvent versus no solvent exposure (95 % CI = 1.5–4.9) (Strom et al. 2005). A study in Shanghai, China, that defined MDS according to WHO 2008 criteria found that cases with high benzene exposure were more likely to be of unspecified MDS histology (OR = 11.1, 95 % CI = 1.3–92.4) or RAEB (OR = 1.4, 95 % CI = 0.2–11.1) than other subtypes (Irons et al. 2010). Other frequent features of highly exposed compared to non-exposed cases were marked erythroid dysplasia in the bone marrow, no evidence of increased ring sideroblasts, near-normal hemoglobin values in the face of marked bone marrow dyserythropoiesis, and a striking increase in multilineage dysplasia with abnormal eosinophils. However, highly benzene-exposed cases were less likely to have any clonal cytogenetic abnormalities (OR = 0.5, 95 % CI = 0.2–1.3) (Irons et al. 2010). Another study found no association between solvent exposure and the number or type of chromosomal abnormalities (Albin et al. 2003).

2.3.2.4 Other Environmental Exposures

There have been several other environmental exposures that have been associated with MDS, usually not in targeted investigations but rather in studies that screened a large number of possible

risk factors. These associations are worth mentioning, if only for generating new research directions for potential causes of MDS. Increased risks have been observed in at least one study for working in the industries: nonmetallic minerals; plumbing, heating, and air conditioning; sales; metals fabricating; or education, welfare, and health or occupation as truck drivers, machine operators, textile workers, and health professionals (Blair et al. 2001; Nisse et al. 2001; West et al. 1995). Specific exposures associated with increased risk of MDS in at least one study are ammonia, diesel, oil, metals, copper compounds, fertilizers, cereal dust, poultry, livestock, infectious agents, cotton and flax dusts, and extremely low-frequency electric and magnetic fields (EMF) (Albin et al. 2003; Farrow et al. 1989; Nisse et al. 2001; West et al. 1995). Other occupational/environmental exposures that should be pursued as potential risk factors for MDS are those with suggestive associations with AML, including engine exhausts (Blair et al. 2001; Howe and Lindsay 1983) and formaldehyde (Baan et al. 2009; Beane Freeman et al. 2009; Hauptmann et al. 2003).

2.3.3 Host Risk Factors for MDS

A person's biological makeup may predispose them to develop MDS; obvious examples are the striking increase with age and the higher incidence in men versus women. Several studies have found significant or nonsignificant increased risk of MDS among persons with a family history of hematopoietic or lymphatic cancer (Nisse et al. 2001; Strom et al. 2005; West et al. 1995), suggesting that genetic factors are likely to play a role in MDS etiology. Various genetic pathways have been investigated with regards to MDS, including detoxification, DNA repair, and immune-related polymorphisms, but individual studies have generally been small and underpowered, and few variants have been investigated in more than one study. An exception is the well-studied glutathione-S-transferase (GST) genes responsible for detoxification of xenobiotics such as polycyclic aromatic hydrocarbons, solvents, and pesticides. GSTT1 was evaluated in a meta-analysis of 13 studies, which found a 1.4-fold increased risk associated with the null genotype (95 % CI = 1.1–1.9), with moderate between-study heterogeneity (Dahabreh et al. 2010). In contrast, there was no association with GSTM1 null genotype in ten studies (Dahabreh et al. 2010). The GSTP1 105Val allele was associated with increased risk of MDS in one study (Fabiani et al. 2009). A study of therapy-related MDS/AML found that patients with variants in detoxification genes were at increased risk of therapy-related malignancy, for either NAD(P)H:quinine oxidoreductase (NQO1; OR = 2.1, 95 % CI = 1.1–4.0) or a polymorphism profile consisting of GSTT1 null in addition to variants in NQO1 and CYP1A1 (Bolufer et al. 2007). Several variants in DNA repair genes (RAD51, XRCC3, XPD) were not associated with MDS incidence in two studies (Baumann Kreuziger and Steensma 2008; Fabiani et al. 2009). An epoetin (EPO) promoter gene variant (rs1617640) associated with decreased EPO expression was significantly associated with increased risk of MDS among 187 cases and 95 controls (OR = 5.0, 95 % CI = 2.0–12.1) (Ma et al. 2010). Polymorphisms associated with increased expression of TNF-alpha and TGF-beta 1 (TGFB1) were more prevalent in a small group of MDS cases ($n = 21$) than among normal populations listed in the NCI SNP500 database (Powers et al. 2007), and another study found a TGFB1 variant (TT at codon 10, exon 1) to be more common in MDS patients with bi- or pancytopenia than those with only anemia ($p = 0.007$) (Gyuali et al. 2005), but found no associations with TNF-alpha.

Persons with autoimmune diseases are at increased risk of developing MDS. A hospital-based case–control study conducted in Greece found that persons with autoimmune diseases were at increased risk of developing de novo MDS, even 10 years or longer after autoimmune disease diagnosis (OR = 3.5, 95 % CI = 1.2–10.3) (Dalamaga et al. 2002). They reported a particular association with autoimmune thyroid disease (OR = 5.7, 95 % CI = 2.0–16.1) (Dalamaga et al. 2008). Autoimmune phenomena are commonly reported in patients with MDS (Enright and Miller 1997); however, it is not known if these symptoms

simply coevolve with MDS or if autoimmunity is potentially causal of MDS. In a large study linking SEER-reported myeloid cancer cases with Medicare records, autoimmune conditions were associated with increased risk of developing MDS (OR = 1.5, 95 % CI = 1.4–1.7) and AML (OR = 1.3, 95 % CI = 1.2–1.4) (Anderson et al. 2009). Specifically, the risk of MDS was associated with rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, and pernicious anemia. There was no association with autoimmune thyroid disease, unlike the study conducted in Greece. A similar type of linkage study conducted in Sweden found increased risk of MDS associated with any autoimmune disease (OR = 2.1, 95 % CI = 1.7–2.6) as well as specifically with rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, immune thrombocytopenic purpura, myasthenia gravis, polymyalgia rheumatica, psoriasis, giant cell arteritis, and aplastic anemia; there were similar findings for AML. These risks were also present when limited to MDS cases diagnosed at least 3 years after diagnosis of the autoimmune condition, although estimates were not always statistically significant (Kristinsson et al. 2011). A history of allergy, another immune-related condition, was not associated with risk of MDS in two case–control studies (Dalamaga et al. 2002; Pekmezovic et al. 2006). Similarly, asthma history was not associated with MDS in the SEER-Medicare linkage study (Anderson et al. 2009).

Bacterial and viral infections have been investigated in relation to MDS. History of upper respiratory infections two or more times per year (vs. none) was associated with increased risk of MDS in a case–control study conducted in Serbia (Pekmezovic et al. 2006). There was a similar observation in the registry-based linkage study conducted in Sweden, in which a history of pneumonia, even 3 or more years before MDS diagnosis, was associated with 30 % increased risk of MDS (OR = 1.3, 95 % CI = 1.0–1.7) (Kristinsson et al. 2011). Other types of infection were also associated with increased MDS risk in the registry study, including herpes zoster and meningitis, and the risk estimate for any infection was 1.3 (95 % CI = 1.1–1.5) (Kristinsson et al. 2011).

Conclusion

The hematopoietic system appears to be vulnerable to neoplastic changes from a variety of lifestyle and environmental exposures. However, accepted causes of MDS are limited at this time to treatment-related chemotherapy and radiation and occupational exposure to benzene. Probable causes, supported by studies of MDS as well as similar findings for AML, are smoking, obesity, and pesticides (without knowledge of specific exposures).

MDS has been particularly challenging to study, for several reasons. As a result of limited historical registration and the difficulty in recruiting controls from the general population, most case–control studies have been based in hospitals (Table 2.2). While these studies have certainly moved the field forward by describing associations with exposures such as smoking and alcohol in multiple studies, as well in conducting exploratory analyses of occupational exposures, the possibility of selection bias from use of patient controls may have produced spurious associations (or masked true effects). An additional issue is that most studies to date (with the exception of Ma et al. 2009) have collected exposure information retrospectively (i.e., after MDS diagnosis), an approach which is subject to biased recall according to case status, as well as generally faulty recall. Nevertheless, very large prospective cohort studies with long follow-up will be required to achieve a large enough number of MDS cases for sufficient power to identify etiologic risk factors. Because of the inherent strengths and weaknesses of both study designs, both prospective cohorts and general population-based retrospective case–control studies are needed to continue to investigate risk factors for MDS. For many of the exposures discussed, further research on broad exposure groupings (e.g., any solvent) or exposure assessment without any distinction of intensity (e.g., ever smoked) will not be useful for clarifying suggested associations. For suspected high-risk exposures and jobs (e.g., pesticides or farmers), future research

should focus on semiquantitative or quantitative exposure assessment (e.g., by on-site measurements or biomarkers) of specific exposures of interest (e.g., specific types of pesticides or application practices) in order to move the state of knowledge forward and to target areas for intervention.

Epidemiologic investigation of disease heterogeneity in MDS has been very limited until now, primarily due to the problem of small numbers within specific subtypes. Changing subtype classifications (e.g., from FAB to WHO) also limit interpretation of subtype-specific results across studies spanning recent decades. Nevertheless, several studies report stronger associations between exposures such as smoking and solvents with RAEB/RAEB-t, subtypes that are more likely to evolve to AML, than with other subtypes including RA (Strom et al. 2005; Irons et al. 2010; Lv et al. 2011). Such studies may elucidate causal associations that are specific to certain subtypes, whereas analysis of MDS as one group would mask associations. Even finer stratification may be achieved by grouping of cases by patterns of cytogenetic abnormalities. Such groupings have proven to be prognostically important and may also be etiologically relevant, for example, if a certain cytotoxic exposure consistently produces a signature pattern of chromosomal abnormalities. Crude case groupings according to the number and specific types of cytogenetic abnormalities have been associated with exposures such as smoking, pesticides, and solvents (Bjork et al. 2009; Rigolin et al. 1998); however, these studies have been limited in terms of both the number of chromosomal abnormalities described and the limited detail of exposure information. Rapidly evolving technologies enabling karyotyping by array have proven more sensitive than traditional metaphase cytogenetics (Maciejewski et al. 2009) and thus may allow refined definition of etiologically relevant subgroups of MDS for epidemiologic studies.

Finally, both descriptive epidemiology studies and studies of disease etiology have until now generally only included MDS cases with confirmed diagnoses that were reported to registries. Several studies with active case-finding (by ICD-9 code or close screening of populations) (Cogle et al. 2011; De Roos et al. 2010a; Guralnik et al. 2004; Tettamanti et al. 2010; Williamson et al. 1994) suggest that a potentially large case group, probably with less severe disease, has been previously unrecognized. Epidemiologic research should expand its scope to include these previously missed MDS cases, with the aim to understand the importance of this case group in terms of its burden on the healthcare system, health outcomes compared to both normal populations and "confirmed" MDS, and disease etiology.

References

Albin M, Bjork J, Welinder H et al (2003) Cytogenetic and morphologic subgroups of myelodysplastic syndromes in relation to occupational and hobby exposures. Scand J Work Environ Health 29:378–387

Anderson LA, Pfeiffer RM, Landgren O et al (2009) Risks of myeloid malignancies in patients with autoimmune conditions. Br J Cancer 100:822–828

Andrys C, Hanovcova I, Chylkova V et al (1997) Immunological monitoring of dry-cleaning shop workers–exposure to tetrachloroethylene. Cent Eur J Public Health 5:136–142

Awidi A, Magableh A, Taimeh Z et al (2009) Primary myelodysplastic syndrome in Jordan: a single-centre experience. Med Princ Pract 18:351–355

Baan R, Grosse Y, Straif K et al (2009) A review of human carcinogens – part F: chemical agents and related occupations. Lancet Oncol 10:1143–1144

Bauduer F, Ducout L, Dastugue N et al (1998) Epidemiology of myelodysplastic syndromes in a French general hospital of the Basque country. Leuk Res 22:205–208

Baumann Kreuziger LM, Steensma DP (2008) RAD51 and XRCC3 polymorphism frequency and risk of myelodysplastic syndromes. Am J Hematol 83: 822–823

Beane Freeman LE, Blair A, Lubin JH et al (2009) Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries: the National Cancer Institute Cohort. J Natl Cancer Inst 101:751–761

Belli C, Acevedo S, Bengio R et al (2002) Detection of risk groups in myelodysplastic syndromes. A multicenter study. Haematologica 87:9–16

Bennett JM, Catovsky D, Daniel MT et al (1982) Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51:189–199

Bjork J, Johansson B, Broberg K et al (2009) Smoking as a risk factor for myelodysplastic syndromes and acute myeloid leukemia and its relation to cytogenetic findings: a case–control study. Leuk Res 33:788–791

Blair A, Freeman LB (2009) Epidemiologic studies in agricultural populations: observations and future directions. J Agromedicine 14:125–131

Blair A, Zheng T, Linos A et al (2001) Occupation and leukemia: a population-based case–control study in Iowa and Minnesota. Am J Ind Med 40:3–14

Bloomfield CD (1986) Chromosome abnormalities in secondary myelodysplastic syndromes. Scand J Haematol Suppl 45:82–90

Blossom SJ, Gilbert KM (2006) Exposure to a metabolite of the environmental toxicant, trichloroethylene, attenuates CD4+ T cell activation-induced cell death by metalloproteinase-dependent FasL shedding. Toxicol Sci 92:103–114

Bolufer P, Collado M, Barragan E et al (2007) Profile of polymorphisms of drug-metabolising enzymes and the risk of therapy-related leukaemia. Br J Haematol 136:590–596

Bowles KM, Warner BA, Baglin TP (2006) Platelet mass has prognostic value in patients with myelodysplastic syndromes. Br J Haematol 135(2):198–200

Brown LM, Blair A, Gibson R et al (1990) Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res 50:6585–6591

Brown LM, Gibson R, Burmeister LF et al (1992) Alcohol consumption and risk of leukemia, non-Hodgkin's lymphoma, and multiple myeloma. Leuk Res 16:979–984

Cartwright RA, Gurney KA, Moorman AV (2002) Sex ratios and the risks of haematological malignancies. Br J Haematol 118:1071–1077

Catenacci DV, Schiller GJ (2005) Myelodysplastic syndromes: a comprehensive review. Blood Rev 19:301–319

Chen B, Zhao WL, Jin J et al (2005) Clinical and cytogenetic features of 508 Chinese patients with myelodysplastic syndrome and comparison with those in Western countries. Leukemia 19:767–775

Cogle CR, Craig BM, Rollison DE et al (2011) Incidence of the myelodysplastic syndromes using a novel claims-based algorithm: high number of uncaptured cases by cancer registries. Blood 117:7121–7125

Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation NRC (2006) Health risks from exposure to low levels of ionizing radiation: BEIR VII Phase 2. The National Academies Press, Washington, D.C

Cowles SR, Bennett JM, Ross CE (1991) Medical surveillance for leukemia at a petrochemical manufacturing complex: four-year summary. J Occup Med 33:808–812

Dahabreh IJ, Giannouli S, Gota V et al (2010) GSTT1 and GSTM1 polymorphisms and myelodysplastic syndrome risk: a systematic review and meta-analysis. Int J Cancer 126:1716–1723

Dalamaga M, Petridou E, Cook FE et al (2002) Risk factors for myelodysplastic syndromes: a case–control study in Greece. Cancer Causes Control 13:603–608

Dalamaga M, Nikolaidou A, Karmaniolas K et al (2007) Circulating adiponectin and leptin in relation to myelodysplastic syndrome: a case–control study. Oncology 73:26–32

Dalamaga M, Karmaniolas K, Papadavid E et al (2008) Association of thyroid disease and thyroid autoimmunity with multiple myeloma risk: a case–control study. Leuk Lymphoma 49:1545–1552

De Roos AJ, Deeg HJ, Davis S (2007) A population-based study of survival in patients with secondary myelodysplastic syndromes (MDS): impact of type and treatment of primary cancers. Cancer Causes Control 18:1199–1208

De Roos AJ, Deeg HJ, Onstad L et al (2010a) Incidence of myelodysplastic syndromes within a nonprofit healthcare system in western Washington state, 2005–2006. Am J Hematol 85:765–770

De Roos AJ, Ulrich CM, Ray RM et al (2010b) Intentional weight loss and risk of lymphohematopoietic cancers. Cancer Causes Control 21:223–236

Du Y, Fryzek J, Sekeres MA et al (2010) Smoking and alcohol intake as risk factors for myelodysplastic syndromes (MDS). Leuk Res 34:1–5

Enright H, Miller W (1997) Autoimmune phenomena in patients with myelodysplastic syndromes. Leuk Lymphoma 24:483–489

Fabiani E, D'Alo F, Scardocci A et al (2009) Polymorphisms of detoxification and DNA repair enzymes in myelodysplastic syndromes. Leuk Res 33:1068–1071

Farrow A, Jacobs A, West RR (1989) Myelodysplasia, chemical exposure, and other environmental factors. Leukemia 3:33–35

Finch SC (2004) Myelodysplasia and radiation. Radiat Res 161:603–606

Fritz A, Percy C, Jack A, Shanmugaratnam K, Sobin L, Parkin DM, Whelan S (2000) International classification of diseases for oncology, 3rd edn. World Health Organization, Geneva, Ref Type: Report

Germing U, Strupp C, Kundgen A et al (2004) No increase in age-specific incidence of myelodysplastic syndromes. Haematologica 89:905–910

Germing U, Aul C, Niemeyer CM et al (2008) Epidemiology, classification and prognosis of adults and children with myelodysplastic syndromes. Ann Hematol 87:691–699

Goldberg H, Lusk E, Moore J et al (1990) Survey of exposure to genotoxic agents in primary myelodysplastic syndrome: correlation with chromosome patterns and data on patients without hematological disease. Cancer Res 50:6876–6881

Golding BT, Watson WP (1999) Possible mechanisms of carcinogenesis after exposure to benzene. IARC Sci Publ (150):75–88

Greenberg P, Cox C, LeBeau MM et al (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89:2079–2088

Griffin JM, Gilbert KM, Lamps LW et al (2000) CD4(+) T-cell activation and induction of autoimmune hepati-

tis following trichloroethylene treatment in MRL+/+ mice. Toxicol Sci 57:345–352
Guralnik JM, Eisenstaedt RS, Ferrucci L et al (2004) Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood 104:2263–2268
Gyuali Z, Balog A, Borbenyi Z et al (2005) Genetic polymorphisms in patients with myelodysplastic syndrome. Acta Microbiol Immunol Hung 52(3-4):463–475
Hauptmann M, Lubin JH, Stewart PA et al (2003) Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. J Natl Cancer Inst 95:1615–1623
Haus O, Kotlarek-Haus S, Potoczek S et al (2006) Myelodysplastic syndromes according to FAB and WHO classification. Single center experience. Neoplasma 53:136–143
Hayes RB, Yin SN, Dosemeci M et al (1997) Benzene and the dose-related incidence of hematologic neoplasms in China. Chinese Academy of Preventive Medicine – National Cancer Institute Benzene Study Group. J Natl Cancer Inst 89:1065–1071 (see comments)
Honda Y, Delzell E, Cole P (1995) An updated study of mortality among workers at a petroleum manufacturing plant. J Occup Environ Med 37:194–200
Howe GR, Lindsay JP (1983) A follow-up study of a ten-percent sample of the Canadian labor force. I. Cancer mortality in males, 1965–73. J Natl Cancer Inst 70: 37–44
Ido M, Nagata C, Kawakami N et al (1996) A case–control study of myelodysplastic syndromes among Japanese men and women. Leuk Res 20:727–731
Irons RD, Gross SA, Le A et al (2010) Integrating WHO 2001–2008 criteria for the diagnosis of myelodysplastic syndrome (MDS): a case-case analysis of benzene exposure. Chem Biol Interact 184:30–38
Irwin J, D'Souza A, Johnson L et al (2011) Myelodysplasia in the Wellington region 2002–2007: disease incidence and treatment patterns. Intern Med J 41:399–407
Jaffe ES (2001) Pathology & genetics, tumours of haematopoietic and lymphoid tissues, World Health Organization classification of tumours. IARC Press, Lyon
Keller-Byrne JE, Khuder SA, Schaub EA (1995) Meta-analysis of leukemia and farming. Environ Res 71:1–10
Khalade A, Jaakkola MS, Pukkala E et al (2010) Exposure to benzene at work and the risk of leukemia: a systematic review and meta-analysis. Environ Health 9:31
Kokouva M, Bitsolas N, Hadjigeorgiou GM et al (2011) Pesticide exposure and lymphohaematopoietic cancers: a case–control study in an agricultural region (Larissa, Thessaly, Greece). BMC Public Health 11:5
Kristinsson SY, Bjorkholm M, Hultcrantz M et al (2011) Chronic immune stimulation might act as a trigger for the development of acute myeloid leukemia or myelodysplastic syndromes. J Clin Oncol 29:2897–2903
Kuendgen A, Matsuda A, Germing U (2007) Differences in epidemiology of MDS between Western and Eastern countries: ethnic differences or environmental influence? Leuk Res 31:103–104
Leone G, Fianchi L, Pagano L et al (2010) Incidence and susceptibility to therapy-related myeloid neoplasms. Chem Biol Interact 184:39–45
Levine EG, Bloomfield CD (1992) Leukemias and myelodysplastic syndromes secondary to drug, radiation, and environmental exposure. Semin Oncol 19:47–84
Lichtman MA (2010) Obesity and the risk for a hematological malignancy: leukemia, lymphoma, or myeloma. Oncologist 15:1083–1101
Lv L, Lin GW, Wang XQ et al (2007) A case–control study of risk factors for myelodysplastic syndromes. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 25:705–709
Lv L, Lin G, Gao X et al (2011) Case–control study of risk factors of myelodysplastic syndromes according to World Health Organization classification in a Chinese population. Am J Hematol 86:163–169
Ma X, Does M, Raza A et al (2007) Myelodysplastic syndromes: incidence and survival in the United States. Cancer 109:1536–1542
Ma X, Lim U, Park Y et al (2009) Obesity, lifestyle factors, and risk of myelodysplastic syndromes in a large US cohort. Am J Epidemiol 169:1492–1499
Ma W, Kantarjian H, Zhang K et al (2010) Significant association between polymorphism of the erythropoietin gene promoter and myelodysplastic syndrome. BMC Med Genet 11:163
Ma X, Wang R, Galili N et al (2011) Cigarette smoking shortens the survival of patients with low-risk myelodysplastic syndromes. Cancer Causes Control 22:623–629
Maciejewski JP, Tiu RV, O'Keefe C (2009) Application of array-based whole genome scanning technologies as a cytogenetic tool in haematological malignancies. Br J Haematol 146:479–488
MacInnis RJ, English DR, Hopper JL et al (2005) Body size and composition and the risk of lymphohematopoietic malignancies. J Natl Cancer Inst 97:1154–1157
Maynadie M, Girodon F, Manivet-Janoray I et al (2011) Twenty-five years of epidemiological recording on myeloid malignancies: data from the specialized registry of hematologic malignancies of Cote d'Or (Burgundy, France). Haematologica 96:55–61
McHale CM, Lan Q, Corso C et al (2008) Chromosome translocations in workers exposed to benzene. J Natl Cancer Inst Monogr (39):74–77
McNally RJ, Rowland D, Roman E et al (1997) Age and sex distributions of hematological malignancies in the U.K. Hematol Oncol 15:173–189
Mele A, Szklo M, Visani G et al (1994) Hair dye use and other risk factors for leukemia and pre-leukemia: a case–control study. Italian Leukemia Study Group. Am J Epidemiol 139:609–619
Moorman AV, Roman E, Cartwright RA et al (2002) Smoking and the risk of acute myeloid leukaemia in cytogenetic subgroups. Br J Cancer 86:60–62
Motohashi N, Nagashima H, Molnar J (1999) Trichloroethylene. II. Mechanism of carcinogenicity of trichloroethylene. In Vivo 13:215–219
Mukiibi JM, Paul B (1994) Myelodysplastic syndromes (MDS) in Central Africans. Trop Geogr Med 46:17–19

Nagata C, Shimizu H, Hirashima K et al (1999) Hair dye use and occupational exposure to organic solvents as risk factors for myelodysplastic syndrome. Leuk Res 23:57–62

Neukirchen J, Schoonen WM, Strupp C et al (2011) Incidence and prevalence of myelodysplastic syndromes: data from the Dusseldorf MDS-registry. Leuk Res 35:1591–1596

Nisse C, Haguenoer JM, Grandbastien B et al (2001) Occupational and environmental risk factors of the myelodysplastic syndromes in the North of France. Br J Haematol 112:927–935

Pekmezovic T, Suvajdzic VN, Kisic D et al (2006) A case–control study of myelodysplastic syndromes in Belgrade (Serbia Montenegro). Ann Hematol 85:514–519

Phekoo KJ, Richards MA, Moller H et al (2006) The incidence and outcome of myeloid malignancies in 2,112 adult patients in southeast England. Haematologica 91:1400–1404

Powers MP, Nishino H, Luo Y et al (2007) Polymorphisms in TGFbeta and TNFalpha are associated with the myelodysplastic syndrome phenotype. Arch Pathol Lab Med 131(12):1789–1793

Preston DL, Kusumi S, Tomonaga M et al (1994) Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma, 1950–1987. Radiat Res 137:S68–S97

Radlund A, Thiede T, Hansen S et al (1995) Incidence of myelodysplastic syndromes in a Swedish population. Eur J Haematol 54:153–156

Richardson D, Sugiyama H, Nishi N et al (2009) Ionizing radiation and leukemia mortality among Japanese Atomic Bomb Survivors, 1950–2000. Radiat Res 172:368–382

Rigolin GM, Cuneo A, Roberti MG et al (1998) Exposure to myelotoxic agents and myelodysplasia: case–control study and correlation with clinicobiological findings. Br J Haematol 103:189–197

Rodger EJ, Morison IM (2011) Myelodysplastic syndrome in New Zealand and Australia. Intern Med J 42(11):1235–1242

Rollison DE, Howlader N, Smith MT et al (2008) Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001–2004, using data from the NAACCR and SEER programs. Blood 112:45–52

Ross JA, Parker E, Blair CK et al (2004) Body mass index and risk of leukemia in older women. Cancer Epidemiol Biomarkers Prev 13:1810–1813

Rubin CM, Larson RA, Anastasi J et al (1990) t(3;21)(q26;q22): a recurring chromosomal abnormality in therapy-related myelodysplastic syndrome and acute myeloid leukemia. Blood 76:2594–2598

Sandler DP, Shore DL, Anderson JR et al (1993) Cigarette smoking and risk of acute leukemia: associations with morphology and cytogenetic abnormalities in bone marrow. J Natl Cancer Inst 85:1994–2003

Sant M, Allemani C, Tereanu C et al (2010) Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. Blood 116:3724–3734

Sekeres MA (2011) Epidemiology, natural history, and practice patterns of patients with myelodysplastic syndromes in 2010. J Natl Compr Canc Netw 9:57–63

Sekeres MA, Schoonen WM, Kantarjian H et al (2008) Characteristics of US patients with myelodysplastic syndromes: results of six cross-sectional physician surveys. J Natl Cancer Inst 100:1542–1551

Shimizu H, Matsushita Y, Aoki K et al (1995) Prevalence of the myelodysplastic syndromes in Japan. Int J Hematol 61:17–22

Singh ZN, Huo D, Anastasi J et al (2007) Therapy-related myelodysplastic syndrome: morphologic subclassification may not be clinically relevant. Am J Clin Pathol 127:197–205

Steensma DP, Bennett JM (2006) The myelodysplastic syndromes: diagnosis and treatment. Mayo Clin Proc 81:104–130

Strom SS, Gu Y, Gruschkus SK et al (2005) Risk factors of myelodysplastic syndromes: a case–control study. Leukemia 19:1912–1918

Surveillance, Epidemiology, and End Results (SEER) Program (2011) www.seer.cancer.gov. SEER*Stat Database: Incidence – SEER 17 Regs Research Data + Hurricane Katrina Impacted Louisiana Cases, Nov 2010 Sub (2000–2008) < Katrina/Rita Population Adjustment > – Linked To County Attributes – Total U.S., 1969–2009 Counties, National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2011 (updated 10/28/2011), based on the November 2010 submission. Ref Type: Report

Tao L, Li Y, Kramer PM et al (2004) Hypomethylation of DNA and the insulin-like growth factor-II gene in dichloroacetic and trichloroacetic acid-promoted mouse liver tumors. Toxicology 196:127–136

Tettamanti M, Lucca U, Gandini F et al (2010) Prevalence, incidence and types of mild anemia in the elderly: the "Health and Anemia" population-based study. Haematologica 95:1849–1856

Tiu RV, Gondek LP, O'Keefe CL et al (2011) Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies. Blood 117:4552–4560

Van Maele-Fabry GV, Duhayon S, Lison D (2007) A systematic review of myeloid leukemias and occupational pesticide exposure. Cancer Causes Control 18:457–478

Vardiman JW, Thiele J, Arber DA et al (2009) The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 114:937–951

West RR, Stafford DA, Farrow A et al (1995) Occupational and environmental exposures and myelodysplasia: a case–control study. Leuk Res 19:127–139

Williamson PJ, Kruger AR, Reynolds PJ et al (1994) Establishing the incidence of myelodysplastic syndrome. Br J Haematol 87:743–745

Zhang L, Rothman N, Li G et al (2007) Aberrations in chromosomes associated with lymphoma and therapy-related leukemia in benzene-exposed workers. Environ Mol Mutagen 48:467–474

Part II

Pathology, Pathophysiology, and Staging

Cytogenetic Diagnosis of Myelodysplastic Syndromes

3

Harold J. Olney and Michelle M. Le Beau

3.1 Introduction

The cytogenetic evaluation of a bone marrow sample from patients with a myelodysplastic syndrome (MDS) is an integral part of clinical care. This analysis not only confirms the diagnosis, but it is invaluable in defining the prognosis and median survival, as well as establishing the risk for progression to an acute myeloid leukemia (AML). On a more fundamental level, cytogenetic analysis has been instrumental in confirming the clonality of these syndromes as well as providing hints into the pathobiology of these entities. This chapter will review the most frequently encountered abnormalities exploring their clinical and genetic features, as well as the techniques of cytogenetic analysis and their applications in MDS.

H.J. Olney, MD, CM (✉)
Department of Medicine and Hematology-Transfusion Medicine, Centre Hospitalier de L'universite De Montreal, 1560 Sherbrooke Street East, Montreal, QC H2L 4M1, Canada
e-mail: hj.olney@umontreal.ca

M.M. Le Beau, PhD
Section of Hematology/Oncology,
Cancer Research Center, University of Chicago,
5841 S. Maryland, MC1140, Chicago, IL 60637, USA
e-mail: mlebeau@bsd.uchicago.edu,

3.2 Diagnosis

The diagnosis of all hematological malignancies, including MDS, begins with the appropriate clinical evaluation combined with expert pathological and genetic analysis. An accurate diagnosis can be crucial in management decisions. MDS is typically first suspected with the identification of cytopenia which can be limited to a single cell line resulting in anemia, neutropenia, or thrombocytopenia on routine blood screening that can have an indolent course but may also present with a symptomatic complication from a profound decrease in one or all three lineages with life-threatening consequences. Dysplasia identified in bone marrow samples may be found in a number of benign and congenital conditions including nutritional disorders, toxic exposures, and infectious states, as well in MDS and acute leukemias (Swerdlow et al. 2008). In highly dysplastic cases of MDS, or when the blast count is elevated, the diagnosis of MDS is relatively straightforward and is characterized by typical laboratory findings discussed earlier in this volume. Given the varied pathological and clinical picture of MDS, however, more sophisticated testing can be useful in establishing the diagnosis.

The key distinguishing feature of these syndromes is the clonal nature of the dysplasia. Initial work with X chromosome inactivation patterns in females, based on isozymes of the enzyme glucose-6-phosphate dehydrogenase, suggested that MDS was a clonal disorder (Prchal et al. 1978).

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_3, © Springer-Verlag Berlin Heidelberg 2013

The most widely available and standardized technique for identifying clonality in MDS, however, is classic cytogenetic analysis. In fact, the World Health Organization has included recurring cytogenetic abnormalities in the classification of several subtypes of MDS with distinct clinical presentations and natural histories as discussed below (Swerdlow et al. 2008). The identification of mutated oncogenes or tumor suppressor genes has been used to confirm the clonal nature of MDS and to provide additional prognostic information (Weimar et al. 1994). Aberrant in vitro growth patterns of stem cells can be characteristic of MDS (Spitzer et al. 1979), yet this evaluation is restricted to laboratories with expertise with this technique and is not routinely available. Immunophenotyping protocols (Wells et al. 2003; Kussick et al. 2005; van de Loosdrecht et al. 2008) and high-resolution microarray techniques (Walker et al. 2002; Gondek et al. 2008), including array comparative genomic hybridization and single-nucleotide polymorphism (SNP) arrays to detect copy-neutral loss of heterozygosity (LOH), may refine future clinical diagnostic and prognostic reasoning. Next-generation sequencing and mass-spectrometry genotyping have permitted the whole-genome sequencing of human malignancies, and its application to MDS holds promise of further understanding of its pathobiology, its diagnosis, and the identification of novel therapeutic targets (Bejar et al. 2011a, b).

Both the newly identified SNP array lesions and mutations of known and novel genes were factored into the IPSS classification refining survival curves of IPSS intermediate-1 patients. The SNP arrays provide additional information allowing for better prognostic resolution (median survival 28 vs. 9 months, $p=0.03$). Thus, the results of these studies suggest that SNP array analysis and mutation screening may have further diagnostic application and complement conventional cytogenetic analysis in risk stratification and the selection of therapy.

3.2.1 Cytogenetic Analysis

The value of cytogenetic analysis in predicting survival and risk of leukemic transformation during a patient's clinical course has been well established (Morel et al. 1993; Toyama et al. 1993; Jotterand and Parlier 1996; Sole et al. 2000; Sole et al. 2005; Haase et al. 2007). Among the few independent variables identified that predict clinical outcomes in MDS, cytogenetic findings form the cornerstone of successful prognostic scoring systems including the most widely adopted International Prognostic Scoring System (Table 3.1a) (Greenberg et al. 1997). Since its adoption, newer classifications have incorporated the degree of cytopenias reflected in transfusion dependency as well as having the advantages of being dynamic throughout the course of the disease (WPSS) (Malcovati et al. 2007) and having been applied to a broader cohort of patients, including therapy-related patients and CMML (Kantarjian et al. 2008). With collaborative efforts using patients from the German-Austrian Working Group, the International Risk Analysis Workshop, the Spanish Cytogenetics Workshop, and the International Cytogenetics Working Group of the MDS Foundation, the prognostic information has been extended to 20 common cytogenetic abnormalities classified into risk quartiles with medial survival ranging from 5.7 to 50.6 months, time until 25 % of patients evolved to AML ranged from 3.4 to 71.9 months (Table 3.1b). Undoubtedly with genetic and epigenetic aberrations being established as key components in the pathogenesis of MDS (as described below), their inclusion in future prognostic modeling will likely be forthcoming.

At the time of diagnosis, recurring chromosomal abnormalities are found in 40–70 % of patients with primary MDS and in 95 % of patients with therapy-related MDS (t-MDS) (Vallespi et al. 1998). The frequency of cytogenetic abnormalities increases with the severity of disease, as does the risk of leukemic transformation with the exception of the del(5q) found in MDS with an isolated del(5q) (the 5q− syndrome). Clonal chromosome abnormalities can be detected in marrow cells of 25 % of patients with Refractory Anemia (RA), 10 % of patients with Refractory Anemia with Ringed Sideroblasts (RARS), 50 % of patients with Refractory Cytopenias with Multilineage

Table 3.1a Cytogenetic abnormalities of the international prognosis scoring system

	Cytogenetic abnormalities	25 % AML progression (years)	Median survival (years)
Favorable risk	Normal karyotype Isolated del(5q) Isolated del(20q) Isolated −Y	5.6	3.8
Intermediate risk	Other abnormalities	1.6	2.4
Poor risk	−7/del(7q) complex karyotypes	0.9	0.8

Table 3.1b Cytogenetic abnormalities of the proposed 4-tier cytogenetic risk stratification system

Risk group	Cytogenetic abnormalities	25 % AML progression (months)	Median survival (months)
Good	Normal del(5q) del(5q) and another abnormality der(1;7)(q10;p10)/t(1;7) del(11q) del(12p) +19 del(20q) −Y	50.6	71.9
Intermediate-1	Two abnormalities (without del(5q) or −7/del(7q)) +8 i(17)(q10) +21 Any other single abnormality	25.7	14.7
Intermediate-2	Two abnormalities with −7/del(7q) t(3q26.2) Complex karyotype (3 abnormalities)	16	9.8
High	Complex karyotype (>3 abnormalities)	5.7	3.4

Dysplasia (RCMD), 50–70 % of patients with Refractory Anemia with Excess Blasts 1, 2 (RAEB-1,2), and 100 % of patients with MDS with isolated del(5q).

Most recurring cytogenetic abnormalities found in MDS are unbalanced, most commonly the result of the loss of a whole chromosome or a deletion of part of a chromosome, but unbalanced translocations and more complex derivative (rearranged) chromosomes can be found (Figs. 3.1 and 3.2, Table 3.2). The most common cytogenetic abnormalities encountered in MDS are del(5q), −7, +8, and del(20q) which have been incorporated into the more robust prognostic scoring systems of MDS. Clones with unrelated abnormalities, one of which typically has a gain of chromosome 8, are seen at a greater frequency (~5 % vs. ~1 %) in patients with MDS than in patients with AML.

A handful of specific cytogenetic abnormalities, including MDS with an isolated del(5q) (the 5q− syndrome) (Van den Berghe and Michaux 1997) and the 17p− syndrome (Jary et al. 1997), are associated with morphologically and clinically distinct subsets of MDS (Table 3.2). Many findings, including rearrangements or deletions of chromosome 5, loss or deletion of chromosome 7, gain of chromosome 8, and complex karyotypes, are common to both MDS and AML (Fig. 3.2). In rare cases, recurring balanced translocations have been reported. Abnormalities characteristic of acute leukemia without a prior myelodysplastic phase, such as the t(15;17), inv(16), and t(8;21),

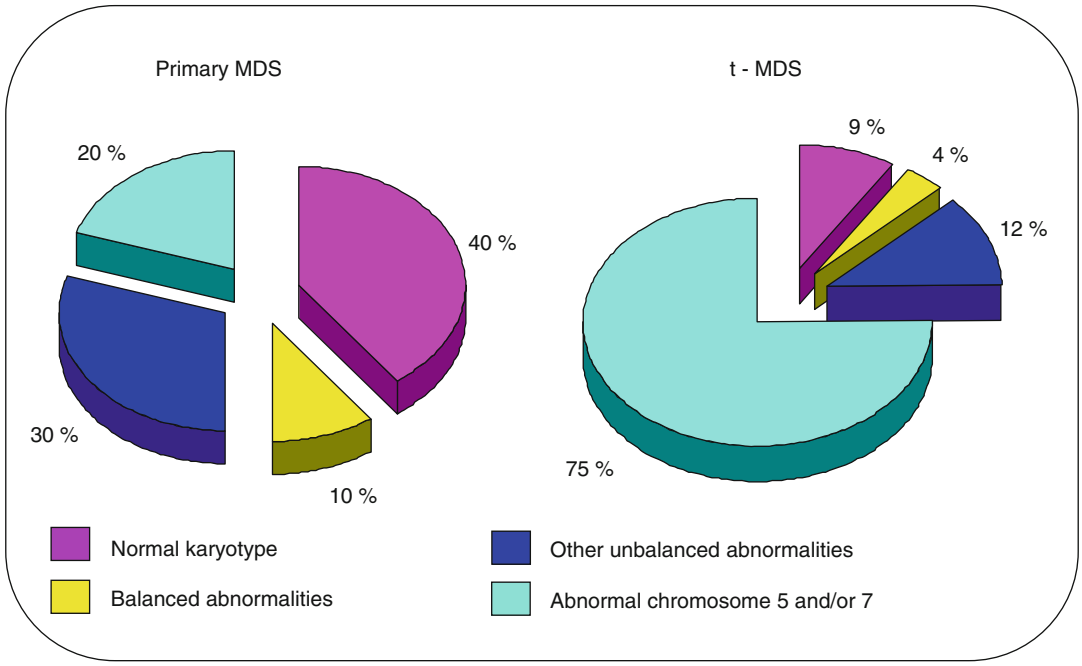

Fig. 3.1 Type of karyotypic abnormalities in MDS

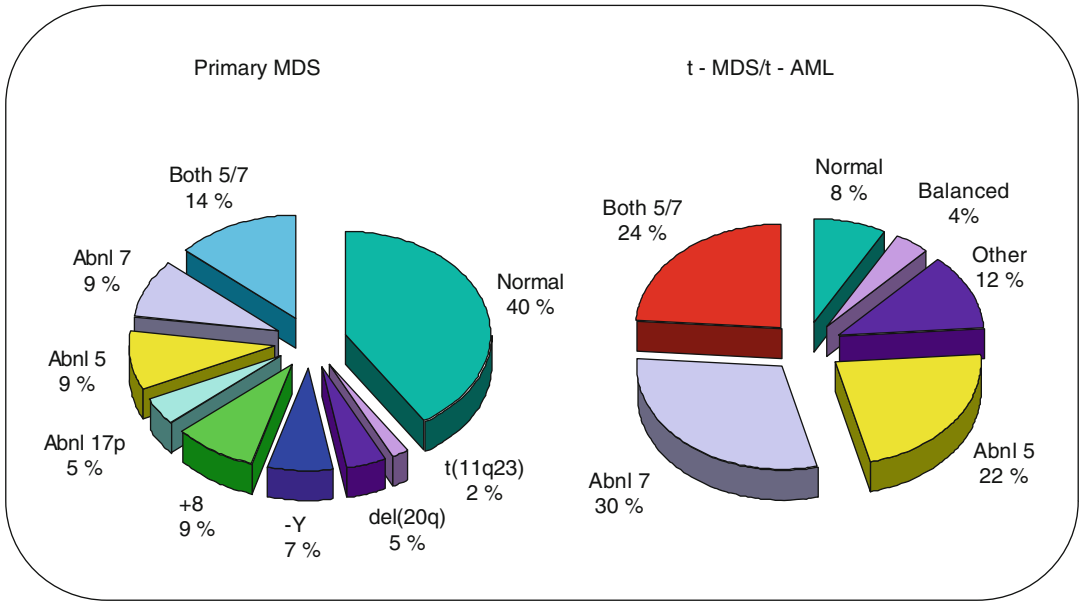

Fig. 3.2 Recurring chromosomal abnormalities in MDS

can be identified rarely in MDS (Rowley 1999). The t(9;22), diagnostic of chronic myelogenous leukemia and a subtype of acute lymphoblastic leukemia (ALL), has only rarely been reported in MDS (Smadja et al. 1989).

3.2.1.1 Specimen Collection

Detailed methods for the cytogenetic analysis of hematological malignant diseases have been described previously (Roulston and Le Beau 1997). Cytogenetic studies are performed on

Table 3.2 Recurring chromosomal abnormalities in myelodysplastic syndromes

Disease[a]	Chromosome abnormality	Frequency (%)	Involved genes[b]		Consequence
MDS	+8	10			
Unbalanced	−7 or del(7q)	10			
	del(5q)/t(5q)	10			
	del(20q)	5–8			
	−Y	5			
	i(17q)/t(17p)	3–5	*TP53*		Loss of function, DNA damage response
	−13/del(13q)	3			
	del(11q)	3			
	del(12p)/t(12p)	3			
	del(9q)	1–2			
	idic(X)(q13)	1–2			
Balanced	t(1;3)(p36.3;q21.2)	1	*MMEL1*	*RPN1*	Deregulation of *MMEL1* – transcriptional activation?
	t(2;11)(p21;q23)/t(11q23)	1		*MLL*	MLL fusion protein – altered transcriptional regulation
	inv(3)(q21q26.2)/t(3;3)(q21;q26.2)	1	*RPN1*	*MDS1/EVI1*	Altered transcriptional regulation by EVI1
	t(6;9)(p23;q34)	1	*DEK*	*NUP214*	Fusion protein – nuclear pore
Therapy-related MDS	−7 or del(7q)	50	*RPL22L1*	*TP53*	Loss of function, DNA damage response
	del(5q)/t(5q)	40–45	*MLL*	*RUNX1*	RUNX1 fusion protein – altered transcriptional regulation
	dic(5;17)(q11.1-13;p11.1-13)	5		*TP53*	Loss of function, DNA damage response
	der(1;7)(q10;p10)	3			
	t(3;21)(q26.2;q22.1)	3	*RPL22L1*	*RUNX1*	RUNX1 fusion protein – altered transcriptional regulation
	t(11;16)(q23;p13.3)/t(11q23)	2	*MLL*	*CREBBP*	MLL fusion protein – altered transcriptional regulation
CMMoL	t(5;12)(q33;p12)	2–5	*PDGFRB*	*ETV6/TEL*	Fusion protein – altered signaling pathways

[a]*MDS* myelodysplastic syndrome, *CMMoL* chronic myelomonocytic leukemia
[b]Genes are listed in order of citation in the karyotype, e.g., for the t(11;16), *MLL* is at 11q23 and *CREBBP* at 16p13.3

spontaneously dividing cells that are typically cultured for short periods (24–72 h). The dividing cells are enriched by arresting them in metaphase using a spindle fiber inhibitor, e.g., Colcemid. Slides are prepared by dropping the cell suspension onto microscope slides, followed by histological staining. Conventional cytogenetic studies can be performed on almost any tissue with actively dividing cells. Bone marrow is the tissue of choice for cytogenetic studies of MDS. Tissue should be collected in cell culture media with heparin to prevent clotting and antibiotics to prevent bacterial contamination, and immediately transported to the laboratory. Specimens should not be collected in tubes containing agents such as EDTA or Sodium Citrate, as these affect cell viability and limit the cells from undergoing mitosis which is essential for analysis.

3.2.1.2 Report and Interpretation of Results

The International System of Human Cytogenetic Nomenclature (ISCN 2009) was developed as the result of several international conferences and is used to describe chromosomal abnormalities in a consistent manner (Shaffer et al. 2009). Using this system, abnormalities can be described with abbreviated terms for the type of rearrangement, followed by the chromosome(s) number, chromosome arm(s), and band(s) involved. A typical cytogenetic analysis includes at least 20 metaphase cells, which provides a good sensitivity for lower-frequency clones. This number may not always be possible and depends on the cellularity, mitotic index, and quality of the specimen. An analysis of less than 20 cells is still informative if a clonal abnormality is detected. The cytogenetic workup should include the analysis of cells from more than one preparation, preferably two short-term cultures, e.g., 24- and 48-h cultures. Genetic mutations accumulate during the progression of a normal cell to a malignant state. Therefore, multiple, related subpopulations of cells derived from a single progenitor may be present in any one specimen. A chromosomal abnormality is considered clonal if a structural abnormality or gain of a chromosome is identified in two or more cells. Chromosome loss can occur as a technical artifact during metaphase cell preparation; thus, a loss of a chromosome is considered to be clonal only when it occurs in three or more cells. The number of cells observed in each clone is listed after the clone description in square brackets [n]. The simplest clone is listed first, and related clones are listed in order of increasing complexity. To describe subclones, the term "idem" followed by any additional changes can be used. It is important to note that "idem" always refers to the abnormalities described in the simplest clone. This type of karyotypic progression is referred to as clonal evolution.

3.2.1.3 Applications of Conventional Cytogenetic Analysis

Cytogenetic analysis should be requested for any patient with a suspected or confirmed MDS. In addition, any abnormality noted at the time of diagnosis can be used as a biological marker to monitor the response to therapy or to detect residual disease in follow-up specimens. Subsequent specimens without this biological marker can be interpreted to represent a cytogenetic remission. Likewise, if the abnormal chromosome(s) is detected in a follow-up specimen, it is indicative of residual disease, relapse, or in the case of karyotypic evolution, disease progression. Increasingly, cytogenetic results will be used to select risk-adapted therapies.

3.2.2 Fluorescence In Situ Hybridization (FISH)

3.2.2.1 Background and Theory

In contrast to classical cytogenetic analysis, fluorescence in situ hybridization (FISH) can evaluate both metaphase and interphase cells in a rapid and efficient manner. The primary advantage of FISH is its simplified analysis permitting the evaluation of an increased number of cells greatly increasing sensitivity. It can also be applied to histologically stained cells allowing a direct correlation of the genetic target's status within morphologically characterized cells. The technique does not, however, evaluate the entire chromosome complement but rather specific

Table 3.3 FISH probes to detect recurring chromosomal abnormalities in MDS

Abnormality	Probe	Format[a]	Vendor[b]
t(11q23)	MLL	Two-color break-apart	Abbott, Kreatech
del(5q)/t(5q)[c]	EGR1D5S23/D5S72	Two-color deletion	Abbott
	CSF1R D5S23/D5S72	Two-color deletion	Abbott
	5q31/5q33	Two-color deletion	Kreatech
−7/del(7q)	D7S522/CEP7	Two-color deletion	Abbott
	D7S486/CEP7	Two-color deletion	Abbott
	CUTL1/7q35	Two-color deletion	Kreatech
del(20q)	D20S108	Single color	Abbott
	20q11.2/q12	Two-color deletion	Kreatech
+8	CEP8, D8Z1	Single color	Abbott, Cytocell, Ltd, Kreatech
del(13q)	D13S25, 13q14	Single color	Abbott, Kreatech
del(11q)	ATM	Single color	Abbott, Kreatech
−17/del(17p)	TP53	Single color	Abbott
	TP53/D17Z1	Two-color deletion	Kreatech
Miscellaneous			
Transplants	CEPX/CEPY, DXZ1, DYZ3	Single color or two color	Abbott, Kreatech

[a]In two-color break-apart probes, DNA sequences from the 5′ and 3′ regions of a single gene are labeled and detected with red and green fluorochromes. In the germ line configuration, a yellow fusion signal is observed, whereas individual red and green signals are observed when the sequences are separated as a result of a translocation

[b]Abbott Molecular Diagnostics (http://www.abbottmolecular.com/home.html); Cytocell, Banbury England (http://www.cytocell.co.uk). Kreatech Diagnostics (http://www.kreatech.com/products.html). Note that there are additional commercial suppliers of FISH probes for diagnostics of hematological malignancies

[c]The *EGR1* gene at 5q31.2 is located within the commonly deleted segment of the del(5q) in AML, whereas the *CSF1R* gene is mapped to 5q33 and detects the del(5q) in the 5q− syndrome

alterations based on probe selection. Not all recurring abnormalities of interest have probes suitable for clinical use, and variation in the cytogenetic abnormality (with either complex rearrangement or differences in breakpoints) may occasionally not be detected with conventional probes.

Fluorescence in situ hybridization is based on the ability of single-stranded DNA to anneal to complementary DNA. In the case of FISH, the target DNA is the nuclear DNA of interphase cells or the DNA of metaphase chromosomes affixed to microscope slides. The test probe is labeled through incorporation of a fluorescently tagged reporter nucleotide. The test probe anneals to its complementary sequences on fixed metaphase chromosomes or interphase nuclei and is visualized by fluorescence microscopy as a brilliantly colored signal at the hybridization site. FISH can be performed using standard cytogenetic cell preparations, bone marrow or peripheral blood smears, or fixed and sectioned tissue.

3.2.2.2 Types of FISH Probes

A variety of probes can be used to detect chromosomal abnormalities by FISH; a partial list of probes available for detection of the recurring abnormalities in MDS is given in Table 3.3. In general, these probes can be divided into three groups: (1) probes that identify a specific chromosome structure, (2) probes that hybridize to multiple chromosomal sequences, and (3) probes that hybridize to unique DNA sequences (Gozzetti and Le Beau 2000).

Centromere Probes

Examples of probes that hybridize to a specific chromosome structure include α- and β-satellite probes. These probes represent tandemly repeated DNA sequences that flank the centromeres of human chromosomes. In most instances, these sequences are distinct, such that an α-satellite probe derived from one chromosome will hybridize to that chromosome only. At present, specific probes are available for chromosomes 1–4, 6–12, 15–18, and 20, as well as the X and Y chromosomes.

Hybridization of chromosome-specific repetitive sequence probes is particularly suitable for the detection of monosomy, trisomy, and other aneuploidies, e.g., −7 or +8 (Moyzis et al. 1987; Jenkins et al. 1992).

Chromosome Painting Probes

Chromosome painting probes utilize cloned DNA libraries derived from whole, flow-sorted human chromosomes (Pinkel et al. 1988). After hybridization and detection, the result is the fluorescent staining or "painting" of an entire chromosome. Chromosome-specific painting probes are available for each of the human chromosomes and are particularly useful in characterizing marker chromosomes (a rearranged chromosome of unknown origin), or additional material of unknown origin translocated to other chromosomes, which are often seen in the complex karyotypes of some MDS patients.

Locus-Specific Probes

Probes that hybridize to unique DNA sequences are usually genomic clones, which vary in size. Probes in this group are particularly useful for detecting structural rearrangements. Using multicolor FISH, recurring translocations can be identified in interphase or metaphase cells by using genomic probes that are derived from the breakpoints (Nederlof et al. 1990; Tkachuk et al. 1990). Similarly, recurring deletions can be detected using genomic probes located within the commonly deleted segment. For example, a locus-specific probe for the *EGR1* gene at 5q31 detected with a green fluorochrome and a locus-specific control probe for the short arm of chromosome 5 detected with a red fluorochrome can be used to identify the del(5q) in MDS.

3.2.2.3 FISH Strategies

Dual-Color/Dual-Fusion Probes

A variety of strategies have been developed for the detection of recurring translocations in interphase cells, each with variable sensitivity (Gozzetti and Le Beau 2000). The selection of a particular probe configuration may vary depending on the application. A common type of probe for detecting translocations with high sensitivity and specificity is a *dual-fusion signal*. In the example of the t(8;21), both probes include large regions spanning the breakpoints in the *RUNX1* and *ETO* loci, such that cells with the t(8;21) have one red and one green signal observed on the normal homologs and yellow fusion signals observed on both the der(21) and der(8) homologs. This type of probe has a low rate of false-positive cells, and the cutoff value for a positive result is ~1 % (Dewald et al. 1998). The results of a recent study suggested that quantitative analysis of 6,000 nuclei could be used to detect MRD with a sensitivity of ~0.2 % (Dewald et al. 1998).

Dual-Color Break-Apart Probes

Two-color break-apart probes are designed such that DNA sequences from the 5′ and 3′ regions of a single gene are differentially labeled and detected with red and green fluorochromes. In the germ line configuration, a yellow fusion signal is observed, whereas separate red and green signals are observed when the sequences are separated as a result of a translocation. This probe configuration offers the advantage that knowledge of the partner gene is not necessary and, therefore, is most useful for loci that are translocated to multiple sites, such as *MLL*. The sensitivity of this type of probe is exceedingly high (cutoff value of ~1.7 %) with very high specificity.

3.2.2.4 Report and Interpretation

When interpreting FISH results, it is important to first note the type of probe strategy used. This provides a general indication of the sensitivity of the specific test performed. Next, it is important to note the specific laboratory reference range for the probe used. The guidelines for clinical FISH analysis establish procedures for probe validation, assay validation, and assay analytical sensitivity (available through http://www.acmg.net). Each laboratory must establish normal and abnormal reference ranges; without this information it is not possible to interpret FISH results accurately. FISH nomenclature, much like chromosome banding nomenclature, is outlined in the ISCN (2009) (Shaffer et al. 2009).

3.2.2.5 Applications of FISH

The applications of FISH in cancer diagnosis have been described in depth in several reviews (Kearney 1999; Gozzetti and Le Beau 2000). In many cases, FISH analysis provides increased sensitivity in that cytogenetic abnormalities have been detected in samples that appeared to be normal by morphological and conventional cytogenetic analysis. FISH is most powerful when the analysis is targeted toward those abnormalities that are known to be associated with a particular disease or are known to occur in a particular patient's tumor. For example, cytogenetic analysis is often performed at the time of diagnosis to identify the chromosomal abnormalities in an individual patient's malignant cells. Thereafter, FISH can be used to detect residual disease or early relapse and to assess the efficacy of therapeutic regimens. It can also be used to follow engraftment of donor cells in stem cell transplant cases, as well as in a number of research applications, including mapping and establishing copy number of different genes.

3.2.2.6 Advantages and Limitations of Cytogenetic and FISH Analysis

The advantages and limitations of cytogenetic and FISH analysis have been described (Gozzetti and Le Beau 2000). Perhaps the most critical parameters for consideration are the sensitivity and specificity of each method and the speed with which each can be accomplished. Cytogenetic analysis has a relatively low sensitivity as compared to FISH (~1:20 cells vs. 1:100). Moreover, cytogenetic analysis requires highly skilled personnel and is labor-intensive; results are not available for 1–3 weeks in all but exceptional cases. In contrast, material for FISH can be processed in 4–24 h, and the analysis of 200–400 cells can be accomplished in 15–20 min. Thus, FISH is a rapid technique that enables one to obtain information on the cytogenetic pattern of tumor cells in a time frame sufficient for the data to be considered in treatment decisions. Both methods can be used to detect numerical and structural chromosomal abnormalities. Cytogenetic analysis requires dividing cells from which metaphase chromosomes can be examined, whereas FISH may be applied to both metaphase cells and interphase cells, allowing for the accurate and informative analysis of those specimens for which no metaphase spreads could be isolated.

The specificity of cytogenetic analysis is very high, and conventional cytogenetic analysis can detect the presence of virtually all chromosomal abnormalities with a single test. In contrast, the most notable limitation of FISH is that the detection of abnormalities is restricted to those loci tested and to those probes that are currently available (Table 3.3). With the exception of several probes marketed by Vysis, Inc. (Abbott Laboratories), most of the commercially available FISH probes have not yet received US Food and Drug Administration approval for diagnostic use. This compels laboratories to continue to use both cytogenetic analysis and FISH methods to analyze the same clinical sample, rather than FISH alone. Other limitations of FISH relate to technical factors. First, the technique has been demonstrated to be highly sensitive for the detection of trisomy but is less sensitive in detecting chromosome loss. The false monosomy rate (presence of only one signal in a diploid cell) can vary from 3 to 9 % of all nucleated cells in preparations from bone marrow or peripheral blood; therefore, the application of FISH to detect monosomy in a small percentage of the cell population, e.g., MRD, is limited. Second, processing of bone marrow and peripheral blood cells is relatively simple; however, processing other tissues, such as paraffin-embedded tissues or frozen sections from lymph node biopsies, is substantially more difficult. Artifacts created by crushing and sectioning of tissue and technical factors related to penetration of the probe into the cell nucleus can give misleading results. In these tissues, the percentage of cells showing false monosomy may be very high (>50 %).

3.3 Recurring Abnormalities

3.3.1 Cytogenetic Findings in MDS

3.3.1.1 Normal Karyotype

In MDS, 30–60 % of patients have a normal karyotype. This group of patients is almost certainly genetically heterogeneous where technical

factors precluded the detection of chromosomally abnormal cells or where leukemogenic alterations occur at the molecular level and are not detectable with standard cytogenetic methods. Nonetheless, despite this heterogeneity, these cases are a standard reference for comparison of outcomes. Patients with a normal karyotype fall within the favorable risk group. The median survival for these patients is 3.8 years, and the time to progression to AML of 25 % of this cohort was 5.6 years (Greenberg et al. 1997).

3.3.1.2 –Y

The clinical and biological significance of the loss of the Y chromosome, –Y, is unknown. The loss of the Y chromosome has been observed in a number of malignant diseases but has also been reported to be a phenomenon associated with aging (Pierre and Hoagland 1972). The United Kingdom Cancer Cytogenetics Group undertook a comprehensive analysis of this abnormality in both normal and neoplastic bone marrows (1992). A –Y could be identified in 7.7 % of patients without a hematologic malignant disease and in 10.7 % of patients with MDS and, thus, was not reliable in documenting a malignant process. In a large series of 215 male patients, Wiktor et al. (2000) found that patients with a hematological disease had a significantly higher percentage of cells with a –Y (52 % vs. 37 %, $p=0.036$). In this series, the presence of –Y in >75 % of metaphase cells accurately predicted a malignant hematological disease. A neutral or favorable prognosis for an isolated –Y was noted by the authors. While loss of a Y chromosome may not be diagnostic of MDS, once the disease is identified by clinical and pathologic means, the International MDS Risk Analysis Workshop found that –Y as the sole cytogenetic abnormality conferred a favorable outcome (Greenberg et al. 1997).

3.3.1.3 del(20q)

A deletion of the long arm of chromosome 20, del(20q), is a common recurring abnormality in malignant myeloid disorders. The del(20q) is seen in approximately 5 % of MDS cases and 7 % of t-MDS cases (Vallespi et al. 1998). Clinical features characterizing MDS patients with a del(20q) include low-risk disease (usually RA), low rate of progression to AML, and prolonged survival (median of 45 months vs. 28 months for other MDS patients) (Wattel et al. 1993). Morphologically, the presence of a del(20q) is associated with prominent dysplasia in the erythroid and megakaryocytic lineages (Kurtin et al. 1996). The International MDS Risk Analysis Workshop noted that patients with a del(20q) observed in association with a complex karyotype identified a poor risk group with a median survival for the entire poor risk group of 9.6 months, whereas the prognosis for patients with an isolated del(20q) was favorable (Greenberg et al. 1997). These data suggest that the del(20q) in MDS may be associated with a favorable outcome when noted as the sole abnormality but with a less favorable prognosis in the setting of a complex karyotype. This phenomenon is analogous to that observed for the del(5q) in MDS (discussed below).

3.3.1.4 Rearrangements of Chromosome 5 or del(5q)

In MDS or AML arising de novo, rearrangements of the long arm of chromosome 5, or a deletion, –5/del(5q), are observed in 10–20 % of patients, whereas in t-MDS/t-AML, it is identified in 40 % of patients (Fig. 3.3) (Vallespi et al. 1998; Godley and Larson 2002; Smith et al. 2003). Although an interstitial deletion is most common, other rearrangements such as unbalanced translocations also occur. Recent studies using FISH and array-based approaches have revealed the presence of chromosome 5 sequences in marker chromosomes, or other rearranged chromosomes, and that –5 does not actually occur. A significant occupational exposure to potential carcinogens is present in many patients with AML or MDS de novo and either del(5q) or a –7/del(7q) (discussed below), suggesting that abnormalities of chromosome 5 or 7 may be a marker of mutagen-induced malignant hematological disease (West et al. 2000).

In primary MDS, abnormalities of chromosome 5 are observed in the 5q– syndrome (described below) or, more commonly, in RAEB 1, 2 of the WHO classification in association with a complex karyotype. Clinically, the

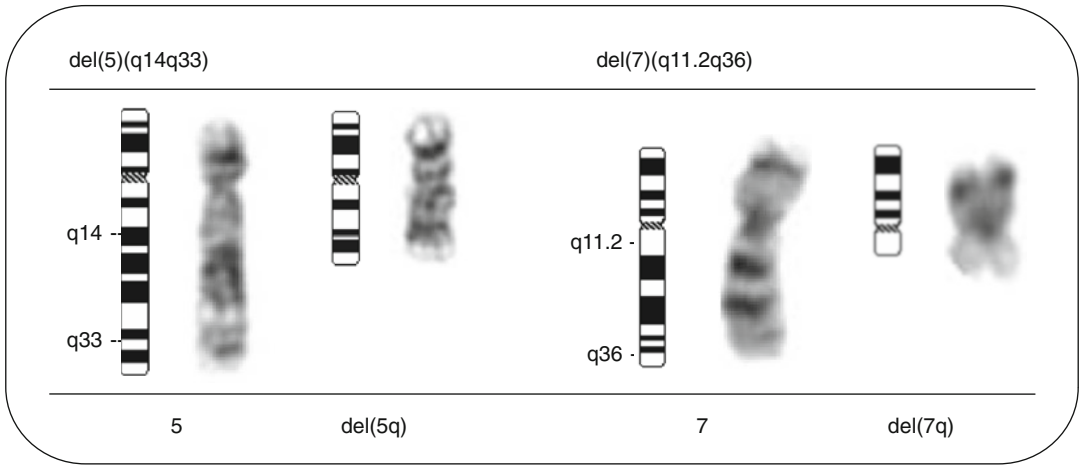

Fig. 3.3 Deletions of 5q and 7q in myeloid neoplasms. In this del(5q), breakpoints occur in q14 and q33, resulting in interstitial loss of the intervening chromosomal material. In this del(7q), breakpoints occur in q11.2 and q36. In both cases, the critical commonly deleted segments are lost. Normal chromosome 5 and 7 homologs are shown for comparison

patients with del(5q) coupled with other cytogenetic abnormalities have a poor prognosis with early progression to leukemia, resistance to treatment, and short survival. Abnormalities of 5q are associated with previous exposure to standard and high-dose alkylating agent therapy, including use in immunosuppressive regimens (Larson et al. 1996; Aul et al. 1998; McCarthy et al. 1998; Pedersen-Bjergaard et al. 2000). A role for exposure to benzene (Hayes et al. 1997) as well as therapeutic ionizing radiation (Fenaux et al. 1989; Rowley and Olney 2002) as risks for MDS is emerging.

3.3.1.5 MDS with an Isolated del(5q) (the 5q– Syndrome)

The 5q– syndrome represents a distinct clinical syndrome characterized by a del(5q) as the sole karyotypic abnormality (Boultwood et al. 1994; Van den Berghe and Michaux 1997). Unlike the male predominance in MDS in general, the 5q– syndrome has an overrepresentation of females (2:1). The initial laboratory findings are usually a macrocytic anemia with a normal or elevated platelet count. The diagnosis is usually RA (in two-thirds) or RAEB-1 (in one-third). On bone marrow examination, abnormalities in the megakaryocytic lineage (particularly micromegakaryocytes) are prominent. These patients have a favorable outcome, in fact the best of any MDS subgroup, with low rates of leukemic transformation and a relatively long survival of several years duration (Boultwood et al. 1994; Greenberg et al. 1997). Loss of a single copy of the *RPS14* gene may be involved in the pathogenesis of this syndrome as described subsequently (Ebert et al. 2008).

3.3.1.6 +8

The incidence of a gain of chromosome 8 in MDS is ~10 %. This abnormality is observed in all MDS subgroups varying with age, gender, and prior treatment with cytotoxic agents or radiation (Morel et al. 1993; Greenberg et al. 1997; Vallespi et al. 1998; Paulsson et al. 2001). The significance of the gain of chromosome 8 in MDS patients is not fully characterized as a risk factor. The situation is complicated in that +8 is often associated with other recurring abnormalities known to have prognostic significance, e.g., del(5q) or –7/del(7q) and may be seen in isolation as a separate clone unrelated to the primary clone in up to 5 % of cases. The presence of cryptic abnormalities at other sites within the genome has also been described in some cases using molecular techniques (Paulsson et al. 2006). The International MDS Risk Analysis Workshop as well as the WPSS ranked this abnormality in the intermediate risk group (Greenberg et al. 1997). Several

groups found that +8 as a sole abnormality had a worse behavior than expected for an intermediate IPSS risk group (Sole et al. 2000; Haase et al. 2007). In a subset of patients, however, a gain of chromosome 8 as the sole abnormality is associated with a better response to immunosuppressive therapy, particularly if the patient is younger, diagnosed with refractory anemia, on HLA typing is DR15 positive, and has a short duration of disease (Sloand and Rezvani 2008). This gain, one of the few examples of gene amplification in MDS, leads to higher levels of expressions of many genes, including *MYC* and several anti-apoptotic genes.

3.3.1.7 Loss of Chromosome 7 or del(7q)

Figure 3.4 A −7/del(7q) is observed as the sole abnormality in approximately 5 % of adult patients with de novo MDS (Toyama et al. 1993; Sole et al. 2000), but in ~50 % of children with de novo MDS (Kardos et al. 2003) and in ~55 % of patients with t-MDS (Fig. 3.3) (Godley and Larson 2002; Smith et al. 2003). As with del(5q), exposure to mutagens, whether occupational (benzene), environmental (smoking), or therapeutic (chemotherapy and radiotherapy as well as immunosuppressive agents), has been associated with −7/del(7q) (Bjork et al. 2000). It can occur in three clinical situations (reviewed in (Luna-Fineman et al. 1995)): (1) de novo MDS and AML, (2) myeloid leukemia associated with constitutional predisposition, and (3) t-MDS/t-AML. The similar clinical and biological features of the myeloid disorders associated with −7/del(7q) suggest that the same gene(s) is altered in each of these contexts. The IPSS considers the −7/del(7q) to be a poor prognosis cytogenetic finding (Greenberg et al. 1997).

"Monosomy 7 Syndrome" has been described in young children. It is characterized by a preponderance of males (~4:1), hepatosplenomegaly, leukocytosis, thrombocytopenia, and poor prognosis (Emanuel 1999; Martinez-Climent and Garcia-Conde 1999). Juvenile myelomonocytic leukemia (JMML, previously known as juvenile chronic myelogenous leukemia), classified by the WHO as a MDS/MPD disease, shares many features with this entity, with −7 observed either at diagnosis or as a new cytogenetic finding associated with disease acceleration on marrow examination (Luna-Fineman et al. 1995). A new paradigm is that −7 cooperates with deregulated signaling via the RAS pathway in the pathogenesis of JMML. Activation of the RAS pathway occurs as a result of mutations in the *NRAS* or *KRAS* genes, inactivating mutations in the gene encoding NF1, a negative regulator of RAS proteins, or activating mutations in the gene encoding the PTPN11/SHP2 phosphatase, a positive regulator of RAS proteins, as well as *RUNX1* mutations, and methylation silencing of *CDKN2B*($p15^{INK4B}$) gene (Christiansen et al. 2003; Loh et al. 2004; Side et al. 2004). In constitutional disorders associated with a predisposition to myeloid neoplasms, including Fanconi anemia, neurofibromatosis type 1, and severe congenital neutropenia, a −7/del(7q) is the most frequent bone marrow cytogenetic abnormality detected.

3.3.1.8 The 17p− Syndrome

Loss of the short arm of chromosome 17 (17p−) has been reported in up to 5 % of patients with MDS. This loss can result from various abnormalities, including simple deletions, unbalanced translocations, dicentric rearrangements (particularly with chromosome 5), or less often −17 or isochromosome formation (Johansson et al. 1993). The dic(5;17)(q11.1-13;p11.1-13) is a frequent recurring rearrangement (Lai et al. 1995; Wang et al. 1997). Approximately one-third of these patients have t-MDS (Merlat et al. 1999), and most have complex karyotypes. The most common additional changes are −7 or loss of 7q and +8.

Morphologically, the 17p− syndrome is associated with a characteristic form of dysgranulopoiesis combining pseudo-Pelger-Huet hypolobulation and the presence of small granules in granulocytes. Clinically, the disease is aggressive with resistance to treatment and short survival. The *TP53* (p53) gene, an important tumor suppressor gene that functions in the cellular response to DNA damage, is located at 17p13.1. In these cases, one allele of *TP53* is typically lost as a result of the abnormality of 17p; an inactivating mutation in the second allele on the remaining, morphologically

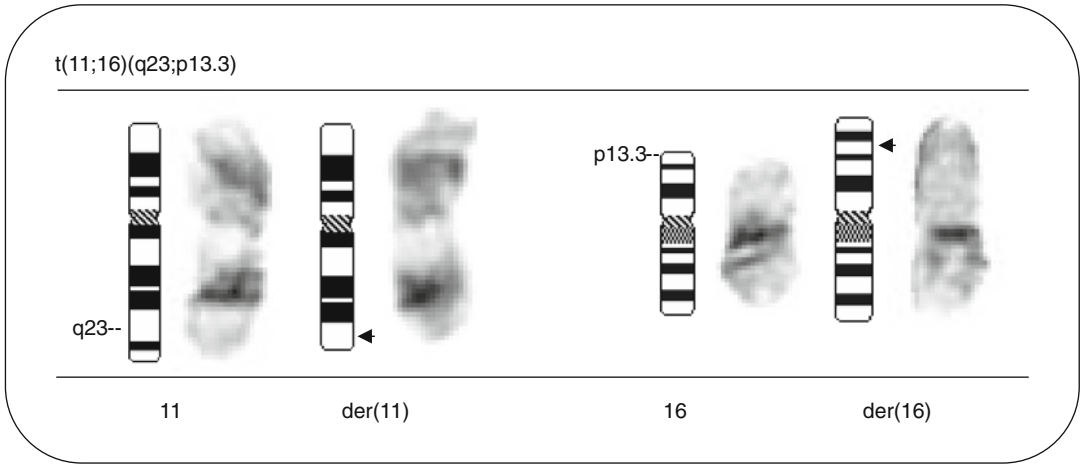

Fig. 3.4 t(11;16)(q23;p13.3). In the t(11;16), breakpoints occur in 11q23 and 16p13.3, followed by a reciprocal exchange of chromosomal material. The 5′ end of the *MLL* gene at 11q23 is fused to the 3′ end of the *CREBBP* gene from 16p13.3 to form the *MLL/CREBBP* fusion gene on the der(11). *Arrowheads* indicate the breakpoints. Normal chromosome 11 and 16 homologs are shown for comparison

normal chromosome 17 occurs in ~70 % of cases (Lai et al. 1995; Wang et al. 1997). Note that the 17p− syndrome is not recognized as a clinically/morphologically distinct entity by the WHO classification (Swerdlow 2008).

3.3.1.9 Translocations of 11q23

The *MLL* (Mixed Lineage Leukemia) gene (also known as *ALL1*, *HTRX*, *HRX*) is involved in rearrangement with 71 partner genes in leukemia (Marschalek 2011). In a European workshop of 550 patients with 11q23 abnormalities, 28 cases (5.1 %) presented with a MDS, and five others with such an abnormality had evolved from t-MDS to t-AML prior to cytogenetic analysis, for a total of 6 % of all examined cases. One fourth of these cases were t-MDS (Bain et al. 1998). Other abnormalities, including complex karyotypes and a −7/del(7q), frequently accompany the 11q23 abnormalities in both primary MDS and t-MDS. No association with FAB subgroup was identified, although RA was overrepresented, and RARS underrepresented as compared to most series of MDS patients. The median survival was short (19 months) with leukemic transformation in ~20 % of cases. The classic association of prior exposure to topoisomerase II inhibitors in their 40 cases of t-MDS and t-AML was not confirmed in this workshop, but this may simply reflect the relatively small number ($n=23$) of cases with full treatment details (Secker-Walker 1998).

Just under 12 % of the 162 patients with 11q23 involvement included in an international workshop on MDS and leukemia following cytotoxic treatment presented with a t-MDS (Bloomfield et al. 2002; Rowley and Olney 2002). One-third (6/19) of these patients had progression to an acute leukemia (5 AML, 1 ALL). This study also did not find a clear association with FAB subtype. The most common translocations were t(9;11)(p22;q23) in six cases, t(11;19)(q23;p13.1) in three cases, and t(11;16)(q23;p13.3) in three cases.

3.3.1.10 t(11;16)

The t(11;16)(q23;p13.3) occurs primarily in t-MDS, but rare cases have presented as t-AML (Fig. 3.4) (Rowley et al. 1997). The t(11;16) is unique among at least 54 recurring translocations of *MLL* in myeloid malignancies (with AML predominating), in that most patients have t-MDS. The *MLL* gene on chromosome 11 is fused with the *CREBBP* (CREB binding protein or *CBP*) gene on chromosome 16. The MLL protein is a histone methyltransferase that assembles in protein complexes that regulate gene transcription,

e.g., *HOX* genes during embryonic development, via chromatin remodelling. CREBBP is an adapter protein involved in transcriptional control via histone acetylation, which mediates chromosome decondensation, thereby facilitating transcription.

3.3.1.11 Complex Karyotypes

Complex karyotypes are variably defined but generally involve the presence of ≥3 chromosomal abnormalities. The majority of cases with complex karyotypes involve unbalanced chromosomal abnormalities leading to the loss of genetic material. Complex karyotypes are observed in ~20 % of patients with primary MDS and in as many as 90 % of patients with t-MDS (Le Beau et al. 1986; Godley and Larson 2002; Smith et al. 2003). Abnormalities involving chromosomes 5, 7, or both are identified in most cases with complex karyotypes. There is general agreement that a complex karyotype carries a poor prognosis (Hamblin and Oscier 1987; Greenberg et al. 1997; Haase et al. 2007; Malcovati et al. 2007).

3.3.1.12 Rare Recurring Translocations

The identification of genes involved in recurring cytogenetic abnormalities has been extremely useful in gaining insights into their normal functions and their role in leukemogenesis (Look 1997; Rowley 2000). The consequence of the recurring translocations is the deregulation of gene expression with increased production of a normal protein product or the generation of a novel fusion gene and production of a fusion protein. To date, all of the recurring translocations cloned in malignant myeloid disorders result in fusion proteins. In MDS, several such translocations have been identified and examined by molecular analysis.

The Platelet-Derived Growth Factor Receptor Beta Translocations

The t(5;12)(q33;p13) is observed in ~1 % of patients with chronic myelomonocytic leukemia (CMMoL). In 1994, the molecular consequences of this translocation were elucidated. The gene encoding the beta chain of the platelet-derived growth factor receptor (*PDGFRB*) is involved on chromosome 5. A novel ETS-like (Erythroblastosis Virus Transforming Sequence) transcription factor, *TEL* (translocated ETS in leukemia, also known as *ETV6*), is the gene affected on chromosome 12. The translocation creates a fusion gene, and the encoded fusion protein contains the 5′ portion of *TEL* and the 3′ portion of *PDGFRB* (Golub et al. 1994). Biochemical studies have revealed that the PDGFRB kinase activity is perturbed contributing to the transformed phenotype. *TEL* encodes a transcriptional repressor and is promiscuously involved in translocations with some 40 genes in hematologic malignancies (Zhang and Rowley 2006). Interest has increased in identifying this translocation which predicts for a response to imatinib mesylate, a selective inhibitor of the tyrosine kinase activity of the PDGFRB protein (Apperley et al. 2002). Similarly, *PDGFRB* participates in other rare translocations involving genes encoding the membrane associated protein HIP1 (Huntington interacting protein 1) in the t(5;7)(q33;q11.2) (Ross et al. 1998); the small GTPase RABPT5 (Rabaptin 5) in the t(5;17)(q33;p13.2) (Magnusson et al. 2001) and H4, a ubiquitous protein of unknown function in the t(5;10)(q33;q21) (Kulkarni et al. 2000) to produce CMMoL; and with CEV14 (clonal evolution-related gene on chromosome 14, also known as TRIP11, thyroid hormone receptor interactor 11) in the t(5;14)(q33;q32) in a case of AML (Abe et al. 1997). A unifying feature of these various translocations is the presence of eosinophilia.

Translocations of 3q

The t(3;21)(q26.2;q22.1) has been linked to acute leukemia arising after cytotoxic therapy. This abnormality was first recognized in chronic myelogenous leukemia in blast crisis (Rubin et al. 1987) and later in t-MDS/t-AML (Rubin et al. 1990). The *EAP* gene (Epstein-Barr small RNAs-associated protein) at 3q26.2 encodes a highly expressed small nuclear protein associated with EBV small RNA (EBER1). *EAP* was found to be fused with the *RUNX1* (runt-related transcription factor, also known as AML1) gene at 21q22, retaining the DNA-binding sequences of *EAP*.

The fusion is out-of-frame; thus, the *RUNX1* gene is truncated and loses its functional activity. Further work has identified two additional genes 400–750 kb centromeric to *EAP*, also at 3q26.2, namely, *MDS1/EVI1* (MDS-associated sequences) and *EVI1* (Ecotropic Virus Insertion site) (Nucifora et al. 1994). Both genes encode nuclear transcription factors containing DNA-binding zinc finger domains, which are identical other than an N-terminal extension of 12 amino acids in the MDS1/EVI1 protein, representing a splicing variant. Each gene has independent and tightly controlled expression during differentiation (Sitailo et al. 1999). The MDS1/EVI1 and EVI1 proteins have opposite functions. EVI1 inhibits G-CSF-mediated differentiation and TGFβ1 growth-inhibitory effects, whereas MDS1/EVI1 has no effect on G-CSF and enhances TGFβ1 growth inhibition (Sitailo et al. 1999). *RUNX1* fuses with *MDS1/EVI1* in-frame, resulting in the loss of the first 12 amino acids, producing a novel EVI1 protein, and a phenotype of arrested differentiation, which leads to apoptosis in vitro (Sood et al. 1999). MDS1/EVI1 serves as a translocation partner with the ribosome binding protein RPN1 (ribophorin 1) (Martinelli et al. 2003) and/or the gene encoding GR6, a poorly characterized protein in fetal development (Pekarsky et al. 1997) in the inv(3)(q21q26.2) or the t(3;3)(q21;q26.2) associated with normal or increased platelet counts as well as *TEL1* (Raynaud et al. 1996) (discussed above) in the t(3;12)(q26.2;p13). EVI1 encodes a transcription factor that contains a zinc finger protein that interacts with a number of transcriptional and epigenetic regulators (CREBBP, CTBP, HDAC, KAT2B (P/CAF), SMAD3, GATA1, GATA2, DNMT3A, and DNMT3B) and mediates chromatin modifications and DNA hypermethylation. Depending on its binding partners, EVI1 can act as a transcriptional activator to promote the proliferation of HSPCs, e.g., when bound to GATA2, or as a transcriptional repressor inhibiting erythroid differentiation, e.g., when bound to GATA1. In addition, EVI1 impairs myelopoiesis by deregulation of multiple transcription factors, including RUNX1 and PU.1 (Laricchia-Robbio et al. 2009). Common features of myeloid diseases associated with abnormalities of 3q are a previous history of cytotoxic exposure, prominent bone marrow dysplasia, and a poor prognosis. Abnormalities of chromosome 7 [–7/del(7q)] are observed in most cases with rearrangements of 3q. In an international workshop on therapy-related hematologic disease, inv(3)/t(3;3) abnormalities were the most frequent of the 3q abnormalities (Block et al. 2002).

3.3.2 Evolution of the Karyotype

Serial evaluations can be informative, particularly when there is a change in the clinical features of a patient. The identification of new abnormalities in the karyotype often coincides with a change in the behavior of the disease, usually to a more aggressive course, and may herald incipient leukemia.

Cytogenetic evolution is the appearance of an abnormal clone where only normal cells have been seen previously, or the progression from the presence of a single clone (often with a simple karyotype) to multiple related, or occasionally unrelated, abnormal clones. The abnormal clones may evolve acquiring additional abnormalities with disease progression, and typically resolve with remission of disease following treatment. In published series, most MDS patients die of bone marrow failure, close to half progress to acute leukemia, and a few die of intercurrent illness.

The natural history of MDS is generally characterized by one of three clinical scenarios: (1) a gradual worsening of pancytopenia where the marrow blast count is found to be increasing, (2) a relatively stable clinical course followed by an abrupt change with a clear leukemic transformation, and (3) a stable course over many years without significant change in the marrow blast counts when reevaluated (Hamblin and Oscier 1987). In the first group, the karyotype typically remains stable, and the progression to leukemia is based on the relatively arbitrary finding of greater than 20 % blasts (30 % in the FAB classification) in the marrow, making the transition

to AML a relatively ill-defined event. In the second group, a change in the karyotype with the gain of secondary clones, and complex karyotypes, is typical. Both the karyotype and the disease tend to remain stable in the third group. Few series with sequential cytogenetic studies have been published, and most series are small with short follow-up periods (Horiike et al. 1988; Geddes et al. 1990; de Souza Fernandez et al. 2000). Nonetheless, karyotypic evolution in MDS is associated with transformation to acute leukemia in about 60 % of cases and reduced survival, particularly for those patients who evolve within a short period of time (less than 100 days) (Geddes et al. 1990).

3.4 The Genetics of the Myelodysplastic Syndromes

3.4.1 Molecular Models for Chromosome Abnormalities in MDS

As described earlier, many of the recurring chromosomal abnormalities in MDS lead to the loss of genetic material. Such loss is the hallmark of tumor suppressor genes, which normally function to control cell growth and/or cell death by regulating the cell cycle, the response to DNA damage, and apoptosis. A simple "two-hit" model involving a single target tumor suppressor gene (Knudson's model) predicts that loss of function of both alleles must occur for the malignant phenotype to be expressed (Knudson 1971). Loss of gene function may occur by chromosomal deletion or loss, point mutations, or methylation of the regulatory elements of the gene (transcriptional silencing). A clinical example to illustrate this principle is the occurrence of MDS or AML following cytotoxic therapy (t-MDS and t-AML, respectively). A relatively long latency period following cytotoxic exposure precedes bone marrow dysfunction. This latency is compatible with a two-step mechanism in which a second mutation of a target gene must occur in a myeloid progenitor cell. Given two normal alleles at the tumor suppressor gene locus initially, one would be mutated as a result of therapy. Subsequent loss of the second allele in a bone marrow stem cell would permit leukemia development. Alternatively, because AML develops in only 5–15 % of patients who are treated for a primary tumor, these individuals may have inherited a predisposing mutant allele; subsequent exposure to cytotoxic therapy may induce the second mutation, giving rise to leukemia. In these cases, characterization of the predisposing mutations will be important in identifying individuals who are at risk of developing t-AML and in the selection of the appropriate therapy for the primary malignant disease.

In an alternative model, loss of only a single copy of a gene may result in a reduction in the level of one or more critical gene products (haploinsufficiency). Several reports implicate haploinsufficiency of the *TP53* and *p27Kip1* genes in the pathogenesis of tumors in mice, where a substantial percentage of tumors developing in heterozygous mice retain a functional copy of *TP53* or *p27Kip1* (Fero et al. 1996; Venkatachalam et al. 1998; Song et al. 1999; French et al. 2001; Rosenbauer et al. 2004). In humans, haploinsufficiency of the *RUNX1* gene results in a familial platelet disorder with a predisposition to AML (Song et al. 1999; Michaud et al. 2002; Nakao et al. 2004). Importantly, the few leukemias available for analysis from affected family members appear to retain one normal *RUNX1* allele. Support for this mechanism in sporadic cases of MDS and AML with point mutations in the *RUNX1* gene is also emerging (Nakao et al. 2004). Despite intensive analysis, homozygous deletions have not been detected in myeloid leukemia cells characterized by deletions of 5q, 7q, or 20q in MDS and AML, an observation that is compatible with a haploinsufficiency model in which loss of one allele of the relevant gene (or genes) alters the cell's fate. Moreover the absence of inactivating mutations in the remaining allele of candidate genes located within the commonly deleted segments of these chromosomes lends further support for the haploinsufficiency model.

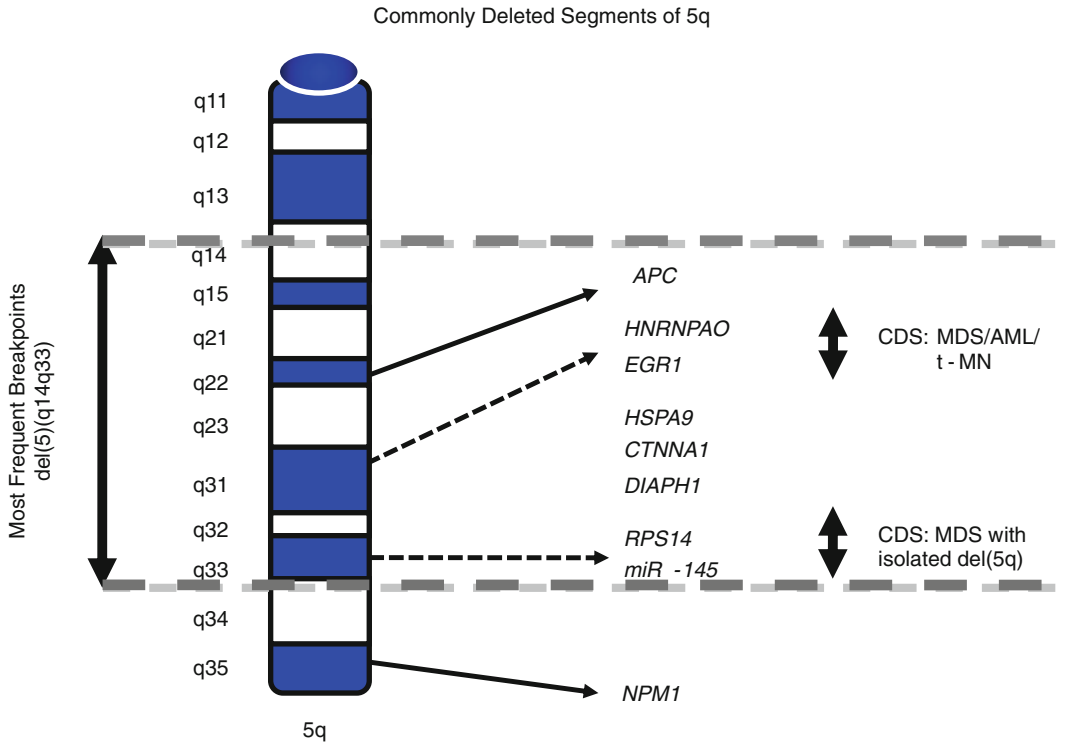

Fig. 3.5 Ideogram of the long arm of chromosome 5 showing chromosome markers and candidate genes within the commonly deleted segments (CDSs) as reported by various investigators. The proximal CDS in 5q31 was identified in MDS, AML, and t-MDS/AML, whereas the distal CDS in 5q33 was identified in MDS with isolated del(5q) (the 5q− syndrome)

3.4.2 Molecular Analysis of the del(5q)

Several groups of investigators have defined a CDS (Figs. 3.4 and 3.5) on the long arm of chromosome 5, band 5q31.2, predicted to contain a myeloid tumor suppressor gene that is involved in the pathogenesis of the more aggressive forms of MDS and AML (Fairman et al. 1995; Zhao et al. 1997). A second, distal CDS of 1.5 Mb within 5q33.1 has been identified in MDS with an isolated del(5q) (Boultwood et al. 2002). Despite intense efforts, the identification of tumor suppressor genes (TSGs) on chromosomes 5 has been challenging, as a result of the fact that the deletions of 5q are typically large and encompass both of these regions. Molecular analysis of the 19 candidate genes within the CDS of 5q31.2 and 44 genes in the 5q33.1 CDS did not reveal inactivating mutations in the remaining alleles nor was there evidence of transcriptional silencing (Lai et al. 2001; Boultwood et al. 2002; Graubert et al. 2009) [Godley and Le Beau 2011, unpublished data]. Moreover, copy-neutral loss of heterozygosity (also known as acquired uniparental disomy) is not seen on 5q in MDS or AML. These observations are compatible with a haploinsufficiency model in which loss of one allele of the relevant gene(s) on 5q perturbs cell fate, rather than the biallelic inactivation of a tumor suppressor gene (Shannon and Le Beau 2008). A number of genes and several miRNAs located on 5q, including *RPS14* (Ebert et al. 2008), miRNA-145 (Kumar et al. 2009; Starczynowski et al. 2010), *EGR1* (Joslin et al. 2007), APC (Wang et al. 2010), *CTNNA1* (Liu et al. 2007), *HSPA9* (Chen et al. 2011), and *DIAPH1* (Eisenmann et al. 2009), have been implicated in the development of myeloid disorders due to a gene dosage effect, and several of these are reviewed below. Together, these studies

support a haploinsufficiency model, in which loss of a single allele of more than one gene on 5q act in concert to alter hematopoiesis, promote self-renewal of HSPCs, induce apoptosis of hematopoietic cells, and disrupt differentiation.

3.4.2.1 RPS14

The gene encoding RPS14, which is required for the processing of 18S pre-rRNA, is located at 5q33.1 and is involved in MDS with an isolated del(5q) (Ebert et al. 2008). Downregulation of *RPS14* in CD34+ bone marrow cells blocks the differentiation and increases apoptosis of erythroid cells via a TP53-dependent mechanism (Barlow et al. 2010). Of interest, the ribosomal processing defect caused by haploinsufficiency of RPS14 in MDS is highly analogous to the functional ribosomal defect seen in Diamond-Blackfan anemia. Other studies have shown that haploinsufficiency of two micro-RNAs, miR-145 and miR-146a, encoded by sequences near the *RPS14* gene, cooperate with loss of RPS14 (Kumar et al. 2009; Starczynowski et al. 2010). The Toll-interleukin-1 receptor domain-containing adaptor protein (TIRAP) and *FLI1* are targets of these miRNAs. Haploinsufficiency of miR-145 may account for several features of MDS with an isolated del(5q), including megakaryocytic dysplasia; however, neither *RPS14* nor miR-145 haploinsufficiency is predicted to confer clonal dominance.

3.4.2.2 APC

APC is a multifunctional tumor suppressor involved in the pathogenesis of colorectal cancer via regulation of the WNT signaling cascade. The *APC* gene is located at 5q22.2 and is deleted in >95 % of patients with a del(5q) (Zhao et al. 1997). Conditional inactivation of a single allele of *Apc* in mice leads to the development of severe macrocytic anemia, a block in erythropoiesis at the early stages of differentiation, and an expansion of the short-term and long-term HSCs (Wang et al. 2010). *Apc* heterozygous myeloid progenitor cells display an increased frequency of apoptosis and decreased in vitro colony-forming capacity, recapitulating several characteristic features of myeloid neoplasms with a del(5q).

3.4.2.3 EGR1

The early growth response 1 gene (*EGR1*) encodes a member of the WT-1 family of Zn finger transcription factors and mediates the cellular response to growth factors, mitogens, and stress stimuli (Baron et al. 2006). Recently, Egr1 has been shown to be a direct transcriptional regulator of many known TSGs, e.g., *Tp53*, *Cdkn1a*/*p21*, *Tgfb*, and *Pten*, and acts as a TSG in several human tumors, including breast non-small cell lung cancer (Baron et al. 2006). *Egr1*-null mice show spontaneous mobilization of HSPCs into the periphery, identifying Egr1 as a transcriptional regulator of stem cell migration (Joslin et al. 2007; Min et al. 2008). Moreover, loss of a single allele of *Egr1* cooperates with mutations induced by an alkylating agent in the development of malignant myeloid diseases (MPD with ineffective erythropoiesis) in mice, indicating that Egr1 is a haploinsufficient myeloid suppressor gene (Joslin et al. 2007)

In summary, the existing data suggest that there are two non-overlapping CDSs in 5q31.2 and 5q33.1. The proximal segment in 5q31.2 is likely to contain a tumor suppressor gene involved in the pathogenesis of both de novo and therapy-related MDS/AML. Band 5q33.1 is likely to contain a second myeloid tumor suppressor gene involved in the pathogenesis of the 5q− syndrome.

3.4.3 Molecular Analysis of −7/del(7q)

A −7/del(7q) is observed as the sole abnormality in ~5 % of adult patients, in ~50 % of children with primary MDS, and in ~55 % of patients with t-MDS (Fig. 3.3) (Luna-Fineman et al. 1995; Sole et al. 2000; Godley and Larson 2002; Smith et al. 2003). As with del(5q), occupational or environmental exposure to mutagens including chemotherapy, radiotherapy, benzene exposure, and smoking, as well as severe aplastic anemia (regularly treated with immunosuppressive agents alone), have been associated with −7/del(7q) (Greenberg et al. 1997). The IPSS considers the −7/del(7q) to be a poor prognostic cytogenetic finding (Greenberg et al. 1997). "Monosomy 7 Syndrome" has been described in young children and is characterized by a preponderance of males

(~4:1), hepatosplenomegaly, leukocytosis, thrombocytopenia, and a poor prognosis (Luna-Fineman et al. 1995). Juvenile myelomonocytic leukemia (JMML) is a MDS/MPD disease in the WHO classification and shares many features with this entity (Luna-Fineman et al. 1995). An emerging paradigm is that −7/del(7q) cooperates with deregulated signaling via the RAS pathway as a result of mutations in the *NRAS* or *KRAS* gene, inactivating mutations in the gene encoding NF1, a negative regulator of RAS proteins, or activating mutations in the gene encoding the PTPN11/SHP2 phosphatase, a positive regulator of RAS proteins, as well as *RUNX1* mutations, and methylation silencing of the *CDKN2B* ($p15^{INK4B}$) gene (Christiansen et al. 2003; Loh et al. 2004; Side et al. 2004).

To date, three CDSs have been identified on 7q; however, the molecular mutations underlying the development of MDS and AML with del(7q) are poorly understood (Johnson et al. 1996; Le Beau et al. 1996; Fischer et al. 1997). Le Beau previously identified two distinct CDSs, a 2.52 Mb CDS within 7q22 spanning the interval containing *LRCC17* and *SRPK2* and a second, less frequent, region in q32-33 (Le Beau et al. 1996). Each of the candidate genes within the CDS at 7q22 has been evaluated for mutations (Curtiss et al. 2005); however, no inactivating mutations have been identified in the remaining allele. Mice with a conditional heterozygous deletion of this region in murine HSPCs had no alterations of hematopoiesis, suggesting that this region does not contain a haploinsufficient myeloid tumor suppressor gene or that mutations in cooperating genes are required (Wong et al. 2010). Recently, Dohner et al. reported the analysis of a large series of patients with abnormalities of 7q using FISH. Whereas most patients had large deletions, they identified an ~2 Mb-deleted segment in proximal q22 that overlaps with the proximal portion of the CDS defined by Le Beau et al. but extends more proximally and includes the *CUTL1*, *RASA4*, *EPO*, and *FBXL13* genes in 7q22.1 (Dohner et al. 2006). The recent recognition of mutations in *EZH2*, a gene located at 7q36.1, is intriguing; however, myeloid neoplasms with *EZH2* mutations typically do not have −7/del(7q), and the del(7q) does not always result in loss of one *EZH2* allele (Ernst et al. 2010; Nikoloski et al. 2010).

3.4.4 Molecular Analysis of the del(20q)

The majority of deletions of 20q are large with loss of most of the long arm, although cytogenetic analysis of the deleted chromosome 20 homologs has revealed that the deletions are variable in size. By using FISH with a panel of probes from 20q, combined with LOH studies, investigators have identified an interstitial CDS of 4 Mb within 20q12 that is flanked by D20S206 proximally and D20S424 distally, containing a number of genes. Despite the availability of detailed physical maps and transcripts maps, the identity of a myeloid tumor suppressor gene on 20q is unknown (Bench et al. 2000; Wang et al. 2000).

Recent studies have implicated the genes encoding topoisomerase I and lethal (3) malignant brain tumor (L3MBTL), which is related to the polycomb group family of transcriptional repressors. Although *L3MBTL* is not mutated in MDS, reduced or absent L3MBTL expression may be relevant in some cases of myeloid leukemia (MacGrogan et al. 2004).

3.4.5 Alterations in Gene Function

A growing body of evidence suggests that mutations of multiple genes mediate the pathogenesis and progression of MDS. The involved genes fall into two main classes, namely, genes encoding hematopoietic transcription factors or proteins that regulate cytokine signaling pathways. There is an increase in the frequency of molecular mutations from low-risk to high-risk MDS, or AML evolving from MDS, emphasizing the role of these mutations in disease progression. A detailed review of these genes is beyond the scope of this chapter and has been provided elsewhere (Olney and Le Beau 2008; Bejar et al. 2011a, b). Table 3.4 provides a partial list and overview of some of

Table 3.4 Genes altered in myelodysplastic syndromes

Gene	Alteration	Associated features	Reference
ASXL1	Mutations in 10–15 % of MDS	Involved in epigenetic regulation of gene expression Mutations are dominant-negative proteins inhibiting function Exclusive of *NPM1* mutations but may be present with mutations of *RUNX1* and *TET2*	Fisher et al. (2006), Gelsi-Boyer et al. (2009), Boultwood et al. (2010)
ATRX	Rare in MDS	Involved in epigenetic modifications of DNA Associated with acquired alpha thalassemia with severe anemia	Steensma et al. (2004)
BCL2	Overexpressed in all FAB subtypes	Encodes a protein product that suppresses apoptosis No correlation with survival Highest levels noted in higher-risk entities where apoptosis is reduced	Lepelley et al. (1995), Parker et al. (2000)
CEBPA	Mutated 1–8 % of MDS, higher in CMML (10–20 %)	Encodes a transcription factor that is essential for granulopoiesis Mutations appear to have no effect on time to overall progression or overall survival	Shih et al. (2005)
CSF1R/FMS	Mutated in 12–20 %, increased with higher-risk MDS	Encodes the macrophage colony-stimulating factor receptor with tyrosine kinase activity Karyotype predominantly normal Increased frequency of transformation to AML and poor survival	Ridge et al. (1990), Padua et al. (1998)
EZH2	Found in 5 % of MDS	Results in defective methyltransferase activity and gene silencing Associated with unfavorable prognosis	Nikoloski et al. (2010)
FLT3	Internal tandem duplication (ITD) in ~10 % of MDS and AML with trilineage dysplasia	Encodes a class III receptor tyrosine kinase playing a role in stem cell differentiation ITD results in constitutive activation of protein Associated with progression to AML and poor prognosis Frequently observed with normal karyotype in AML	Horiike et al. (1997), Kiyoi et al. (1998)
GCSFR	Point mutations identified	Encodes the G-CSF receptor Severe congenital neutropenia (SCN) patients with G-CSF receptor defects can progress to MDS and/or AML Mutation alone is not sufficient for transformation Progression to leukemia in SCN associated with loss of chromosome 7 and *NRAS/KRAS* mutations	Tidow et al. (1998)
HLA-DR15 (DR2)	Overrepresentation in MDS of RA subtype (36 % vs. 21 % in normal blood donors)	T cell-mediated autoimmune mechanism implicated in some forms of MDS Correlated with response to immunosuppression of carefully defined MDS	Sauntharajah et al. (2002)

IDH1, *IDH2*	Rarely found in MDS	Enzymes catalyzing decarboxylation of isocitrate to alpha ketoglutarate important in CNS tumors Associated with more advanced disease and with progression to AML	Kosmider et al. (2010), Thol et al. (2010)
JAK2^{V617F}	Mutated in 2–5 % of MDS, 60 % of RARS-T	Encodes a tyrosine kinase component of various cytokine signaling pathways Activation mutations results in constitutive signaling Mutation in 60 % of patients with RARS with thrombocytosis, an unclassified MDS/MPD Does not appear to alter prognosis	Zipperer et al. (2008)
KIT	Overexpressed; no mutations found	Encodes the stem cell factor receptor May provide an autocrine growth pathway	Arland et al. (1994), Siitonen et al. (1994)
MDR1	Expressed in ~60 %	Encodes a transmembrane drug efflux pump May be involved in resistance of MDS to drug therapy Associated with monosomy 7	Zochbauer et al. (1994)
MDM2	Overexpressed in ~70 %	Encodes a protein product (murine double minute-2) which abrogates the function of the TP53 tumor suppressor protein via ubiquitination and degradation of TP53 Gene amplification not detected Associated with unfavorable cytogenetic abnormalities Shorter remission duration	Bueso-Ramos et al. (1995), Faderl et al. (2000)
MLL	Internal tandem duplication in 3 % of MDS	Encodes a histone methyltransferase that assembles in protein complexes that regulate gene transcription via chromatin remodelling Increased mutation frequency in AML following MDS	Bacher et al. (2007)
MPL	Overexpressed in ~45 % of CMMoL and ~40 % of RAEB-1,2 patients; underexpressed (~50 % of normal levels) in most MDS patients, especially RA	Encodes the thrombopoietin receptor Higher expression in RAEB-1,2 associated with poor prognosis, increased progression to AML Correlated with dysmegakaryocytopoiesis	Bouscary et al. (1995), Ogata and Tamura (2000)
NF1	Loss and mutations identified, particularly in pediatric MDS/MPS	Encodes neurofibromin, a tumor suppressor gene product, that functions as a GTPase activating (GAP) protein to downregulate RAS function High incidence of MDS and AML in children with neurofibromatosis type I No structural alteration in homologous allele in adults with loss of one chromosome 17	Shannon et al. (1994), Gallagher et al. (1997)
NPM1	Mutation rare in MDS, 5 %	Encodes a protein with diverse functions in the cell, including chromatin remodelling, gene stability, ribosome biogenesis, DNA duplication, and transcriptional regulation Mutations typically involve exon 12 with C terminus alterations and aberrant protein localization to the cytoplasm	Pedersen-Bjergaard et al. (2007)

(continued)

Table 3.4 (continued)

Gene	Alteration	Associated features	Reference
NRAS	Mutated in 10–15 %; overexpressed in RA, RARS	Encodes a GTPase component of various cytokine signal transduction pathways Activating mutations result in constitutive signaling Associated with a monocytic component Increased risk of progression to AML Overexpression may represent an early event in the multi-step process of transformation	Padua and West (2000)
CDKN2B/p15^{INK4B}	Decreased expression via gene silencing by DNA methylation in 68 % of t-MDS/t-AML	Closely associated with deletion or loss of 7q Independently associated with poor survival	Christiansen et al. (2003)
PTPN11	Somatic missense mutations in 33 % of JMML patients	A non-receptor tyrosine phosphatase that functions as a positive regulator of RAS proteins, mutations activate the phosphatase activity Mutations of *NRAS/KRAS1*, *NF1* and *PTPN11* are mutually exclusive	Loh et al. (2005)
RPS14	Underexpressed in 5q− syndrome and a proportion of non-del(5q) patients	Universal structural protein of the 40S ribosomal subunit that is essential for the cleavage of the 30S precursor rRNA Haploinsufficiency seems to cause apoptosis in rapidly dividing cells	Ebert et al. (2008), Czibere et al. (2009)
RUNX1/AML1	Mutated in 10–15 % MDS	Encodes the DNA-binding subunit of the heterodimer core-binding factor complex Point mutations in the Runt (DNA binding) domain result in loss of function and a dominant-negative effect Associated with activating mutations of the RAS pathway, -7/del(7q) Increased progression to AML and a shorter overall survival	Niimi et al. (2006), Chen et al. (2007)
Telomerase (including *TERT*, *TR*, and *TP1*)	Increased activity late in disease, particularly *TERT*	Enzyme complex responsible for chromosome telomere maintenance and replication Variable levels of activity Abnormal telomere maintenance may be an early indication of genetic instability Telomeres shortened with disease progression	Counter et al. (1995), Norrback and Roos (1997), Xu et al. 1998; Li et al. (2000)
TET2	Mutations found in 20 % of MDS	Appears to control balance between survival, apoptosis, and differentiation Associated with a better prognosis	Kosmider et al. (2009), Langemeijer et al. (2009)

TP53	Mutated in 5–25 %; higher frequency in t-MDS	Encodes G1, S, and G2 checkpoint protein product which monitors integrity of genome; arrests cell cycle in response to DNA damage Loss of wild-type allele Associated with weak BCL2 expression Observed as both early and late genetic event in MDS Associated with rapid progression and poor outcome seen with loss of 17p or del(5q) suggesting pathogenic exposure to carcinogens Significantly differentiates worse prognosis within each IPSS subgroup	Misawa and Horiike (1996), Christiansen et al. (2001), Kita-Sasai et al. (2001)
WT1	Associated with overexpression	Overexpressed in 65 % of bone marrow specimens and 78 % of peripheral blood specimens compared to normal cells, including all RAEB and t-AML samples Correlated with blast counts and cytogenetic abnormalities Significantly correlated with IPSS score	Cilloni and Saglio (2004)

the salient features of genes implicated in the pathogenesis of MDS.

One of the paradoxes associated with MDS is the presence of peripheral cytopenias, frequently involving all three lineages (granulocytic, erythroid, and megakaryocytic), with the presence of a hypercellular bone marrow where cells in both the peripheral blood and bone marrow exhibit varying degrees of dysmorphic features. Many genes are involved in the tightly regulated and complex process of apoptosis (programmed cell death), which plays an important role in maintaining normal homeostasis by removing immature and damaged cells. Although some of the findings are conflicting, there is consensus on a number of points. Measurements of cell cycle kinetics demonstrate an increase in the proliferation of all hematopoietic cell lineages, particularly the myeloid cell lines (Raza et al. 1995; Parker et al. 2000). This proliferation is balanced by an increase in apoptosis in MDS. It is well documented that altered cytokine levels play a pivotal role in this process (Westwood and Mufti 2003). The proapoptotic tumor necrosis factor alpha (TNFα), transforming growth factor alpha (TGFα), interferon gamma (IFNγ), and interleukin-1 beta (IL1β) are increased in MDS (Reza et al. 1999; Yoshida and Mufti 1999; Allampallam et al. 2002). They may function to suppress the growth of hematopoietic progenitors and induce expression of the FAS receptor which, when appropriately triggered, can initiate the apoptotic pathways. The prominent role of some cytokines has been examined in clinical studies. Strategies to neutralize TNFα by decreasing its production with pentoxifylline or thalidomide and with soluble TNFα receptors (to competitively bind the excess TNF) have resulted in clinical responses in a minority of MDS patients (Raza et al. 1996, 2001; Turk et al. 1996).

DNA regions with origin from only one parent, uniparental disomy (UPD), have been detected with SNP arrays in 15–50 % of MDS patients across all subtypes (Mohamedali et al. 2007; Gondek et al. 2008; Heinrichs et al. 2009). These areas were found both in hematologic tissues as well as buccal tissue, confirming a constitutional origin. Additional amplifications and deletions, however, are acquired in the clonal hematopoietic cells suggesting possible genetic instability and an increased risk of individuals carrying UPD regions for the development of MDS.

3.4.6 Genetic Findings in MDS

Recently, it has been demonstrated that the integration of mutation analysis into diagnostic classification and prognostic scoring systems in MDS has the potential to stratify a diverse disease into discrete subsets with more consistent clinical phenotypes and prognosis (Langemeijer et al. 2009; Bejar et al. 2011a, b). For example, mutations in *RUNX1*, *TP53*, and *NRAS* were associated with severe thrombocytopenia and increased blast percentage. In multivariate analysis, mutations in 5 genes, occurring in one-third of patients, retained independent prognostic significance: *TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1*, and predicted poor overall survival. Mutations of these genes stratified low- and intermediate-1 and intermediate-2 IPSS risk groups into two risk groups each, identifying patients within these subgroups with a poorer prognosis who may require a more intensive therapeutic approach. The genes most commonly mutated in MDS are *TET2*, *ASXL1*, *EZH2*, *RUNX1*, and *TP53*, which are described briefly below.

An emerging paradigm in MDS is the high frequency of mutations in genes involved in the regulation of transcription via chromatin modifications (*IDH1/2*, *TET2*, *EZH2*, *ASXL1*) and the intriguing observation that mutations often occur in more than one gene in the same patient, implying functional cooperation (note that *IDH1/2* and *TET2* mutations are mutually exclusive). The most frequently mutated gene in MDS is *TET2* (20 %); point mutations are observed in all cytogenetic subsets (Langemeijer et al. 2009; Bejar et al. 2011a, b). TET2 converts 5-methylcytosine to 5-hydroxymethylcytosine, thereby altering the epigenetic mark created by DNA methyltransferases (Ko et al. 2010). Recent studies suggest no impact of *TET2* mutations on overall survival in MDS (Kosmider et al. 2009; Langemeijer et al. 2009).

ASXL1 mutations are observed in 10–15 % of MDS (Gelsi-Boyer et al. 2009; Bejar et al. 2011a, b). ASXL1 is a member of the polycomb family of chromatin-binding proteins and is involved in the epigenetic regulation of gene expression (typically repression). Mutated proteins are predicted to function as dominant-negative proteins inhibiting the function of the wild-type proteins as well as other members of the polycomb complex. The prognostic significance of ASXL1 mutations in MDS is not yet known.

EZH2 (enhancer of zeste homolog 2) mutations occur in 5 % of MDS (Ernst et al. 2010; Nikoloski et al. 2010; Bejar et al. 2011a, b). EZH2 encodes a histone methyltransferase that trimethylates histone 3 at lysine 27, an epigenetic mark that confers gene silencing. In MDS, the mutations lead to loss of the catalytic activity and are predicted to increase HSC expansion (Majewski et al. 2010). Although *EZH2* is located at 7q36.1, loss or mutation of *EZH2* does not appear to be the sole driver of myeloid neoplasms associated with −7/del(7q).

Point mutations in the runt-related transcription factor 1 (RUNX1) have been reported in AML and MDS (12 %), particularly in MDS secondary to treatment with cytotoxic therapy, and increase with the severity of the disease (Chen et al. 2007). RUNX1, also known as CBFA2 or AML1, encodes the DNA-binding subunit of the heterodimeric core-binding factor (CBF) complex, which is essential for definitive hematopoiesis (Speck and Gilliland 2002). *RUNX1* mutations impair DNA binding and act as dominant-negative proteins and are associated with activating mutations of the RAS pathway, −7/del(7q), and a shorter overall survival (Chen et al. 2007). Germ line mutations of *RUNX1* cause a rare human disease called familial platelet disorder; affected individuals have an MDS-like phenotype with thrombocytopenia, and/or dysfunctional platelets, and a predisposition to progress to AML (Song et al. 1999).

The *TP53* tumor suppressor gene encodes an essential checkpoint protein that monitors the integrity of the genome and arrests cell cycle progression in response to DNA damage. Mutations of *TP53* (exons 4–8) or loss of an allele, typically as a result of a cytogenetic abnormality of 17p, are observed in MDS (5–10 %) and t-MDS (25–30 %), particularly in patients who have received alkylating agent therapy (Christiansen et al. 2001). *TP53* mutations may occur as either an early or late event in the course of the disease and are associated with rapid progression, and a poor outcome. In t-MDS, *TP53* mutations are associated with del(5q)/t(5q) and a complex karyotype.

JAK2^{V617F} is a constitutively active cytoplasmic tyrosine kinase that is able to activate JAK-STAT signaling and mediate transformation to cytokine-independent growth in MPN and has been identified in rare cases of MDS (2–5 %) and CMML (3 %) (Steensma et al. 2005). An exception is RARS-T, in which 60 % of patients have the *JAK2*^{V617F} mutation (Zipperer et al. 2008). RARS-T patients with *JAK2*^{V617F} mutations present with higher WBCs and platelet counts.

The role of epigenetic changes in the pathogenesis and treatment of MDS is becoming increasingly important. Transcriptional silencing via DNA methylation of the *CDKN2B* (*p15*^{INK4B}) gene increases with progression from RA to RAEB-T, is observed in a high percentage of patients with t-MDS, and is associated with −7/del(7q) and a poor prognosis (Christiansen et al. 2003). Recent genome-wide studies have demonstrated that increases in promoter hypermethylation are predictive of survival in MDS, even when age, sex, and IPSS risk groups are considered. Moreover, increases in promoter methylation are seen during progression to AML (Shen et al. 2010). These observations form the rationale for use of demethylating agents in MDS. Similarly, inhibition of histone-modifying enzymes represents another rational target for MDS therapy. The RAS gene family has been extensively studied in MDS. RAS proteins are a critical component of signaling pathways from cell-surface receptors to the nucleus and result in the control of cellular proliferation, differentiation, and cell death (Rebollo and Martinez 1999). The RAS signaling cascade is downstream of a number of activated cytokine receptors, including the FLT3, IL3, and GM-CSF receptors; thus, this signaling pathway plays a pivotal role in hematopoiesis.

Mutant RAS proteins retain an active GTP-bound form, promoting constitutive activation which is seen in a high frequency of hematological malignancies. RAS mutations are present in 10–15 % of MDS cases; the most frequent mutation is a single base change at codon 12 of the protein, but codons 13 and 61 are also frequently mutated (Neubauer et al. 1994; Gallagher et al. 1997). These mutations are associated with a poor prognosis, with higher incidence of transformation to AML and shorter survival. Patients with both abnormal karyotypes and *NRAS* mutations have the highest likelihood of transformation (Neubauer et al. 1994; Tien et al. 1994; de Souza Fernandez et al. 1998; Padua et al. 1998; Beaupre and Kurzrock 1999; Bacher et al. 2007). Many therapeutic molecules, including the farnesyltransferase inhibitors and imatinib, interrupt various steps in the RAS signaling pathways (Apperley et al. 2002; Kurzrock et al. 2003).

Mutations of the FMS-like tyrosine kinase 3 (*FLT3*) gene, including both point mutations within the tyrosine kinase domain and internal tandem duplications (ITD), are among the most common genetic changes seen in AML, occurring in 15–35 % of cases. *FLT3*-ITD mutations are associated with a poor prognosis, particularly in cases with loss of the remaining wild-type *FLT3* allele. In MDS, *FLT3*-ITD mutations are rare in RA and RARS but increase to about 3 and 12 % in RAEB and AML following MDS (Bacher et al. 2007). Thus, *FLT3*-ITD may represent a secondary event associated with progression rather than an initiating event.

Conclusions

Cytogenetic analysis of patient bone marrow remains fundamental to the diagnosis and prognosis of MDS. Numerous recurrent abnormalities guide the clinician in orienting the patient toward appropriate therapeutic options. The most widely used clinical tools, the IPSS and the WPSS, both incorporate these abnormalities into the classification of patients. Increasingly, molecularly identified gene abnormalities are being shown to convey independent prognostic information and occasionally permit targeted therapy. The research techniques permitting this identification are moving closer to the bedside and are unraveling the genetic complexity of MDS with the promise of elucidating the pathogenesis of these disorders. This work, more importantly, moves us closer to refining the prognostic scoring systems and identifying targets for novel therapeutic agents.

References

Abe A, Emi N et al (1997) Fusion of the platelet-derived growth factor receptor beta to a novel gene CEV14 in acute myelogenous leukemia after clonal evolution. Blood 90(11):4271–4277

Allampallam K, Shetty V et al (2002) Biological significance of proliferation, apoptosis, cytokines, and monocyte/macrophage cells in bone marrow biopsies of 145 patients with myelodysplastic syndrome. Int J Hematol 75(3):289–297

Apperley JF, Gardembas M et al (2002) Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N Engl J Med 347(7):481–487

Arland M, Fiedler W et al (1994) Absence of point mutations in a functionally important part of the extracellular domain of the c-kit proto-oncogene in a series of patients with acute myeloid leukemia (AML). Leukemia 8(3):498–501

Aul C, Bowen DT et al (1998) Pathogenesis, etiology and epidemiology of myelodysplastic syndromes. Haematologica 83(1):71–86

Bacher U, Haferlach T et al (2007) A comparative study of molecular mutations in 381 patients with myelodysplastic syndrome and in 4130 patients with acute myeloid leukemia. Haematologica 92(6):744–752

Bain BJ, Moorman AV et al (1998) Myelodysplastic syndromes associated with 11q23 abnormalities. European 11q23 Workshop participants. Leukemia 12(5):834–839

Barlow JL, Drynan LF et al (2010) New insights into 5q− syndrome as a ribosomopathy. Cell Cycle 9(21):4286–4293

Baron V, Adamson ED et al (2006) The transcription factor Egr1 is a direct regulator of multiple tumor suppressors including TGFbeta1, PTEN, p53, and fibronectin. Cancer Gene Ther 13(2):115–124

Beaupre DM, Kurzrock R (1999) RAS and leukemia: from basic mechanisms to gene-directed therapy. J Clin Oncol 17(3):1071–1079

Bejar R, Levine R et al (2011a) Unraveling the molecular pathophysiology of myelodysplastic syndromes. J Clin Oncol 29(5):504–515

Bejar R, Stevenson K et al (2011b) Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 364(26):2496–2506

Bench AJ, Nacheva EP et al (2000) Chromosome 20 deletions in myeloid malignancies: reduction of the common

deleted region, generation of a PAC/BAC contig and identification of candidate genes. UK Cancer Cytogenetics Group (UKCCG). Oncogene 19(34): 3902–3913
Bjork J, Albin M et al (2000) Smoking and myelodysplastic syndromes. Epidemiology 11(3):285–291
Block AW, Carroll AJ et al (2002) Rare recurring balanced chromosome abnormalities in therapy-related myelodysplastic syndromes and acute leukemia: report from an international workshop. Genes Chromosomes Cancer 33(4):401–412
Bloomfield CD, Archer KJ et al (2002) 11q23 balanced chromosome aberrations in treatment-related myelodysplastic syndromes and acute leukemia: report from an international workshop. Genes Chromosomes Cancer 33(4):362–378
Boultwood J, Lewis S et al (1994) The 5q-syndrome. Blood 84(10):3253–3260
Boultwood J, Fidler C et al (2002) Narrowing and genomic annotation of the commonly deleted region of the 5q− syndrome. Blood 99(12):4638–4641
Boultwood J, Perry J et al (2010) Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. Leukemia 24(5):1062–1065
Bouscary D, Prudhomme C et al (1995) c-mpl expression in hematologic disorders. Leuk Lymphoma 17(1–2):19–26
Bueso-Ramos CE, Manshouri T et al (1995) Multiple patterns of MDM-2 deregulation in human leukemias: implications in leukemogenesis and prognosis. Leuk Lymphoma 17(1–2):13–18
Chen CY, Lin LI et al (2007) 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 139(3):405–414
Chen TH, Kambal A et al (2011) Knockdown of Hspa9, a del(5q31.2) gene, results in a decrease in hematopoietic progenitors in mice. Blood 117(5):1530–1539
Christiansen DH, Andersen MK et al (2001) Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. J Clin Oncol 19(5):1405–1413
Christiansen DH, Andersen MK et al (2003) Methylation of p15INK4B is common, is associated with deletion of genes on chromosome arm 7q and predicts a poor prognosis in therapy-related myelodysplasia and acute myeloid leukemia. Leukemia 17(9):1813–1819
Cilloni D, Saglio G (2004) WT1 as a universal marker for minimal residual disease detection and quantification in myeloid leukemias and in myelodysplastic syndrome. Acta Haematol 112(1–2):79–84
Counter CM, Gupta J et al (1995) Telomerase activity in normal leukocytes and in hematologic malignancies. Blood 85(9):2315–2320
Curtiss NP, Bonifas JM et al (2005) Isolation and analysis of candidate myeloid tumor suppressor genes from a commonly deleted segment of 7q22. Genomics 85(5):600–607
Czibere A, Bruns I et al (2009) Low RPS14 expression is common in myelodysplastic syndromes without 5q− aberration and defines a subgroup of patients with prolonged survival. Haematologica 94(10):1453–1455
de Souza Fernandez T, Menezes de Souza J et al (1998) Correlation of N-ras point mutations with specific chromosomal abnormalities in primary myelodysplastic syndrome. Leuk Res 22(2):125–134
de Souza Fernandez T, Ornellas MH et al (2000) Chromosomal alterations associated with evolution from myelodysplastic syndrome to acute myeloid leukemia. Leuk Res 24(10):839–848
Dewald GW, Wyatt WA et al (1998) Highly sensitive fluorescence in situ hybridization method to detect double BCR/ABL fusion and monitor response to therapy in chronic myeloid leukemia. Blood 91(9): 3357–3365
Dohner K, Habdank M et al (2006) Molecular characterization of distinct hotspot regions on chromosome 7q in myeloid leukemias. Blood 108
Ebert BL, Pretz J et al (2008) Identification of RPS14 as a 5q− syndrome gene by RNA interference screen. Nature 451(7176):335–339
Eisenmann KM, Dykema KJ et al (2009) 5q− myelodysplastic syndromes: chromosome 5q genes direct a tumor-suppression network sensing actin dynamics. Oncogene 28(39):3429–3441
Emanuel PD (1999) Myelodysplasia and myeloproliferative disorders in childhood: an update. Br J Haematol 105(4):852–863
Ernst T, Chase AJ et al (2010) Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet 42(8):722–726
Faderl S, Kantarjian HM et al (2000) The prognostic significance of p16(INK4a)/p14(ARF) locus deletion and MDM-2 protein expression in adult acute myelogenous leukemia. Cancer 89(9):1976–1982
Fairman J, Chumakov I et al (1995) Physical mapping of the minimal region of loss in 5q− chromosome. Proc Natl Acad Sci U S A 92(16):7406–7410
Fenaux P, Lucidarme D et al (1989) Favorable cytogenetic abnormalities in secondary leukemia. Cancer 63(12): 2505–2508
Fero ML, Rivkin M et al (1996) A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. Cell 85(5):733–744
Fischer K, Frohling S et al (1997) Molecular cytogenetic delineation of deletions and translocations involving chromosome band 7q22 in myeloid leukemias. Blood 89(6):2036–2041
Fisher CL, Randazzo F et al (2006) Characterization of Asxl1, a murine homolog of additional sex combs, and analysis of the Asx-like gene family. Gene 369:109–118
French JE, Lacks GD et al (2001) Loss of heterozygosity frequency at the Trp53 locus in p53-deficient (+/−) mouse tumors is carcinogen-and tissue-dependent. Carcinogenesis 22(1):99–106

Gallagher A, Darley RL et al (1997) The molecular basis of myelodysplastic syndromes. Haematologica 82(2): 191–204

Geddes AA, Bowen DT et al (1990) Clonal karyotype abnormalities and clinical progress in the myelodysplastic syndrome. Br J Haematol 76(2):194–202

Gelsi-Boyer V, Trouplin V et al (2009) Mutations of polycomb-associated gene ASXL1 in myelodysplastic syndromes and chronic myelomonocytic leukaemia. Br J Haematol 145(6):788–800

Godley LA, Larson R (2002) The syndrome of therapy-related myelodysplasia and myeloid leukemia. The myelodysplastic syndromes: pathobiology and clinical management. J.M. Bennett/Marcel Dekker Inc, New York, pp 136–176

Golub TR, Barker GF et al (1994) Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell 77(2):307–316

Gondek LP, Tiu R et al (2008) Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS, MDS/MPD, and MDS-derived AML. Blood 111(3): 1534–1542

Gozzetti A, Le Beau MM (2000) Fluorescence in situ hybridization: uses and limitations. Semin Hematol 37(4):320–333

Graubert TA, Payton MA et al (2009) Integrated genomic analysis implicates haploinsufficiency of multiple chromosome 5q31.2 genes in de novo myelodysplastic syndromes pathogenesis. PLoS One 4(2):e4583

Greenberg P, Cox C et al (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89(6):2079–2088

Haase D, Germing U et al (2007) New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. Blood 110(13):4385–4395

Hamblin TJ, Oscier DG (1987) The myelodysplastic syndrome–a practical guide. Hematol Oncol 5(1):19–34

Hayes RB, Yin SN et al (1997) Benzene and the dose-related incidence of hematologic neoplasms in China. Chinese Academy of Preventive Medicine–National Cancer Institute Benzene Study Group. J Natl Cancer Inst 89(14):1065–1071

Heinrichs S, Kulkarni RV et al (2009) Accurate detection of uniparental disomy and microdeletions by SNP array analysis in myelodysplastic syndromes with normal cytogenetics. Leukemia 23(9):1605–1613

Horiike S, Taniwaki M et al (1988) Chromosome abnormalities and karyotypic evolution in 83 patients with myelodysplastic syndrome and predictive value for prognosis. Cancer 62(6):1129–1138

Horiike S, Yokota S et al (1997) Tandem duplications of the FLT3 receptor gene are associated with leukemic transformation of myelodysplasia. Leukemia 11(9):1442–1446

Jary L, Mossafa H et al (1997) The 17p− syndrome: a distinct myelodysplastic syndrome entity? Leuk Lymphoma 25(1–2):163–168

Jenkins RB, Le Beau MM et al (1992) Fluorescence in situ hybridization: a sensitive method for trisomy 8 detection in bone marrow specimens. Blood 79(12): 3307–3315

Johansson B, Mertens F et al (1993) Cytogenetic deletion maps of hematologic neoplasms: circumstantial evidence for tumor suppressor loci. Genes Chromosomes Cancer 8(4):205–218

Johnson EJ, Scherer SW et al (1996) Molecular definition of a narrow interval at 7q22.1 associated with myelodysplasia. Blood 87(9):3579–3586

Joslin JM, Fernald AA et al (2007) Haploinsufficiency of EGR1, a candidate gene in the del(5q), leads to the development of myeloid disorders. Blood 110(2):719–726

Jotterand M, Parlier V (1996) Diagnostic and prognostic significance of cytogenetics in adult primary myelodysplastic syndromes. Leuk Lymphoma 23(3–4):253–266

Kantarjian H, O'Brien S et al (2008) Proposal for a new risk model in myelodysplastic syndrome that accounts for events not considered in the original International Prognostic Scoring System. Cancer 113(6):1351–1361

Kardos G, Baumann I et al (2003) Refractory anemia in childhood: a retrospective analysis of 67 patients with particular reference to monosomy 7. Blood 102(6):1997–2003

Kearney L (1999) The impact of the new fish technologies on the cytogenetics of haematological malignancies. Br J Haematol 104(4):648–658

Kita-Sasai Y, Horiike S et al (2001) International prognostic scoring system and TP53 mutations are independent prognostic indicators for patients with myelodysplastic syndrome. Br J Haematol 115(2):309–312

Kiyoi H, Towatari M et al (1998) Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product. Leukemia 12(9):1333–1337

Knudson AG Jr (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 68(4):820–823

Ko M, Huang Y et al (2010) Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature 468(7325):839–843

Kosmider O, Gelsi-Boyer V et al (2009) TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs). Blood 114(15):3285–3291

Kosmider O, Gelsi-Boyer V et al (2010) Mutations of IDH1 and IDH2 genes in early and accelerated phases of myelodysplastic syndromes and MDS/myeloproliferative neoplasms. Leukemia 24(5):1094–1096

Kulkarni S, Heath C et al (2000) Fusion of H4/D10S170 to the platelet-derived growth factor receptor beta in BCR-ABL-negative myeloproliferative disorders with a t(5;10)(q33;q21). Cancer Res 60(13):3592–3598

Kumar M, Narla A et al (2009) Coordinate loss of a microRNA mir145 and a protein-coding gene RPS14 cooperate in the pathogenesis of 5q-syndrome. Blood 114:947

Kurtin PJ, Dewald GW et al (1996) Hematologic disorders associated with deletions of chromosome 20q: a clinicopathologic study of 107 patients. Am J Clin Pathol 106(5):680–688

Kurzrock R, Kantarjian HM et al (2003) Farnesyltransferase inhibitor R115777 in myelodysplastic syndrome: clinical

and biologic activities in the phase 1 setting. Blood 102(13):4527–4534

Kussick SJ, Fromm JR et al (2005) Four-color flow cytometry shows strong concordance with bone marrow morphology and cytogenetics in the evaluation for myelodysplasia. Am J Clin Pathol 124(2):170–181

Lai JL, Preudhomme C et al (1995) Myelodysplastic syndromes and acute myeloid leukemia with 17p deletion. An entity characterized by specific dysgranulopoiesis and a high incidence of P53 mutations. Leukemia 9(3):370–381

Lai F, Godley LA et al (2001) Transcript map and comparative analysis of the 1.5-Mb commonly deleted segment of human 5q31 in malignant myeloid diseases with a del(5q). Genomics 71(2):235–245

Langemeijer SM, Kuiper RP et al (2009) Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet 41(7):838–842

Laricchia-Robbio L, Premanand K et al (2009) EVI1 impairs myelopoiesis by deregulation of PU.1 function. Cancer Res 69(4):1633–1642

Larson RA, LeBeau MM et al (1996) Myeloid leukemia after hematotoxins. Environ Health Perspect 104 (Suppl 6):1303–1307

Le Beau MM, Albain KS et al (1986) Clinical and cytogenetic correlations in 63 patients with therapy-related myelodysplastic syndromes and acute nonlymphocytic leukemia: further evidence for characteristic abnormalities of chromosomes no. 5 and 7. J Clin Oncol 4(3):325–345

Le Beau MM, Espinosa R 3rd et al (1996) Cytogenetic and molecular delineation of a region of chromosome 7 commonly deleted in malignant myeloid diseases. Blood 88(6):1930–1935

Lepelley P, Soenen V et al (1995) bcl-2 expression in myelodysplastic syndromes and its correlation with hematological features, p53 mutations and prognosis. Leukemia 9(4):726–730

Li B, Yang J et al (2000) Telomerase activity in preleukemia and acute myelogenous leukemia. Leuk Lymphoma 36(5–6):579–587

Liu TX, Becker MW et al (2007) Chromosome 5q deletion and epigenetic suppression of the gene encoding alpha-catenin (CTNNA1) in myeloid cell transformation. Nat Med 13(1):78–83

Loh ML, Vattikuti S et al (2004) Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. Blood 103(6):2325–2331

Loh ML, Martinelli S et al (2005) Acquired PTPN11 mutations occur rarely in adult patients with myelodysplastic syndromes and chronic myelomonocytic leukemia. Leuk Res 29(4):459–462

Look AT (1997) Oncogenic transcription factors in the human acute leukemias. Science 278(5340):1059–1064

Loss of the Y chromosome from normal and neoplastic bone marrows. United Kingdom Cancer Cytogenetics Group (UKCCG) (1992) Genes Chromosomes Cancer 5(1):83–88

Luna-Fineman S, Shannon KM et al (1995) Childhood monosomy 7: epidemiology, biology, and mechanistic implications. Blood 85(8):1985–1999

MacGrogan D, Kalakonda N et al (2004) Structural integrity and expression of the L3MBTL gene in normal and malignant hematopoietic cells. Genes Chromosomes Cancer 41(3):203–213

Magnusson MK, Meade KE et al (2001) Rabaptin-5 is a novel fusion partner to platelet-derived growth factor beta receptor in chronic myelomonocytic leukemia. Blood 98(8):2518–2525

Majewski IJ, Ritchie ME et al (2010) Opposing roles of polycomb repressive complexes in hematopoietic stem and progenitor cells. Blood 116(5):731–739

Malcovati L, Germing U et al (2007) Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 25(23):3503–3510

Marschalek R (2011) Mechanisms of leukemogenesis by MLL fusion proteins. Br J Haematol 152(2):141–154

Martinelli G, Ottaviani E et al (2003) Association of 3q21q26 syndrome with different RPN1/EVI1 fusion transcripts. Haematologica 88(11):1221–1228

Martinez-Climent JA, Garcia-Conde J (1999) Chromosomal rearrangements in childhood acute myeloid leukemia and myelodysplastic syndromes. J Pediatr Hematol Oncol 21(2):91–102

McCarthy CJ, Sheldon S et al (1998) Cytogenetic abnormalities and therapy-related myelodysplastic syndromes in rheumatic disease. Arthritis Rheum 41(8): 1493–1496

Merlat A, Lai JL et al (1999) Therapy-related myelodysplastic syndrome and acute myeloid leukemia with 17p deletion. A report on 25 cases. Leukemia 13(2): 250–257

Michaud J, Wu F et al (2002) In vitro analyses of known and novel RUNX1/AML1 mutations in dominant familial platelet disorder with predisposition to acute myelogenous leukemia: implications for mechanisms of pathogenesis. Blood 99(4):1364–1372

Min IM, Pietramaggiori G et al (2008) The transcription factor EGR1 controls both the proliferation and localization of hematopoietic stem cells. Cell Stem Cell 10:380–391

Misawa S, Horiike S (1996) TP53 mutations in myelodysplastic syndrome. Leuk Lymphoma 23(5–6):417–422

Mohamedali A, Gaken J et al (2007) Prevalence and prognostic significance of allelic imbalance by single-nucleotide polymorphism analysis in low-risk myelodysplastic syndromes. Blood 110(9):3365–3373

Morel P, Hebbar M et al (1993) Cytogenetic analysis has strong independent prognostic value in de novo myelodysplastic syndromes and can be incorporated in a new scoring system: a report on 408 cases. Leukemia 7(9):1315–1323

Moyzis RK, Albright KL et al (1987) Human chromosome-specific repetitive DNA sequences: novel markers for genetic analysis. Chromosoma 95(6):375–386

Nakao M, Horiike S et al (2004) Novel loss-of-function mutations of the haematopoiesis-related transcription factor, acute myeloid leukaemia 1/runt-related transcription factor 1, detected in acute myeloblastic leukaemia and myelodysplastic syndrome. Br J Haematol 125(6):709–719

Nederlof PM, van der Flier S et al (1990) Multiple fluorescence in situ hybridization. Cytometry 11(1):126–131

Neubauer A, Greenberg P et al (1994) Mutations in the ras proto-oncogenes in patients with myelodysplastic syndromes. Leukemia 8(4):638–641

Niimi H, Harada H et al (2006) Hyperactivation of the RAS signaling pathway in myelodysplastic syndrome with AML1/RUNX1 point mutations. Leukemia 20(4):635–644

Nikoloski G, Langemeijer SM et al (2010) Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet 42(8): 665–667

Norrback KF, Roos G (1997) Telomeres and telomerase in normal and malignant haematopoietic cells. Eur J Cancer 33(5):774–780

Nucifora G, Begy CR et al (1994) Consistent intergenic splicing and production of multiple transcripts between AML1 at 21q22 and unrelated genes at 3q26 in (3;21)(q26;q22) translocations. Proc Natl Acad Sci U S A 91(9):4004–4008

Ogata K, Tamura H (2000) Thrombopoietin and myelodysplastic syndromes. Int J Hematol 72(2):173–177

Olney HJ, Le Beau MM (2008) Myelodysplastic syndromes. In: Heim S, Mitelman F (eds) Cancer cytogenetics, 3rd edn. Wiley, Hoboken

Padua RA, West RR (2000) Oncogene mutation and prognosis in the myelodysplastic syndromes. Br J Haematol 111(3):873–874

Padua RA, Guinn BA et al (1998) RAS, FMS and p53 mutations and poor clinical outcome in myelodysplasias: a 10-year follow-up. Leukemia 12(6):887–892

Parker JE, Mufti GJ et al (2000) The role of apoptosis, proliferation, and the Bcl-2-related proteins in the myelodysplastic syndromes and acute myeloid leukemia secondary to MDS. Blood 96(12):3932–3938

Paulsson K, Sall T et al (2001) The incidence of trisomy 8 as a sole chromosomal aberration in myeloid malignancies varies in relation to gender, age, prior iatrogenic genotoxic exposure, and morphology. Cancer Genet Cytogenet 130(2):160–165

Paulsson K, Heidenblad M et al (2006) High-resolution genome-wide array-based comparative genome hybridization reveals cryptic chromosome changes in AML and MDS cases with trisomy 8 as the sole cytogenetic aberration. Leukemia 20(5):840–846

Pedersen-Bjergaard J, Andersen MK et al (2000) Therapy-related acute myeloid leukemia and myelodysplasia after high-dose chemotherapy and autologous stem cell transplantation. Blood 95(11):3273–3279

Pedersen-Bjergaard J, Andersen MT et al (2007) Genetic pathways in the pathogenesis of therapy-related myelodysplasia and acute myeloid leukemia. Hematology Am Soc Hematol Educ Program 392–397

Pekarsky Y, Rynditch A et al (1997) Activation of a novel gene in 3q21 and identification of intergenic fusion transcripts with ecotropic viral insertion site I in leukemia. Cancer Res 57(18):3914–3919

Pierre RV, Hoagland HC (1972) Age-associated aneuploidy: loss of Y chromosome from human bone marrow cells with aging. Cancer 30(4):889–894

Pinkel D, Landegent J et al (1988) Fluorescence in situ hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci U S A 85(23):9138–9142

Prchal JT, Throckmorton DW et al (1978) A common progenitor for human myeloid and lymphoid cells. Nature 274(5671):590–591

Raynaud SD, Baens M et al (1996) Fluorescence in situ hybridization analysis of t(3; 12)(q26; p13): a recurring chromosomal abnormality involving the TEL gene (ETV6) in myelodysplastic syndromes. Blood 88(2):682–689

Raza A, Gezer S et al (1995) Apoptosis in bone marrow biopsy samples involving stromal and hematopoietic cells in 50 patients with myelodysplastic syndromes. Blood 86(1):268–276

Raza A, Mundle S et al (1996) Novel insights into the biology of myelodysplastic syndromes: excessive apoptosis and the role of cytokines. Int J Hematol 63(4):265–278

Raza A, Meyer P et al (2001) Thalidomide produces transfusion independence in long-standing refractory anemias of patients with myelodysplastic syndromes. Blood 98(4): 958–65

Rebollo A, Martinez AC (1999) Ras proteins: recent advances and new functions. Blood 94(9):2971–2980

Reza S, Dar S et al (1999) Biologic characteristics of 164 patients with myelodysplastic syndromes. Leuk Lymphoma 33(3–4):281–287

Ridge SA, Worwood M et al (1990) FMS mutations in myelodysplastic, leukemic, and normal subjects. Proc Natl Acad Sci U S A 87(4):1377–1380

Rosenbauer F, Wagner K et al (2004) Acute myeloid leukemia induced by graded reduction of a lineage-specific transcription factor, PU.1. Nat Genet 36(6):624–630

Ross TS, Bernard OA et al (1998) Fusion of Huntingtin interacting protein 1 to platelet-derived growth factor beta receptor (PDGFbetaR) in chronic myelomonocytic leukemia with t(5;7)(q33;q11.2). Blood 91(12): 4419–4426

Roulston D, Le Beau M (1997) Cytogenetic analysis of hematologic malignant disease. The AGT Cytogenetics Laboratory Manuel. M. Barch. Lippincott-Raven, Philadelphia, pp 325–374

Rowley JD (1999) The role of chromosome translocations in leukemogenesis. Semin Hematol 36(4 Suppl 7):59–72

Rowley JD (2000) Molecular genetics in acute leukemia. Leukemia 14(3):513–517

Rowley JD, Olney HJ (2002) International workshop on the relationship of prior therapy to balanced chromosome aberrations in therapy-related myelodysplastic syndromes and acute leukemia: overview report. Genes Chromosomes Cancer 33(4):331–345

Rowley JD, Reshmi S et al (1997) All patients with the T(11;16)(q23;p13.3) that involves MLL and CBP have treatment-related hematologic disorders. Blood 90(2):535–541

Rubin CM, Larson RA et al (1987) Association of a chromosomal 3;21 translocation with the blast phase of chronic myelogenous leukemia. Blood 70(5):1338–1342

Rubin CM, Larson RA et al (1990) t(3;21)(q26;q22): a recurring chromosomal abnormality in therapy-related myelodysplastic syndrome and acute myeloid leukemia. Blood 76(12):2594–2598

Sauntharajah Y, Nakamura R et al (2002) HLA-DR15 (DR2) is overrepresented in myelodysplastic syndrome and aplastic anemia and predicts a response to immunosuppression in myelodysplastic syndrome. Blood 100(5):1570–1574

Secker-Walker LM (1998) General Report on the European Union Concerted Action Workshop on 11q23, London, UK, May 1997. Leukemia 12(5):776–778

Shaffer LG, Slovak ML et al (2009) ISCN 2009: an International System for Human Cytogenetic Nomenclature (2009): recommendations of the International Standing Committee on Human Cytogenetic Nomenclature. Karger, Basel

Shannon KM, Le Beau MM (2008) Cancer: hay in a haystack. Nature 451(7176):252–253

Shannon KM, O'Connell P et al (1994) Loss of the normal NF1 allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. N Engl J Med 330(9):597–601

Shen L, Kantarjian H et al (2010) DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes. J Clin Oncol 28(18):3098

Shih LY, Huang CF et al (2005) Heterogeneous patterns of CEBPalpha mutation status in the progression of myelodysplastic syndrome and chronic myelomonocytic leukemia to acute myelogenous leukemia. Clin Cancer Res 11(5):1821–1826

Side LE, Curtiss NP et al (2004) RAS, FLT3, and TP53 mutations in therapy-related myeloid malignancies with abnormalities of chromosomes 5 and 7. Genes Chromosomes Cancer 39(3):217–223

Siitonen T, Savolainen ER et al (1994) Expression of the c-kit proto-oncogene in myeloproliferative disorders and myelodysplastic syndromes. Leukemia 8(4):631–637

Sitailo S, Sood R et al (1999) Forced expression of the leukemia-associated gene EVI1 in ES cells: a model for myeloid leukemia with 3q26 rearrangements. Leukemia 13(11):1639–1645

Sloand EM, Rezvani K (2008) The role of the immune system in myelodysplasia: implications for therapy. Semin Hematol 45(1):39–48

Smadja N, Krulik M et al (1989) Cytogenetic and molecular studies of the Philadelphia translocation t(9;22) observed in a patient with myelodysplastic syndrome. Leukemia 3(3):236–238

Smith SM, Le Beau MM et al (2003) Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. Blood 102(1):43–52

Sole F, Espinet B et al (2000) Incidence, characterization and prognostic significance of chromosomal abnormalities in 640 patients with primary myelodysplastic syndromes. Grupo Cooperativo Espanol de Citogenetica Hematologica. Br J Haematol 108(2):346–356

Sole F, Luno E et al (2005) Identification of novel cytogenetic markers with prognostic significance in a series of 968 patients with primary myelodysplastic syndromes. Haematologica 90(9):1168–1178

Song WJ, Sullivan MG et al (1999) Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat Genet 23(2):166–175

Sood R, Talwar-Trikha A et al (1999) MDS1/EVI1 enhances TGF-beta1 signaling and strengthens its growth-inhibitory effect but the leukemia-associated fusion protein AML1/MDS1/EVI1, product of the t(3;21), abrogates growth-inhibition in response to TGF-beta1. Leukemia 13(3):348–357

Speck NA, Gilliland DG (2002) Core-binding factors in haematopoiesis and leukaemia. Nat Rev Cancer 2(7):502–513

Spitzer G, Verma DS et al (1979) Subgroups of oligoleukemia as identified by in vitro agar culture. Leuk Res 3(1):29–39

Starczynowski DT, Kuchenbauer F et al (2010) Identification of miR-145 and miR-146a as mediators of the 5q− syndrome phenotype. Nat Med 16(1):49–58

Steensma DP, Higgs DR et al (2004) Acquired somatic ATRX mutations in myelodysplastic syndrome associated with alpha thalassemia (ATMDS) convey a more severe hematologic phenotype than germline ATRX mutations. Blood 103(6):2019–2026

Steensma DP, Dewald GW et al (2005) The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both "atypical" myeloproliferative disorders and myelodysplastic syndromes. Blood 106(4): 1207–1209

Swerdlow SH, Campo E et al (eds) (2008) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. IARC, Lyon

Thol F, Weissinger EM et al (2010) IDH1 mutations in patients with myelodysplastic syndromes are associated with an unfavorable prognosis. Haematologica 95(10):1668–1674

Tidow N, Kasper B et al (1998) Clinical implications of G-CSF receptor mutations. Crit Rev Oncol Hematol 28(1):1–6

Tien HF, Wang CH et al (1994) Cytogenetic studies, ras mutation, and clinical characteristics in primary myelodysplastic syndrome. A study on 68 Chinese patients in Taiwan. Cancer Genet Cytogenet 74(1):40–49

Tkachuk DC, Westbrook CA et al (1990) Detection of bcr-abl fusion in chronic myelogeneous leukemia by in situ hybridization. Science 250(4980):559–562

Toyama K, Ohyashiki K et al (1993) Clinical and cytogenetic findings of myelodysplastic syndromes showing hypocellular bone marrow or minimal dysplasia, in comparison with typical myelodysplastic syndromes. Int J Hematol 58(1–2):53–61

Turk BE, Jiang H et al (1996) Binding of thalidomide to alpha1-acid glycoprotein may be involved in its inhibition of tumor necrosis factor alpha production. Proc Natl Acad Sci U S A 93(15):7552–7556

Vallespi T, Imbert M et al (1998) Diagnosis, classification, and cytogenetics of myelodysplastic syndromes. Haematologica 83(3):258–275

van de Loosdrecht AA, Westers TM et al (2008) Identification of distinct prognostic subgroups in low- and intermediate-1-risk myelodysplastic syndromes by flow cytometry. Blood 111(3):1067–1077

Van den Berghe H, Michaux L (1997) 5q−, twenty-five years later: a synopsis. Cancer Genet Cytogenet 94(1):1–7

Venkatachalam S, Shi YP et al (1998) Retention of wild-type p53 in tumors from p53 heterozygous mice: reduction of p53 dosage can promote cancer formation. EMBO J 17(16):4657–4667

Walker J, Flower D et al (2002) Microarrays in hematology. Curr Opin Hematol 9(1):23–29

Wang P, Spielberger RT et al (1997) dic(5;17): a recurring abnormality in malignant myeloid disorders associated with mutations of TP53. Genes Chromosomes Cancer 20(3):282–291

Wang PW, Eisenbart JD et al (2000) Refinement of the smallest commonly deleted segment of chromosome 20 in malignant myeloid diseases and development of a PAC-based physical and transcription map. Genomics 67(1):28–39

Wang J, Fernald AA et al (2010) Haploinsufficiency of Apc leads to ineffective hematopoiesis. Blood 115(17):3481–3488

Wattel E, Lai JL et al (1993) De novo myelodysplastic syndrome (MDS) with deletion of the long arm of chromosome 20: a subtype of MDS with distinct hematological and prognostic features? Leuk Res 17(11):921–926

Weimar IS, Bourhis JH et al (1994) Clonality in myelodysplastic syndromes. Leuk Lymphoma 13(3–4):215–221

Wells DA, Benesch M et al (2003) Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. Blood 102(1):394–403

West RR, Stafford DA et al (2000) Cytogenetic abnormalities in the myelodysplastic syndromes and occupational or environmental exposure. Blood 95(6):2093–2097

Westwood NB, Mufti GJ (2003) Apoptosis in the myelodysplastic syndromes. Curr Hematol Rep 2(3):186–192

Wiktor A, Rybicki BA et al (2000) Clinical significance of Y chromosome loss in hematologic disease. Genes Chromosomes Cancer 27(1):11–16

Wong JC, Zhang Y et al (2010) Use of chromosome engineering to model a segmental deletion of chromosome band 7q22 found in myeloid malignancies. Blood 115(22):4524–4532

Xu D, Gruber A et al (1998) Telomerase activity and the expression of telomerase components in acute myelogenous leukaemia. Br J Haematol 102(5):1367–1375

Yoshida Y, Mufti GJ (1999) Apoptosis and its significance in MDS: controversies revisited. Leuk Res 23(9):777–785

Zhang Y, Rowley JD (2006) Chromatin structural elements and chromosomal translocations in leukemia. DNA Repair (Amst) 5(9–10):1282–1297

Zhao N, Stoffel A et al (1997) 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 94(13):6948–6953

Zipperer E, Wulfert M et al (2008) MPL 515 and JAK2 mutation analysis in MDS presenting with a platelet count of more than 500 x 10(9)/l. Ann Hematol 87(5):413–415

Zochbauer S, Gsur A et al (1994) MDR1 gene expression in myelodysplastic syndrome and in acute myeloid leukemia evolving from myelodysplastic syndrome. Anticancer Res 14(3B):1293–1295

MDS Stem Cell Biology

4

Sarah M. Greenblatt, H. Joachim Deeg,
and Stephen D. Nimer

The clinical heterogeneity of patients with myelodysplastic syndromes suggests that there must also be a broad range of pathogenetic abnormalities that underlie this disorder. It is clear that the molecular abnormalities associated with MDS are vast, as are the functional abnormalities within the hematopoietic compartment. This implies that there may also be significant differences in the cell of origin and varying degrees of aberrant communication with the microenvironment in individual patients. It is only through recent advances in flow cytometry, DNA sequencing, and high throughput analysis of the genome, proteome, and RNA expression profiles that we can make these general but profound statements about the spectrum of abnormalities in MDS patients.

S.M. Greenblatt
Sylvester Comprehensive Cancer Center,
University of Miami Miller School of Medicine,
1550 NW 10th Avenue, BRB 742C,
Miami, FL 33136, USA
e-mail: s.greenblatt@med.miami.edu

H.J. Deeg, MD
Division of Clinical Research, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N, D1-100,
Seattle, WA 98109-1024, USA

University of Washington School of Medicine,
Seattle, WA, USA
e-mail: jdeeg@fhcrc.org

S.D. Nimer, MD (✉)
Sylvester Comprehensive Cancer Center,
University of Miami Miller School of Medicine,
1550 NW 10th Avenue, FOX 200,
Miami, FL 33136, USA
e-mail: snimer@med.miami.edu

Given our understanding of the genetic basis of cancer, attention has been primarily focused on identifying defects within the hematopoietic stem/progenitor cell compartment of MDS patients, with the understanding that these defects can give rise to secondary abnormalities in stem cell-stem cell niche interactions and/or immune function. Recent studies have greatly advanced our understanding of the genetic defects found in MDS patients, yet how these abnormalities contribute to the clinical phenotype of MDS is still unclear. The recent identification of splicing factor mutations in all subtypes of MDS, but most impressively, their association with SF3B1 mutations in refractory anemia with ring sideroblasts (RARS), demonstrates that heretofore unknown cellular mechanisms may greatly impact normal hematopoietic processes (Malcovati et al. 2011; Yoshida et al. 2011). However, how the MDS clone persists and how it replaces the normal HSCs within the bone marrow of MDS patients remain a critical and poorly understood phenomenon.

Although the role of "stem cells" has been established in many hematopoietic malignancies, the definition of the disease-initiating population in MDS remains controversial. It is clear that this population shares many features with normal hematopoietic stem cells (HSCs), including the capacity for long-term self-renewal; however, it may lack the multipotentiality that is a critical defining characteristic of HSCs. While MDS-initiating cells display myeloid multipotentiality, abnormalities within the lymphoid compartment are rarely observed; thus, the cell involved may

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_4,

not be a true stem cell. However, there is certainly evidence that the transforming events in the hematopoietic malignancies can promote lineage-specific differentiation and inhibit the differentiation of alternative lineages. In support of this notion is the observation that many of the genetic changes identified in MDS occur in genes encoding transcription factors or genes that control other aspects of their regulation. Thus, the diminished differentiation potential seen in MDS may reflect the disruption of lineage-specific differentiation programs and not the failure of initiating events to occur in HSCs or multipotent progenitors.

The clonal nature of MDS is not disputed and clearly highlights the similarities between MDS and other stem cell propagated hematopoietic malignancies such as AML. Clonality in MDS has historically been established by isoenzyme or cytogenetic analysis (Raskind et al. 1984). Fluorescence in situ hybridization (FISH) studies have improved upon metaphase cytogenetic analysis, which is limited by the ability to obtain metaphase chromosomes and can detect chromosomal abnormalities only in approximately 50 % of MDS cases. In addition, the use of high-density genomic analysis such as single nucleotide polymorphism arrays (SNP-A) and comparative genomic hybridization (CGH) has identified previous unknown sites of genomic DNA deletion, amplification, and loss of heterozygosity in samples from MDS patients (Sugimoto et al. 1993; Christiansen et al. 2001; Mohamedali et al. 2007; Gondek et al. 2008). These techniques have provided additional clonal markers that can be useful in determining how MDS-initiating cells contribute to various hematopoietic lineages and evolve in those patients that progress to AML.

Previous work has shown that the cytogenetic and genetic abnormalities characterizing MDS are rarely found in the lymphoid cell compartment. While cases have been documented in which clonal abnormalities are found in both the myeloid and B-lymphoid lineages, there is no evidence that clonal T cells are generated from the MDS-initiating cell population (White et al. 1994; Thanopoulou et al. 2004). Furthermore, although clonal cytogenetic abnormalities have been found within the dendritic cell population, most of these have been shown to be myeloid derived (Micheva et al. 2004; Ma et al. 2007). While this lineage restriction challenges the assumption that the MDS-initiating cell is a pluripotent stem cell with both myeloid and lymphoid capability, it is also possible that the genetic and epigenetic abnormalities that occur in the MDS stem cell block the ability of these cells to commit to the B-cell lineage. This hypothesis is supported by the accumulating evidence that MDS patients have significant defects in B-cell development (Srivannaboon et al. 2001; Sternberg et al. 2005). When the impairment of lymphoid differentiation is overcome by viral transformation or by transplantation of patient bone marrow-derived MDS cells into immunodeficient mice, clonal cytogenetic abnormalities are detected in both myeloid and B-cell lineages. However, the data supporting B-cell involvement in MDS is somewhat contradictory. Thus, while a B-lymphoblastic cell line was established by transforming peripheral blood mononuclear cells from a patient with MDS containing a 20q deletion identical to that seen in myeloid metaphases from the patient, no B or T cells from the same patient were found to carry the 20q deletion when evaluated using PCR-based assays (White et al. 1994). However, others have reported a 13q deletion identical to that of the myeloid cell population in EBV-transformed B cells derived from two patients with acquired sideroblastic anemia (Lawrence et al. 1987; Asimakopoulos et al. 1996), and when bone marrow cells from an MDS patient with trisomy 8 were injected into NOD/SCID β2-micoglobulin null mice, a similar proportion of trisomy 8-positive granulocytic and B-lymphoid cells were found (Thanopoulou et al. 2004). Thus, while the MDS-initiating abnormality may occur in a pluripotent stem cell, the inhibition of lymphoid differentiation may limit detection of the genetic abnormality in the circulating lymphoid compartment.

Another explanation for the lack of clonal lymphoid cells derived from the MDS-initiating cells may be a myeloid lineage bias in the HSCs. In the murine system, recent studies have challenged the notion that stem cells break off into "lymphoid only" and "myeloid only" progenitors;

Pax5-negative B-cell precursors can switch lineages and develop into functional macrophages (Nutt et al. 1999; Heavey et al. 2003). Similarly, early thymic precursors have both T lineage and myeloid lineage capability (Weerkamp et al. 2006). These studies suggest that the HSCs possess a myeloid lineage bias and that both B- and T-lymphoid precursors retain some myeloid differentiation capacity (Bell and Bhandoola 2008; Wada et al. 2008). Limiting dilution transplantation assays using murine HSCs have revealed significant heterogeneity in the behavior of individually tracked HSCs, with HSC subtypes differing in their ability to complete lymphoid differentiation (Benz et al. 2012). However, whether a similar lymphoid restricted population of HSCs exists in humans remains to be determined. If so, the myeloid restriction in MDS may be due to the transformation of cells with a preexisting limited lymphoid differentiation potential.

The hallmark of murine HSCs is their ability to reconstitute multilineage hematopoiesis in lethally irradiated recipients. Much effort has been placed into the development of surrogate transplantation assays for human HSCs. A variety of in vitro methods are used to establish the hierarchy of human stem/progenitor cells including colony-forming assays and long-term culture initiating cell (LTC-IC) assays, which utilize semisolid media or supporting stroma. The in vitro culture of MDS-derived clones has proved challenging due to the inability of immature progenitors to sustain long-term colony formation. Thus, recent studies have relied on the xenotransplantation of human cells into immunodeficient mice, initially using NOD/SCID mice (Lapidot et al. 1994; Thanopoulou et al. 2004). Over time, more immunodeficient strains have been established, and generally the subset of cells capable of engrafting in these mice varies with the level of immunodeficiency. For example, only CD34+CD38− cells will engraft in NOD/SCID mice, while more cell types are capable of engrafting in the NOD/SCID mice that additionally lack β2 microglobulin or IL-2R common γ-chain (Kollet et al. 2000; Mcdermott et al. 2010). The engraftment potential of human MDS cells in NOD/SCID β2-micoglobulin null mice was evaluated in a study by Thanopoulou et al. Bone marrow cells from 9 of 11 different MDS patients showed transient repopulation of the immunodeficient mouse strain. The engrafting cells retained the original cytogenetic abnormalities characterizing the patient's disease but failed to differentiate into B-lymphoid or erythroid cells. However, CD41+ cells, which include megakaryocytes and platelets, were also not detected in these mice suggesting that the microenvironment may not be conducive to the growth of more than one lineage.

Despite the limitations of these assays, specific cell surface markers have been identified that enrich for primitive human HSCs. For example, more primitive cells lack the cell surface markers associated with terminally differentiated hematopoietic cells, known as lineage markers. CD34 was the first cell surface marker to be recognized on primitive human HSCs, and the CD34+ cells have been further divided into CD38-positive and CD38-negative fractions. It is clear that CD34+CD38− human bone marrow or cord blood cells are more primitive than CD34+CD38+ cells. Differences exist between CD34+ cells isolated from different sources; normal bone marrow contains approximately 1 % CD34+ mononuclear cells, and around 1 % of these are CD38−, while umbilical cord blood collections consist of approximately 1 % of the CD34+ cells, 4 % of which are CD38−. Recent work suggests that the Thy-1 marker (CD90), lineage markers (Lin), and CD45RA can be used to identify the more primitive human cells in cord blood. Isolation and molecular characterization of this Lin-CD34+CD38−CD90+ population in MDS patients has revealed that MDS HSCs have a higher percentage of karyotypic abnormalities compared to total bone marrow cells and that this population may persist even in cases of morphological remission. This primitive population was significantly expanded in high-risk MDS patients compared to normal controls (Will et al. 2012) Furthermore, analysis of the more committed progenitor populations in these patients demonstrated a bias toward the expansion of common myeloid progenitors (CMPs) in the lower-risk MDS patients compared to an expansion of

granulocyte monocyte progenitors (GMPs) and a decrease in megakaryocyte/erythrocyte progenitors (MEPs) in the high-risk patients. While HSCs can be identified by cell surface expression, murine and human HSCs have also been isolated based on their ability to efflux fluorescent dyes such as Hoechst 3342. Hoechst-negative cells form a characteristic pattern when analyzed by FACS called the side population, which is enriched for CD34− HSCs (Goodell et al. 1996). An improved ability to isolate and characterize these stem and progenitor populations in various subtypes of MDS should further our understanding of how changes in specific progenitor populations relate to disease progression in the different risk groups.

Although immunophenotyping of AML or MDS is useful for identifying aberrant cell surface expression and detecting minimal residual disease, it is not yet possible to isolate leukemia or MDS-initiating cells solely based on immunophenotype. Efforts to identify aberrant surface markers specific to malignant HSCs have suggested that the expression of IL-3 receptor alpha chain (IL-3Rα/CD123) may distinguish LSCs from their normal hematopoietic counterparts. IL-3Rα is a component of the high-affinity receptor for IL-3, and CD34+CD123+ AML cells are able to engraft and propagate the leukemia in immunodeficient NOD/SCID mice (Jordan et al. 2000). Enhanced expression of IL-3Rα on AML blasts correlates with a poor prognosis, which may relate to their "stem cell-like" properties (Testa et al. 2002). Blasts expressing elevated IL-3Rα levels exhibit higher cycling activity, increased STAT5 phosphorylation, and relative resistance to apoptosis under growth factor-limiting conditions, features that may explain the ability to evade treatment effects. Whether CD123 or other markers that may identify leukemic blasts such as the C-type lectin-like molecule-1 (CLL-1) (Van Rhenen et al. 2007) or the angiotensin-converting enzyme (ACE/CD143) (Jokubaitis et al. 2008) can distinguish the MDS-initiating cells from their normal HSPC counterparts remains to be determined.

Given the lack of a cell surface expression profile that defines MDS-initiating cells, many studies have focused on identifying gene expression changes and common pathways involved in the pathogenesis of MDS, using patient samples or murine models that recapitulate the features of MDS. Gene expression profiles of the CD34+ fraction of MDS or AML samples obtained from patients with specific FAB or WHO subtypes can begin to characterize whether a specific HSC population is "stem cell"-like. However, the heterogeneity in MDS, including the intrinsic differences between hematopoietic stem cells and progenitor cells, makes it particularly challenging to decipher MDS by assessing relative levels of total cellular RNAs.

Despite these challenges, gene expression profiling in MDS has revealed abnormalities in the pathways regulating HSC self-renewal, differentiation, and quiescence (Chen et al. 2004; Nilsson et al. 2007; Will et al. 2012). The increased apoptosis seen in the early hematopoietic cell compartment in MDS has led to studies of TNFα and β signaling, two negative regulators of hematopoiesis, which could contribute to the observed hematopoietic abnormalities found in MDS patients. Early studies utilizing CD34+ MDS cells revealed a gene expression profile similar to that of the IFNγ response in normal CD34+ HSCs (Pellagatti et al. 2006). IFNγ negatively regulates hematopoietic self-renewal and promotes apoptosis of erythroid progenitor cells (Yang et al. 2005). These changes may provide a potential explanation for the observations that MDS patients often display hypercellularity in the bone marrow yet have peripheral blood cytopenias. A comparison of 5q− stem cells with normal HSCs demonstrated a number of overexpressed genes that regulate stem cell behavior including BMI1, ID1, DNMT3A, and MYC (Nilsson et al. 2007). CEBPA, a master regulator of granulopoiesis, was also upregulated in the 5q− HSCs (CD34+CD38−), but not in the CD38+ progenitors. In general, a large-scale comparison of the gene expression profiles of CD34+ MDS cells revealed that the late stages of the disease are characterized by dysregulation of the DNA damage response and checkpoint pathways.

Many of the mutations identified in MDS patients have been genetically introduced into

mice and have been shown to recapitulate the features of MDS, most commonly defects in myeloid differentiation. Evi1 is found in rare translocations with RUNX1 and is overexpressed in approximately 30 % of MDS patients. Overexpression of Evi1 in mice impaired erythropoiesis and decreased expression of receptors that regulate myeloid proliferation, although with a long latency (Buonamici et al. 2004). Additionally, Evi1 has been shown to interact with GATA1 and RUNX1 and to block PU.1-dependent promoter activation (Senyuk et al. 2007). The association of Evi1 with multiple transcriptional regulators may explain defects in both myelopoiesis and lymphopoiesis that are present in MDS. The Nup98-HoxD13 mouse model recapitulates many of the features of MDS including peripheral blood cytopenias, bone marrow dysplasia, and the eventual progression to leukemia (Lin et al. 2005). Translocations involving Nup98 have been shown to result in the dysregulation of clustered Hox genes, which are important regulators of hematopoietic differentiation and self-renewal. While these translocations occur rarely in MDS, HoxA9 in particular has been shown to be significantly upregulated in MDS patients, and its overexpression is a prognostic indicator for transformation to AML (Lam and Aplan 2001). Another common observation in CD34+ MDS stem cells is disruption of the Wnt pathway; inhibitors of this pathway have been shown to be downregulated in high-risk MDS and may contribute to the inhibition of differentiation. For instance, loss of function of the Wnt inhibitor Apc has been modeled by introducing similar mutations in mice and yields an MDS-like disease (Lane et al. 2010). Lack of APC may be important in MDS patients, as it is located in the region of chromosome 5 that is frequently deleted in 5q− patients. In summary, it is clear that recurrent mutations or abnormalities in gene expression due to changes in DNA methylation or other means result in abnormal regulation of HSC function. It is clear that these changes will be used to predict patient outcome and hopefully ultimately lead to improved MDS therapy.

Transcription regulation can also occur at the level of mRNA processing. Whole exome sequencing of MDS patients has recently identified recurrent somatic mutations in numerous components of the spliceosome. Defects in individual spliceosome components have been associated with specific characteristics of MDS; for example, SF3B1, the most commonly mutated spliceosomal gene in MDS, is associated with the presence of ring sideroblasts (Papaemmanuil et al. 2011). MDS cases with these mutations are also accompanied by higher platelet and white blood cell counts and lower bone marrow blast counts. It is also clear that defective nuclear transport of mRNA or protein can contribute to malignant cell behavior through the involvement of dysregulated nuclear pore proteins, such as Nup98 and NUP214. Furthermore, genes that regulate ribosomal biogenesis may play a role in the pathogenesis of MDS, based on studies of Diamond-Blackfan anemia and 5q− MDS, which have implicated the ribosomal proteins RPS19/RPS24 and RPS14, respectively (Gazda et al. 2006; Choesmel et al. 2007; Pellagatti et al. 2008).

Our new understanding of the critical roles that microRNAs can play in cellular regulation has clearly illustrated that the amount of functional protein in the cell can be regulated even without changes in the level of RNA (Rhyasen and Starczynowski 2012). An elegant demonstration of this came from experiments characterizing the role of miRNA 17-5p, 20a, and 106a in the control of the translation of AML1 (Fontana et al. 2007). Downregulation of this miRNA complex, which is directed against the 3'UTR of the AML1 RNA, is correlated with the induction of monocytic differentiation, as it allows translation of AML1 mRNA into AML1 protein, which then activates expression of the M-CSF receptor, allowing the cell to respond to M-CSF and develop along the monocytic lineage. MicroRNAs have also been implicated in 5q− MDS, where haploinsufficiency for *miR-145* and *miR-146* on chromosome 5 can cause thrombocytosis, mild neutropenia, and megakaryocytic dysplasia in mice. The loss of these two miRNAs may provide a potential explanation for the clonal selection of 5q− cells (Starczynowski et al. 2011). Beyond this regulatory step there is a variety of

posttranslational modifications that may also be critical for proper hematopoietic cell development. These events include phosphorylation events, ubiquitination, acetylation, methylation, and sumoylation. Mutations in genes that encode epigenetic regulatory proteins are among the most common genetic lesions in MDS, and these include TET2, IDH1, DNMT3a, ASXL2, and EZH2. Overall, genome-wide increases in promoter hypermethylation appear to predict survival and progression to AML, even when accounting for patient age, sex, and risk group (Jiang et al. 2009; Shen et al. 2010). Other enzymes affect methylation of lysine residues in histone and nonhistone proteins including transcription factors such as AML1 and elongation factors such as SPT5 and various splicing factors. Acetylation is another important modification, as acetylation of the p53 protein by histone acetyltransferases alters its transcriptional activating properties and is essential for its ability to trigger apoptosis in cells (Tang et al. 2008). Taken together, these mutations in genetic and epigenetic regulators of MDS have underscored the importance of mRNA processing, transport, and posttranslational modifications in this disease.

While the importance of stem cells in the origin of MDS is now recognized, the clinical significance of these cells remains unclear. A study of the clonal architecture of matched patient samples during MDS and the subsequent progression to AML indicated that the preexisting MDS-initiating clone persisted at the time of the secondary AML (Walter et al. 2012). An understanding of how the biology of the initiating clone relates to the resistance to treatment will be critical to developing improved therapy for MDS. Some of the first experiments to study the role of stem cell populations in the therapeutic response have focused on a subset of MDS patients with interstitial deletions involving the long arm of chromosome 5 (5q−). Treatment of these patients with lenalidomide often results in a complete cytological remission; however, there is a high rate of relapse. Samples collected from 5q− patients before and after treatment were examined to study the role of stem cells in this disease and indicated that while lenalidomide effectively reduced the number of 5q−CD34+CD38+CD90+ progenitors in patients who obtained partial or complete remissions, a fraction of the 5q−CD34+CD38−CD90+ stem cells remained resistant to the drug (Tehranchi et al. 2010). This drug resistance was shown to be due to cellular quiescence and an increased expression of multidrug resistance efflux pumps. Over time, these clones, representing a phenotypically and functionally distinct stem cell population, expanded leading to eventual lenalidomide resistance and clinical relapse. A similar study by Will et al. identified a high percentage of cytogenetic abnormalities in Lin−CD34+CD38− MDS HSCs derived from patients with lower-risk or higher-risk MDS. This cell population was evaluated before and after epigenetic therapy with 5-azacytidine and the HDAC inhibitor vorinostat. Although patients in this study achieved complete morphologic remissions, the frequency of HSCs remained elevated, with a high percentage of cells containing the original cytogenetic abnormality. In this case also, morphologic relapse was preceded by several months by an expansion of this cell population, further suggesting that relapse and disease progression in MDS patients may result from an inability to target specific subsets of MDS-initiating cells. Clearly, the identification of minor stem cell populations during disease progression may be useful in developing targeted therapy for this disease. As expansion of IL-3Rα/CD123 may be able to distinguish between malignant stem cells and normal HSPCs, targeting the CD123+ cells may prove useful. One approach, conjugating IL-3 to diphtheria toxin to generate a fusion protein capable of targeting cells expressing the receptor, looks promising, based on results using NOD/SCID mice transplanted with leukemic blasts (Frankel et al. 2000; Frankel et al. 2008). The fusion protein appeared to preferentially target cells with high expression of IL-3R, suggesting it may effectively target a leukemic stem cell-like population. It is clear that MDS and AML are distinct diseases, despite some overlap, with different cell surface profiles, mutation spectra, and the regions of genetic loss or gain. As we better define these features, we will be able to rationally

choose therapies based on genetic or gene expression profiles of the MDS cells. A better understanding of the interactions of MDS HSCs with the bone marrow microenvironment will be critical, as well, to making therapeutic progress.

4.1 The Microenvironment and Its Interaction with the MDS Clone(s)

Hematologic malignancies are generally thought to arise from inherited or acquired genetic abnormalities in HSCs. However, there is growing evidence, which suggests that the marrow microenvironment contributes to the pathogenesis or progression of these neoplasms. While this is not surprising in light of the data supporting the role of the marrow microenvironment in regulating normal hematopoiesis, insights gained from recent studies suggest that focusing treatment strategies on microenvironmental defects may be effective in these disorders.

The marrow microenvironment represents a complex structure composed of both HSC-derived and non-hematopoietic cells, as well as an extracellular matrix, which can concentrate soluble and membrane bound factors that support and regulate normal hematopoiesis. HSCs have also been shown to associate with bone marrow osteoblasts in the endosteal niche. Some of the first experiments to demonstrate that a supportive microenvironment is critical for normal hematopoiesis involved studies in which mice were engineered to lack expression of the c-kit cell surface receptor or its ligand, stem cell factor (Russell 1979). HSCs from W/W^v mice are deficient in c-kit expression, but their hematopoietic defects can be cured by bone marrow transplantation from a normal donor (Huang 1990; Williams 1990). In contrast, the Sl/Sl^d mouse, which develops a severe anemia due to lack of stem cell factor expression on stroma cells, is not cured by HSC transplantation; instead, the defect can be corrected by implanting a normal spleen into the peritoneum of the mouse (McCulloch et al. 1965; Copeland et al. 1990). These experiments highlight the importance of stromal cell interactions in regulating the survival of normal HSCs.

In vitro culture studies of the HSC-microenvironment interaction have relied heavily on long-term marrow culture systems, as first described by Dexter. Cells from marrow aspirates form a complex adherent layer of stromal cells at the bottom of the culture flask that is able to support hematopoiesis for weeks (Dexter et al. 1977; Gartner and Kaplan 1980). The adherent layer is composed of fibroblast-like cells, adipocytes, endothelial cells, macrophages, lymphocytes, osteoclasts, and extracellular matrix (Greenberger 1991; Mayani et al. 1992). Although osteoblasts are not always considered part of the adherent stromal layer of the Dexter culture, they are present in the medullary cavity and recently have been shown to play an important role in normal hematopoiesis and HSC homeostasis (Calvi et al. 2003; Zhang et al. 2003; Taichman 2005; Visnjic et al. 2004). This in vitro system has provided considerable insight into the regulation of hematopoiesis, including the role of contact-dependent and independent interactions, which regulate stem cell fate decisions (Chabannon and Torok-Storb 1992).

One important question in MDS is whether the malignant clone contributes non-hematopoietic cells to the microenvironment. Bone marrow transplantation experiments in mice and humans have shown that the components of the microenvironment that are derived from hematopoietic precursors are replaced by donor, while stromal cells and other similar cell types remain of host origin (Lennon and Micklem 1986; Laver et al. 1987; Simmons et al. 1987; Perkins and Fleischman 1988). In some hematopoietic disorders, such as CML, stromal macrophages can be detected that originate from a Bcr-Abl-positive clone. These stromal elements may contribute to disease progression by promoting the expansion of leukemic progenitors and suppressing normal hematopoiesis (Bhatia et al. 1995). In MDS, it has been reported that mesenchymal "stem" cells can contain the same cytogenetic abnormalities as the myeloid cells in patients (Lennon and Micklem 1986; Laver et al. 1987). Clonal cytogenetic abnormalities have also been found within

"monocyte-derived dendritic cells" and may explain the abnormalities in dendritic cell number and function that have been reported in MDS (Ma et al. 2007). However, similar defects in dendritic cell maturation have also been noted in non-clonal populations.

The fact that hematopoietic cell transplantation (HCT) is curative for many patients with hematopoietic neoplasms strongly supports the concept that functional defects in the microenvironment are reversible. Nevertheless, in the setting of allogeneic HCT for MDS, there are examples where engraftment of donor cells fails, even after repeated attempts. Furthermore, numerous cases of donor-derived leukemia have been reported (Marsh et al. 1989; Witherspoon et al. 1985; Lawler et al. 2002; Mc Cann et al. 1994). One explanation for this phenomenon could be that although the HSCs are replaced, defects in stroma persist. One case of note described a male patient with paroxysmal nocturnal hemoglobulinuria (PNH), who received an HCT from his syngeneic twin, but later experienced a recurrence of the disease (Nafa et al. 1998). Surprisingly, the PNH clone present at the time of the recurrence had a different mutation than the clone present at the time of the original diagnosis, which suggests that the marrow microenvironment can influence the selection of cells that preferentially grow.

Indeed, several mouse models have recently been described, which show that primary stromal abnormalities can induce a malignancy in the hematopoietic compartment. In one model, conditional loss of IkBα, the inhibitor of NF-κB, was conditionally deleted, leading to a disorder similar to chronic myelomonocytic leukemia (CMML) (Rupec et al. 2005). This disease appeared to be dependent on the loss of IkBα in the microenvironment, because when IkBα was conditionally deleted in the myeloid population alone, the mice failed to develop disease. Similarly, Walkely et al. demonstrated that conditional deletion of the retinoblastoma gene (Rb) resulted in a myeloproliferative disorder in mice. The disease was dependent on deletion of Rb in both the hematopoietic cells and the microenvironment. Additionally, transplantation of normal cells into a RARγ-deficient microenvironment resulted in a myeloproliferative disorder (Walkley et al. 2007). Together, these studies demonstrate that a single microenvironmental defect can generate a myeloid neoplasm over time. A report by Raaijmakers et al. 2010 showed that inactivation of the miRNA processing endonuclease Dicer in murine pro-osteoblasts resulted in the development of a disease with features of MDS, further demonstrating that disruption of the hematopoietic niche can contribute to the development of the hematopoietic disorder. These experiments suggest that hematologic disorders may arise due to dysregulation of signaling between bone marrow osteoblasts and the HSCs.

The microenvironment may also play a prominent role in sustaining hematologic malignancies (Duhrsen and Hossfeld 1996; Scadden 2007). It has been postulated that disruption of the signaling events that govern HSC quiescence within the stem cell niches may lead to increased HSC proliferation, thereby increasing their susceptibility to acquire additional genetic abnormalities. Studies in mice have shown that leukemic cells are capable of hijacking hematopoietic niches for support, displacing normal hematopoietic precursors. Furthermore, both the MDS clone and the microenvironment can contribute to severe dysregulation of cytokine production that occurs in the MDS marrow. Although the marrow stroma appears morphologically normal, this dysregulation of cytokine expression may result in changes in homing and the ability of HSCs to undergo the normal interactions with stroma components that facilitate their maturation or maintenance. In fact, a characteristic finding in MDS is the atypical localization of immature progenitors, where these cells are located in a central, medullary location rather than the more terminal, trabecular endosteum.

Paracrine growth factor signaling is found in several hematopoietic malignancies, most notably multiple myeloma (MM), where plasma cell-derived and contact-dependent signals induce functional alterations in the stroma that then support the growth of the malignant clone (Podar et al. 2007). MM cells have been shown to release DKK1, a soluble inhibitor of Wnt signaling that

contributes to osteolytic bone disease by inhibiting osteoblast differentiation (Qiang et al. 2008). Recognition of these MM clone/stromal interactions has led to their successful targeting, with drugs like thalidomide or lenalidomide. Similarly, the malignant clone in primary myelofibrosis (PMF) induces a polyclonal fibrotic reaction through the release of cytokines into the stroma that play a critical role in the pathophysiology of the disease. In MDS, one study showed that clonally marked monocytes contributed to the locally high levels of TNFα, while failing to respond to normal stromal signals (Deeg et al. 2000). Another example is the failure of MDS monocytes to upregulate the matrix metalloproteinase, MMP9, an enzyme that is involved in the cleavage of SDF1 from the microenvironment, facilitating the egress of hematopoietic cells from the marrow to the peripheral blood (Heissig et al. 2002; Iwata et al. 2007). One could speculate that the lack of inducible MMP9 levels in MDS monocytes could contribute to the hypercellularity often seen in this disease. Thus, in a variety of diseases, stromal function can be altered by signals coming from the malignant clone, resulting in further support of the propagation of the clone. It is also evident that agents that target these dysregulated signals in the microenvironment can be very effective in treating these disorders.

Another mechanism by which the microenvironment can contribute to disease progression is through loss of normal protective function. As described in other chapters, MDS patients generally present with ineffective hematopoiesis and high rates of apoptosis in HPCs, with 30–40 % of patients progressing to an acute leukemia, a stage at which the malignant HSCs are generally resistant to apoptosis and cytotoxic therapy. While many investigators have studied the transition of MDS to AML, little is known about the mechanisms that lead to progressive cytopenias in patients who do not have a progressive increase in blasts or worsening dysplasia. The prolonged myelosuppression seen in patients with MDS suggests a limited hematopoietic reserve and implies that patients with MDS likely lose normal HSCs over time, leading to an accumulation of MDS HSCs, which may be competing for stem cell niches. The FoxO transcription factors, p38 MAPK, and ATM have all been implicated in the loss of HSCs due to oxidative stress. The p38 pathway is of particular interest, as p38 inhibitors have been shown to decrease the levels of apoptosis observed in MDS progenitor cells.

In primary cultures, intrinsically apoptosis-resistant MDS HPSs can acquire apoptosis sensitivity when cultured with bone marrow stroma. It appears that these co-culture conditions lead to changes in gene expression as well as methylation patterns in MDS marrow stoma. These alterations within the stroma can then modify the clone via dysregulation of transcription factors and miRs. For example, the transcription factor TWIST1, an inhibitor of p53 function, is upregulated in HSCs in advanced MDS (Li et al. 2010). Contact with stroma, however, results in downregulation of TWIST1, increased expression of p53, and enhanced sensitivity to apoptosis. Importantly, these stroma-dependent contact alterations occurred only with MDS-derived CD34+HSCs and not in CD34+ cells isolated from healthy bone marrow. The comparison of MDS stroma to stroma derived from normal bone marrow has shown that numerous gene promoters are significantly hypermethylated in MDS, with the most significantly differentially methylated genes involving components of the TNF signaling pathways (Figueroa et al. 2009). Preliminary data indicate that altered methylation patterns in stroma are accompanied by significant changes in protein expression, further supporting the concept that the stroma is involved in the pathophysiology of MDS.

Considering all available data, there is evidence that functional abnormalities in the microenvironment are related to their interactions with clonal hematopoietic cells. However, it is possible that intrinsic defects do occur within the stroma and these may alter the function of clonal HSCs, leading to their clonal outgrowth. In general, treatment strategies have focused on the eradication of the clonal hematopoietic stem or progenitor cells; however, observations in patients with MM suggest that focusing therapeutic strategies on the microenvironment could someday be effective. For instance, targeting of CD44 in

AML has been shown to eliminate AML stem cells via interference with homing, triggering differentiation, and enhancing apoptotic responses (Jin et al. 2006). Similarly, interactions between CXCR4 and its ligand SDF-1 may promote the mobilization of MDS HSCs from the marrow (Zhang et al. 2012). The efficacy of immunomodulatory agents, including thalidomide and lenalidomide, and the use of hypomethylating agents may lead to the successful targeting of primary defects in HSCs and changes in stromal methylation patterns (Yang et al. 2006; Raj et al. 2007). The future identification of primary stromal defects might also identify patients who are poor candidates for allogeneic HCT but who may respond favorably to therapies targeting the stromal compartment.

References

Asimakopoulos FA, Holloway TL, Nacheva EP et al (1996) Detection of chromosome 20q deletions in bone marrow metaphases but not peripheral blood granulocytes in patients with myeloproliferative disorders or myelodysplastic syndromes. Blood 87:1561–1570

Bell JJ, Bhandoola A (2008) The earliest thymic progenitors for T cells possess myeloid lineage potential. Nature 452:764–767

Benz C, Copley MR, Kent DG et al (2012) Hematopoietic stem cell subtypes expand differentially during development and display distinct lymphopoietic programs. Cell Stem Cell 10:273–283

Bhatia R, Mcglave PB, Dewald GW et al (1995) Abnormal function of the bone marrow microenvironment in chronic myelogenous leukemia: role of malignant stromal macrophages. Blood 85:3636–3645

Buonamici S, Li D, Chi Y et al (2004) EVI1 induces myelodysplastic syndrome in mice. J Clin Invest 114:713–719

Calvi LM, Adams GB, Weibrecht KW et al (2003) Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 425:841–846

Chabannon C, Torok-Storb B (1992) Stem cell-stromal cell interactions. Curr Top Microbiol Immunol 177:123–136

Chen G, Zeng W, Miyazato A et al (2004) Distinctive gene expression profiles of CD34 cells from patients with myelodysplastic syndrome characterized by specific chromosomal abnormalities. Blood 104:4210–4218

Choesmel V, Bacqueville D, Rouquette J et al (2007) Impaired ribosome biogenesis in Diamond-Blackfan anemia. Blood 109:1275–1283

Christiansen DH, Andersen MK, Pedersen-Bjergaard J (2001) Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. J Clin Oncol 19:1405–1413

Copeland NG, Gilbert DJ, Cho BC et al (1990) Mast cell growth factor maps near the steel locus on mouse chromosome 10 and is deleted in a number of steel alleles. Cell 63:175–183

Deeg HJ, Beckham C, Loken MR et al (2000) Negative regulators of hemopoiesis and stroma function in patients with myelodysplastic syndrome. Leuk Lymphoma 37:405–414

Dexter TM, Allen TD, Lajtha LG (1977) Conditions controlling the proliferation of haemopoietic stem cells in vitro. J Cell Physiol 91:335–344

Duhrsen U, Hossfeld DK (1996) Stromal abnormalities in neoplastic bone marrow diseases. Ann Hematol 73:53–70

Figueroa ME, Skrabanek L, Li Y et al (2009) MDS and secondary AML display unique patterns and abundance of aberrant DNA methylation. Blood 114:3448–3458

Fontana L, Pelosi E, Greco P et al (2007) MicroRNAs 17-5p-20a-106a control monocytopoiesis through AML1 targeting and M-CSF receptor upregulation. Nat Cell Biol 9:775–787

Frankel AE, Mccubrey JA, Miller MS et al (2000) Diphtheria toxin fused to human interleukin-3 is toxic to blasts from patients with myeloid leukemias. Leukemia 14:576–585

Frankel A, Liu JS, Rizzieri D et al (2008) Phase I clinical study of diphtheria toxin-interleukin 3 fusion protein in patients with acute myeloid leukemia and myelodysplasia. Leuk Lymphoma 49:543–553

Gartner S, Kaplan HS (1980) Long-term culture of human bone marrow cells. Proc Natl Acad Sci U S A 77:4756–4759

Gazda HT, Kho AT, Sanoudou D et al (2006) Defective ribosomal protein gene expression alters transcription, translation, apoptosis, and oncogenic pathways in Diamond-Blackfan anemia. Stem Cells 24:2034–2044

Gondek LP, Tiu R, O'keefe CL et al (2008) Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS, MDS/MPD, and MDS-derived AML. Blood 111:1534–1542

Goodell MA, Brose K, Paradis G et al (1996) Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 183:1797–1806

Greenberger JS (1991) The hematopoietic microenvironment. Crit Rev Oncol Hematol 11:65–84

Heavey B, Charalambous C, Cobaleda C et al (2003) Myeloid lineage switch of Pax5 mutant but not wild-type B cell progenitors by C/EBPalpha and GATA factors. EMBO J 22:3887–3897

Heissig B, Hattori K, Dias S et al (2002) Recruitment of stem and progenitor cells from the bone marrow niche

requires MMP-9 mediated release of kit-ligand. Cell 109:625–637
Huang E, Nocka K, Beier DR et al (1990) The hematopoietic growth factor KL is encoded by the Sl locus and is the ligand of the c-kit receptor, the gene product of the W locus. Cell 63:225–233
Iwata M, Pillai M, Ramakrishnan A et al (2007) Reduced expression of inducible gelatinase B/matrix metalloproteinase-9 in monocytes from patients with myelodysplastic syndrome: correlation of inducible levels with the percentage of cytogenetically marked cells and with marrow cellularity. Blood 109:85–92
Jiang Y, Dunbar A, Gondek LP et al (2009) Aberrant DNA methylation is a dominant mechanism in MDS progression to AML. Blood 113:1315–1325
Jin L, Hope KJ, Zhai Q et al (2006) Targeting of CD44 eradicates human acute myeloid leukemic stem cells. Nat Med 12:1167–1174
Jokubaitis VJ, Sinka L, Driessen R et al (2008) Angiotensin-converting enzyme (CD143) marks hematopoietic stem cells in human embryonic, fetal, and adult hematopoietic tissues. Blood 111: 4055–4063
Jordan CT, Upchurch D, Szilvassy SJ et al (2000) The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. Leukemia 14:1777–1784
Kollet O, Peled A, Byk T et al (2000) beta2 microglobulin-deficient (B2m(null)) NOD/SCID mice are excellent recipients for studying human stem cell function. Blood 95:3102–3105
Lam DH, Aplan PD (2001) NUP98 gene fusions in hematologic malignancies. Leukemia 15:1689–1695
Lane SW, Sykes SM, Al-Shahrour F et al (2010) The Apc(min) mouse has altered hematopoietic stem cell function and provides a model for MPD/MDS. Blood 115:3489–3497
Lapidot T, Sirard C, Vormoor J et al (1994) A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 367:645–648
Laver J, Jhanwar SC, O'reilly RJ et al (1987) Host origin of the human hematopoietic microenvironment following allogeneic bone marrow transplantation. Blood 70:1966–1968
Lawler M, Locasciulli A, Longoni D et al (2002) Leukaemic transformation of donor cells in a patient receiving a second allogeneic bone marrow transplant for severe aplastic anaemia. Bone Marrow Transplant 29:453–456
Lawrence HJ, Broudy VC, Magenis RE et al (1987) Cytogenetic evidence for involvement of B lymphocytes in acquired idiopathic sideroblastic anemias. Blood 70:1003–1005
Lennon JE, Micklem HS (1986) Stromal cells in long-term murine bone marrow culture: FACS studies and origin of stromal cells in radiation chimeras. Exp Hematol 14:287–292
Li X, Marcondes AM, Gooley TA et al (2010) The helix-loop-helix transcription factor TWIST is dysregulated in myelodysplastic syndromes. Blood 116:2304–2314
Lin YW, Slape C, Zhang Z et al (2005) NUP98-HOXD13 transgenic mice develop a highly penetrant, severe myelodysplastic syndrome that progresses to acute leukemia. Blood 106:287–295
Ma L, Ceuppens J, Kasran A et al (2007) 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 31:1373–1382
Malcovati L, Papaemmanuil E, Bowen DT et al (2011) Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood 118:6239–6246
Marsh JC, Harhalakis N, Dowding C et al (1989) Recurrent graft failure following syngeneic bone marrow transplantation for aplastic anaemia. Bone Marrow Transplant 4:581–585
Mayani H, Guilbert LJ, Janowska-Wieczorek A (1992) Biology of the hemopoietic microenvironment. Eur J Haematol 49:225–233
Mc Cann SR, Lawler M, Gardiner N et al (1994) Donor leukemia following allogeneic bone marrow transplantation. Leukemia 8(Suppl 1):S133–S135
Mcculloch EA, Siminovitch L, Till JE et al (1965) The cellular basis of the genetically determined hemopoietic defect in anemic mice of genotype Sl-Sld. Blood 26:399–410
Mcdermott SP, Eppert K, Lechman ER et al (2010) Comparison of human cord blood engraftment between immunocompromised mouse strains. Blood 116: 193–200
Micheva I, Thanopoulou E, Michalopoulou S et al (2004) Defective tumor necrosis factor alpha-induced maturation of monocyte-derived dendritic cells in patients with myelodysplastic syndromes. Clin Immunol 113:310–317
Mohamedali A, Gaken J, Twine NA et al (2007) Prevalence and prognostic significance of allelic imbalance by single-nucleotide polymorphism analysis in low-risk myelodysplastic syndromes. Blood 110:3365–3373
Nafa K, Bessler M, Deeg HJ et al (1998) New somatic mutation in the PIG-A gene emerges at relapse of paroxysmal nocturnal hemoglobinuria. Blood 92:3422–3427
Nilsson L, Eden P, Olsson E et al (2007) The molecular signature of MDS stem cells supports a stem-cell origin of 5q myelodysplastic syndromes. Blood 110:3005–3014
Nutt SL, Heavey B, Rolink AG et al (1999) Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. Nature 401:556–562
Papaemmanuil E, Cazzola M, Boultwood J et al (2011) Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Eng J Med 365:1384–1395
Pellagatti A, Cazzola M, Giagounidis AA et al (2006) Gene expression profiles of CD34+ cells in myelodysplastic syndromes: involvement of interferon-stimulated genes and correlation to FAB subtype and karyotype. Blood 108:337–345
Pellagatti A, Hellstrom-Lindberg E, Giagounidis A et al (2008) Haploinsufficiency of RPS14 in 5q− syndrome is associated with deregulation of ribosomal- and translation-related genes. Br J Haematol 142:57–64
Perkins S, Fleischman RA (1988) Hematopoietic microenvironment. Origin, lineage, and transplantability of the

stromal cells in long-term bone marrow cultures from chimeric mice. J Clin Invest 81:1072–1080
Podar K, Richardson PG, Hideshima T et al (2007) The malignant clone and the bone-marrow environment. Best Pract Res Clin Haematol 20:597–612
Qiang YW, Chen Y, Stephens O et al (2008) Myeloma-derived Dickkopf-1 disrupts Wnt-regulated osteoprotegerin and RANKL production by osteoblasts: a potential mechanism underlying osteolytic bone lesions in multiple myeloma. Blood 112:196–207
Raaijmakers MH, Mukherjee S, Guo S et al (2010) Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature 464:852–857
Raj K, John A, Ho A et al (2007) CDKN2B methylation status and isolated chromosome 7 abnormalities predict responses to treatment with 5-azacytidine. Leukemia 21:1937–1944
Raskind WH, Tirumali N, Jacobson R et al (1984) Evidence for a multistep pathogenesis of a myelodysplastic syndrome. Blood 63:1318–1323
Rhyasen GW, Starczynowski DT (2012) Deregulation of microRNAs in myelodysplastic syndrome. Leukemia 26:13–22
Rupec RA, Jundt F, Rebholz B et al (2005) Stroma-mediated dysregulation of myelopoiesis in mice lacking I kappa B alpha. Immunity 22:479–491
Russell ES (1979) Hereditary anemias of the mouse: a review for geneticists. Adv Genet 20:357–459
Scadden DT (2007) The stem cell niche in health and leukemic disease. Best Pract Res Clin Haematol 20:19–27
Senyuk V, Sinha KK, Li D et al (2007) Repression of RUNX1 activity by EVI1: a new role of EVI1 in leukemogenesis. Cancer Res 67:5658–5666
Shen L, Kantarjian H, Guo Y et al (2010) DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes. J Clin Oncol 28:605–613
Simmons PJ, Przepiorka D, Thomas ED et al (1987) Host origin of marrow stromal cells following allogeneic bone marrow transplantation. Nature 328:429–432
Srivannaboon K, Conley ME, Coustan-Smith E et al (2001) Hypogammaglobulinemia and reduced numbers of B-cells in children with myelodysplastic syndrome. J Pediatr Hematol Oncol 23:122–125
Starczynowski DT, Morin R, Mcpherson A et al (2011) Genome-wide identification of human microRNAs located in leukemia-associated genomic alterations. Blood 117:595–607
Sternberg A, Killick S, Littlewood T et al (2005) Evidence for reduced B-cell progenitors in early (low-risk) myelodysplastic syndrome. Blood 106:2982–2991
Sugimoto K, Hirano N, Toyoshima H et al (1993) Mutations of the p53 gene in myelodysplastic syndrome (MDS) and MDS-derived leukemia. Blood 81:3022–3026
Taichman RS (2005) Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. Blood 105:2631–2639
Tang Y, Zhao W, Chen Y et al (2008) Acetylation is indispensable for p53 activation. Cell 133:612–626
Tehranchi R, Woll PS, Anderson K et al (2010) Persistent malignant stem cells in del(5q) myelodysplasia in remission. N Eng J Med 363:1025–1037
Testa U, Riccioni R, Militi S et al (2002) Elevated expression of IL-3Ralpha in acute myelogenous leukemia is associated with enhanced blast proliferation, increased cellularity, and poor prognosis. Blood 100: 2980–2988
Thanopoulou E, Cashman J, Kakagianne T et al (2004) Engraftment of NOD/SCID-beta2 microglobulin null mice with multilineage neoplastic cells from patients with myelodysplastic syndrome. Blood 103: 4285–4293
Van Rhenen A, Van Dongen GA, Kelder A et al (2007) The novel AML stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. Blood 110:2659–2666
Visnjic D, Kalajzic Z, Rowe DW et al (2004) Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. Blood 103:3258–3264
Wada H, Masuda K, Satoh R et al (2008) Adult T-cell progenitors retain myeloid potential. Nature 452: 768–772
Walkley CR, Olsen GH, Dworkin S et al (2007) A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. Cell 129:1097–1110
Walter MJ, Shen D, Ding L et al (2012) Clonal architecture of secondary acute myeloid leukemia. N Eng J Med 366:1090–1098
Weerkamp F, Baert MR, Brugman MH et al (2006) Human thymus contains multipotent progenitors with T/B lymphoid, myeloid, and erythroid lineage potential. Blood 107:3131–3137
White NJ, Nacheva E, Asimakopoulos FA et al (1994) Deletion of chromosome 20q in myelodysplasia can occur in a multipotent precursor of both myeloid cells and B cells. Blood 83:2809–2816
Will B, Zhou L, Vogler TO et al (2012) Stem and progenitor cells in myelodysplastic syndromes show aberrant stage-specific expansion and harbor genetic and epigenetic alterations. Blood 120:2076–2086
Williams DE, Eisenman J, Baird A et al (1990) Identification of a ligand for the c-kit proto-oncogene. Cell 63:167–174
Witherspoon RP, Schubach W, Neiman P et al (1985) Donor cell leukemia developing six years after marrow grafting for acute leukemia. Blood 65:1172–1174
Yang L, Dybedal I, Bryder D et al (2005) IFN-gamma negatively modulates self-renewal of repopulating human hemopoietic stem cells. J Immunol 174: 752–757
Yang AS, Doshi KD, Choi SW et al (2006) DNA methylation changes after 5-aza-2′-deoxycytidine therapy in patients with leukemia. Cancer Res 66:5495–5503
Yoshida K, Sanada M, Shiraishi Y et al (2011) Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 478:64–69
Zhang J, Niu C, Ye L et al (2003) Identification of the haematopoietic stem cell niche and control of the niche size. Nature 425:836–841
Zhang Y, Zhao H, Zhao D et al (2012) SDF-1/CXCR4 axis in myelodysplastic syndromes: correlation with angiogenesis and apoptosis. Leuk Res 36:281–286

The Biology of Myelodysplastic Syndrome Associated with Isolated del(5q)

5

Martin Jadersten and Aly Karsan

5.1 Introduction

The 5q− syndrome was first described by Van den Berghe et al. in 1974 in three patients with refractory anemia characterized by erythroid hypoplasia, hypolobulated megakaryocytes, normal to elevated platelet counts, and an interstitial deletion on chromosome arm 5q (del[5q]). Isolated del(5q) was the second chromosomal abnormality recognized to be linked to a specific type of malignancy, the first being the Philadelphia chromosome t(9;22) described in 1960. The 5q− syndrome was acknowledged as a separate disease entity in the WHO classification of 2001 (Jaffe et al. 2001) but was renamed myelodysplastic syndrome (MDS) associated with isolated del(5q) in the 2008 version (Swerdlow et al. 2008). Del(5q) also occurs in the patients with more advanced MDS due to blast increase or additional karyotypic changes, as well as in acute myeloid leukemia (AML) and therapy-related MDS. In high-risk patients the presence of del(5q) is associated with adverse prognosis, in sharp contrast to the favorable outcome seen in 5q− syndrome. Intensive efforts have been made to investigate the molecular pathogenesis behind del(5q) MDS, and currently several genes on 5q are thought to be involved in the manifestations of the disease. Recent breakthroughs provide important insights into key aspects of the disease biology and pave the way for treatments effectively targeting the malignant del(5q) clone. This chapter focuses mainly on the classical low-risk 5q− syndrome but also covers more advanced types of MDS with del(5q).

M. Jadersten (✉)
Karolinska University Hospital,
Huddinge Hematology Center M54,
Stockholm SE-141 86, Sweden
e-mail: martin.jadersten@ki.se

A. Karsan, MD (✉)
Department of Genome Sciences,
British Columbia Cancer Research Centre,
675 West 10th Ave., Vancouver, BC V5Z 1L3, Canada
e-mail: akarsan@bcgsc.ca

5.2 Clinical Features

5.2.1 Clinical Presentation

MDS with isolated del(5q) has an insidious onset, with gradually progressing macrocytic anemia, coupled with normal or elevated platelet counts. The bone marrow is characteristically normo- or hypercellular, with erythroid hypoplasia and numerous hypolobulated megakaryocytes (Fig. 5.1). The neutrophil counts are generally normal. Rapidly decreasing granulocyte or platelet counts may reflect disease progression and warrant a repeated marrow investigation. Marrow fibrosis may develop at later stages of the disease.

5.2.2 Cytogenetics

Standard karyotyping shows an isolated interstitial del(5q) that should encompass 5q32–33. The

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_5, © Springer-Verlag Berlin Heidelberg 2013

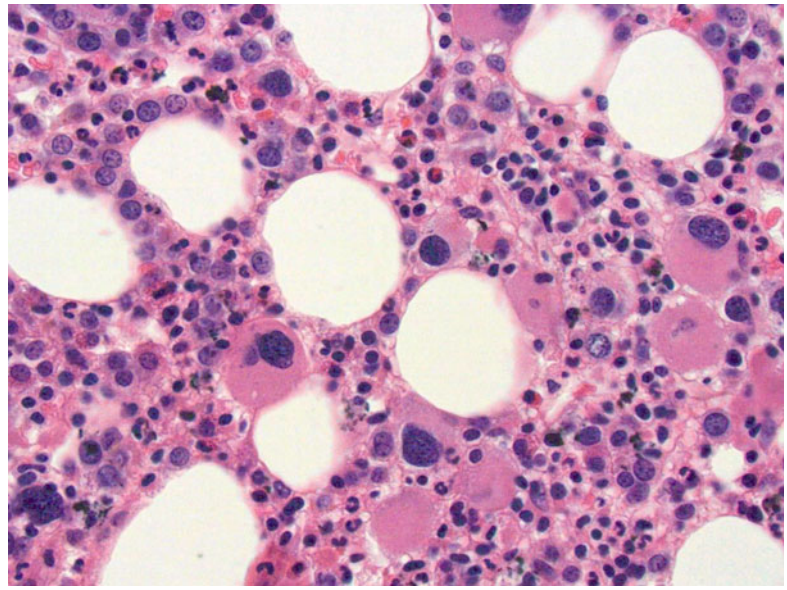

Fig. 5.1 Characteristic bone marrow morphology of MDS with isolated del(5q) demonstrating numerous hypolobulated megakaryocytes

deletion can also be detected by fluorescent in situ hybridization (FISH), which may be of value if karyotyping is unsuccessful. However, karyotyping remains the gold standard for diagnosis and follow-up since it is more sensitive than FISH and has the ability to identify additional abnormalities which may indicate clonal evolution and adverse outcome (Gohring et al. 2011).

5.2.3 Diagnostic Criteria

The WHO 2008 classification defines MDS associated with isolated del(5q) as de novo MDS with less than 5 % bone marrow blasts and isolated del(5q) including the region 5q32–33. Additional criteria are less than 1 % blasts in the peripheral blood and absence of Auer rods, while characteristic marrow morphology or thrombocytosis is not required.

5.2.4 Prognosis

The prognosis is favorable, with median survival of 6–9 years and cumulative probability of evolution to acute myeloid leukemia (AML) of 9–17 % at 5 years (Giagounidis et al. 2004; Giagounidis et al. 2006; Mallo et al. 2011). This relatively favorable outcome is restricted to patients with the classical low-risk 5q− syndrome and does not include patients with additional risk factors.

5.2.5 High-Risk MDS with del(5q)

Additional adverse features such as more than one additional karyotypic abnormality, blast increase ≥5 %, or platelet counts below 100×10^9/l are associated with inferior outcome (Giagounidis et al. 2004; Mallo et al. 2011). The presenting symptoms and the prognosis vary greatly depending on the severity of the alterations. Patients with one additional karyotypic abnormality or blasts elevated to 5–9 % constitute an intermediate-risk group. In MDS with more than 10 % blasts or with therapy-related MDS or AML, del(5q) most frequently occurs in conjunction with complex karyotype and is tightly linked to presence of TP53 mutation (Fidler et al. 2004; Pedersen-Bjergaard et al. 2008) and exceedingly poor outcome.

5.3 Biology of del(5q) MDS

5.3.1 Genes Involved in the Pathogenesis

5.3.1.1 Commonly Deleted Region on Chromosome Arm 5q

There are two distinct commonly deleted regions on 5q (Fig. 5.2). The more distal commonly deleted region (CDR) of 5q− syndrome is defined by a 1.5-megabase region at 5q32–33, containing

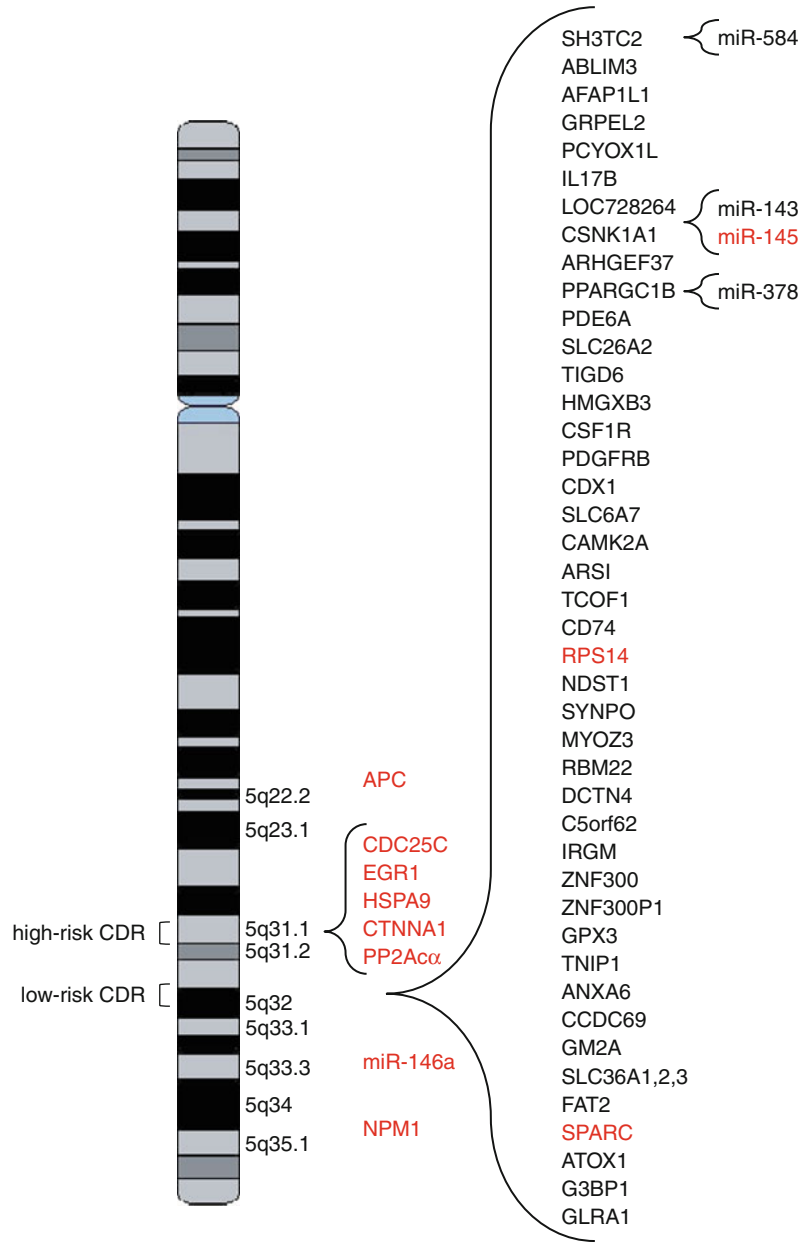

Fig. 5.2 Genes and microRNAs within the low-risk *commonly deleted region* (CDR) at chromosome 5q32–33. The high-risk CDR is also shown; however, only the genes directly implicated in the pathogenesis are listed. Genes potentially disease related, located within or outside the two CDRs, are highlighted in *red*

around 40 coding genes and four microRNAs (Boultwood et al. 2002). No mutations of genes located within the CDR have been identified on the retained allele. This suggests that altered gene dosage may play a role, and recent evidence supports the concept of haploinsufficiency. Functional studies using an RNA interference screen of all genes within the CDR identified ribosomal protein S14 (RPS14) as a haploinsufficient gene responsible for the erythroid failure (Ebert et al. 2008). However, RPS14 haploinsufficiency alone does not explain the megakaryocytic dysplasia and the tendency to thrombocytosis, nor the clonal dominance of del(5q) MDS cells. Examination of noncoding genes at 5q31–5q35 revealed reduced expression of miR-145 and miR-146a in marrow cells from patients with del(5q) MDS (Starczynowski et al. 2010). Depletion of these two microRNAs in mice resulted in variable neutropenia, thrombocytosis, and hypolobulated megakaryocytes with reduced endomitosis in the marrow. These two key

pathogenic mechanisms will be discussed in further detail below.

In patients with high-risk MDS or AML with del(5q), a unique CDR has been delineated at 5q31, centromeric of the low-risk CDR (Zhao et al. 1997; Horrigan et al. 2000). This suggests that other genes are likely to be involved in high-risk disease. It is important to point out that both CDRs are deleted in the majority of low- and high-risk del(5q) MDS patients, indicating that other factors or cooperating mutations elsewhere may be required for disease development.

5.3.1.2 Ribosomal Stress Causes Anemia

Although ribosomes are expressed in all cell types, the dominating feature of ribosomal gene haploinsufficiency is impaired erythropoiesis (Narla and Ebert 2010). This is observed in Diamond-Blackfan anemia, where germline mutations in RPS19 or other ribosomal genes are frequent (Draptchinskaia et al. 1999; Narla and Ebert 2010). In 5q− syndrome, RPS14 is the only gene within the low-risk CDR for which haploinsufficiency has been shown to impair erythropoiesis (Ebert et al. 2008). RPS14 encodes a structural protein of the 40S ribosomal subunit, and its deficiency can cause defects in ribosomal biogenesis and activity (Ebert et al. 2008). The erythroid defect is rescued in vitro by forced expression of RPS14 in hematopoietic stem/progenitor cells from 5q− syndrome patients (Ebert et al. 2008). Haploinsufficiency of RPS14 in mice results in macrocytic anemia and dyserythropoiesis (Barlow et al. 2010). Interestingly, multiple ribosomal genes are downregulated in CD34+ cells of patients with del(5q) MDS, which is consistent with the impaired erythropoiesis being a result of a ribosomal processing defect (Pellagatti et al. 2008). Ribosomal stress due to reduced expression of RPS14 in hematopoietic stem/progenitor cells activates the p53 pathway, inducing the downstream targets p21 and BAX, primarily in the erythroid cell compartment. The mechanism of the observed increase in p53 levels is thought to be increased expression of another ribosomal protein RPL11, which binds the p53 ubiquitin ligase HDM2, thereby preventing p53 ubiquitination and degradation. This results in increased apoptosis and cell cycle arrest (Dutt et al. 2011). Consistent with this finding, crossing mice hemizygous for Rps14 with p53-deficient mice rescues the erythroid progenitor cell defect (Barlow et al. 2010).

5.3.1.3 Reduced miR-145 and miR-146a Expression Induces Innate Immune Signaling

MicroRNAs are small noncoding RNAs, around 22 nucleotides in length, that regulate gene expression by interacting with the 3′ untranslated regions (UTR) of messenger RNAs (mRNAs). MicroRNAs target numerous mRNAs and may induce mRNA degradation and repress protein translation. By interfering with specific cellular pathways, several microRNAs have been shown to have oncogenic properties (Garzon et al. 2009). Knockdown of miR-145 and miR-146a resulted in thrombocytosis and hypolobulated megakaryocytes in the marrow, thus mimicking key features of the 5q− syndrome (Starczynowski et al. 2010). Mice transplanted with marrow depleted for miR-145 and miR-146 succumb to a myeloproliferative/leukemic disorder (Starczynowski et al. 2011). These two microRNAs target genes involved in the innate immune response pathway, including TIRAP (miR-145) and TRAF6 (miR-146a). Transplantation of TRAF6-transduced bone marrow into wild-type mice recapitulated the hematologic phenotype seen with depletion of miR-145/miR-146a including progression to AML or bone marrow failure, suggesting that ectopic activation of innate immune signaling in the hematopoietic stem/progenitor population is a pathogenic feature of del(5q) MDS (Starczynowski et al. 2010). Depletion of miR-145/miR-146a with activation of innate immune signaling results in NF-κB activation and upregulation of IL-6, which is also seen in patients with del(5q) MDS. The platelet and granulocytic defects driven by TRAF6-mediated activation of innate immune signaling and NF-κB are abrogated in mouse marrow cells lacking IL-6, but a similar proportion of mice still develop myeloid neoplasia (Starczynowski et al. 2010). Thus, while the paracrine effects of IL-6 likely explain the thrombocytosis and neutropenia,

clonal dominance of the MDS cells in the marrow appears to be secondary to cell autonomous effects of miR-145/miR-146a haploinsufficiency and deregulated immune signaling.

Recent data indicates that FLI1, another validated target repressed by miR-145, may be of importance for the characteristic megakaryocyte dysplasia and thrombocytosis. FLI1 is an ETS-family transcription factor involved in megakaryocytic differentiation, and its loss results in thrombocytopenia. FLI1 has been shown to be overexpressed in patients with 5q− syndrome, while non-del(5q) MDS patients have levels similar to those of healthy controls. Inhibition of miR-145 or overexpression of FLI1 improves megakaryocyte formation, as seen in patients with 5q− syndrome, while overexpression of miR-145 or inhibition of FLI1 has the reciprocal effect. This suggests that the thrombocytosis observed in patients with 5q− syndrome is due to both cell extrinsic and intrinsic factors. Downregulation of miR-145 or upregulation of FLI1 increased the number of human CD34+ progenitor cells, and knockdown of miR-145 partially rescued the negative effect of RPS14 silencing on the proliferation of progenitor cells in vitro. This further strengthens the evidence that miR-145 is important for clonal dominance (Kumar et al. 2011).

5.3.1.4 Other Candidate Genes Within the CDR

The matricellular protein SPARC is encoded within the low-risk CDR at 5q32–33. It has diverse functions, varying depending on cell type and location. In several malignancies, SPARC acts as a tumor suppressor gene by inhibiting proliferation, angiogenesis, and adhesion to adjacent stroma (Framson and Sage 2004). In del(5q) MDS, SPARC has been shown to be haploinsufficient due to the 5q deletion (Pellagatti et al. 2007), and it is conceivable that this leads to stronger adhesion of the del(5q) hematopoietic stem/progenitor cells to the supporting bone marrow stroma. When CD34+ hematopoietic progenitors are treated with the clinically active drug lenalidomide in vitro, SPARC is upregulated to normal or supranormal levels, thus reversing its haploinsufficiency (Pellagatti et al. 2007). It remains to be demonstrated whether reduced expression of the tumor suppressor gene SPARC plays a role in the pathogenesis of the disease.

5.3.1.5 Genes Implicated in High-Risk del(5q) MDS

Proposed candidate genes within the high-risk CDR at 5q31 include EGR1, HSPA9, and CTNNA1. EGR1-deficient mice treated with an alkylating agent frequently developed myeloproliferative neoplasias coupled with ineffective erythropoiesis and thrombocytopenia (Joslin et al. 2007). Knockdown of HSPA9 in primary human hematopoietic cells or in a murine model impaired erythroid maturation and suppressed hematopoietic cell expansion (Chen et al. 2011). Thus, haploinsufficiency of HSPA9 may play a role in the ineffective hematopoiesis observed in del(5q) MDS but fails to explain the clonal dominance. Forced expression of CTNNA1 in the myeloid cell line HL-60 resulted in reduced proliferation and increased apoptosis, suggesting that haploinsufficiency of this tumor suppressor may provide a growth advantage contributing to the clonal advantage of the del(5q) cells CTNNA1 (Liu et al. 2007).

Several genes outside the two CDRs have also been implicated in myeloid disorders. The gene APC resides more centromeric at 5q21–22 but is generally part of the deletion. APC deficiency leads to ineffective hematopoiesis and loss of HSC quiescence, conceivably contributing to the observed pathology (Lane et al. 2010; Wang et al. 2010). NPM1 is located telomeric of the CDR (at 5q35) and is rarely part of the del(5q) in low-risk MDS, while it is deleted in 40 % of high-risk MDS with del(5q) (La Starza et al. 2010; Pellagatti et al. 2011). Haploinsufficiency of NPM1 is tightly linked to genomic instability and complex karyotype in MDS and AML, likely due to impaired centrosome duplication and inhibition of the tumor suppressors ARF1 and TP53 (Falini et al. 2007). Mice that are heterozygous for Npm1 develop a hypercellular marrow with prominent erythroid and megakaryocytic dysplasia. Moreover, they were prone to develop malignancies, in particular myeloid leukemias, that were associated with

centrosome amplification and chromosomal abnormalities (Grisendi et al. 2005; Sportoletti et al. 2008). In contrast, patients with de novo AML and NPM1 mutations are characterized by normal karyotype (Falini et al. 2005), likely attributable to retained ability to duplicate the centrosome by the mutated NPM1 (Falini et al. 2007). Recently, a transgenic mouse model overexpressing mutated NPM1 resulted in myeloproliferation, suggesting that the NPM1 mutation per se contributes to clonal advantage (Cheng et al. 2010).

5.3.2 Originates in Hematopoietic Stem Cells

In MDS with del(5q) more than 90 % of the CD34+CD38−CD90+ hematopoietic stem cells (HSC) carry the deletion (Nilsson et al. 2000). Further, del(5q) may appear in B and NK cells (Jaju et al. 2000; Nilsson et al. 2000; Kiladjian et al. 2006), and the disease may transform to acute lymphoblastic leukemia. Thus, the disease is considered to originate at the pluripotent HSC level. Despite the fact that the bone marrow is normo- or hypercellular and the stem cell compartment is dominated by del(5q) cells, the marrow fails to release sufficient blood cells into circulation. One contributing factor may be that del(5q) HSCs contain limited long-term culture-initiating assay activity and fail to reconstitute transplanted mice over the long term, reflecting the observed ineffective hematopoiesis (Nilsson et al. 2000). In colony-forming assays in particular, the erythroid colonies are reduced, potentially due to the RPS14 haploinsufficiency (Tehranchi et al. 2010). Despite this impaired function, the gene expression profile of del(5q) and normal HSCs is highly similar. Intriguing exceptions include upregulation of the critical stem cell renewal factor BMI1 and the Notch-signaling inhibitor DLK1 in the HSC fraction and downregulation of the myeloid transcription factor CEBPA at the progenitor stage. The differentially expressed genes differed substantially between HSCs (CD34+CD38−CD90+) and progenitor cells (CD34+CD38+CD90−), stressing the importance of studying homogenous and relevant populations when drawing conclusions about the mechanisms of the disease (Nilsson et al. 2007).

5.3.3 Defects in the Bone Marrow Microenvironment

Evidence suggests that the bone marrow stroma is deficient in del(5q) MDS, resulting in impaired ability to support growth of normal hematopoietic progenitors. The cytokine profile in low-risk MDS marrow plasma is altered, including increased level of TNF-α which is likely to suppress hematopoiesis (Gersuk et al. 1998). It is possible that these alterations are caused by macrophages or other cells that are part of the del(5q) malignant clone and that this may favor the expansion of the malignant over normal cells. There are contradicting data regarding the presence of genetic alterations in stromal cells from patients with del(5q) MDS. Several groups have reported cytogenetic aberrations in mesenchymal cells; however, the genetic changes were generally unrelated to those observed in the MDS cells (Blau et al. 2007; Lopez-Villar et al. 2009). Other groups do not observe genetic alterations in the stromal cells (Soenen-Cornu et al. 2005; Ramakrishnan et al. 2006). Also, allogeneic stem cell transplantation may cure del(5q) MDS, arguing against an inherent stromal defect.

5.4 Novel Prognostic Markers

5.4.1 TP53 Mutation Associated with Leukemic Transformation

Mutations in the tumor suppressor gene TP53 occur in 5–10 % of patients with low-risk MDS and in 10–15 % with high-risk disease. TP53 mutations were until recently thought to be rare in low-risk del(5q) MDS, which stands in contrast to the high frequency observed in MDS patients with complex karyotype that includes del(5q), where around 80 % are mutated (Fidler et al. 2004; Pedersen-Bjergaard et al. 2008). A recent study using deep sequencing demonstrated that 18 % of patients with low-risk del(5q) MDS had TP53

mutation (Jadersten et al. 2011). Interestingly, in more than half of the mutated patients, the clone size was below 20 % and would thus probably not have been detected by conventional Sanger sequencing. TP53 mutation was significantly associated with increased risk of leukemic transformation, in agreement with results in other types of MDS (Padua et al. 1998). When deep sequencing becomes part of the routine workup in MDS, then TP53 mutation is likely to constitute an important prognostic factor.

5.5 Lenalidomide Treatment of del(5q) MDS

5.5.1 Potent Clinical Effect

Lenalidomide has unparalleled efficacy in transfusion-dependent low-risk MDS with del(5q), with 67 % reaching transfusion independence and 45 % complete cytogenetic remission (List et al. 2006). Important side effects include grade III/IV neutropenia and thrombocytopenia. The median response duration is around 2 years. A recent study demonstrated that del(5q) HSCs are relatively insensitive to the effects of lenalidomide. In patients with karyotypic complete cytogenetic remission and no del(5q) detectable by FISH in the CD34+CD38+CD90− progenitor compartment, del(5q) cells were still detectable although at lower levels in all patients studied in the CD34+CD38−CD90+ HSCs fraction. This relative insensitivity of the del(5q) HSCs to lenalidomide suggests that this therapy is unlikely to be curative (Tehranchi et al. 2010). However, it is important to stress that this does not rule out that a large proportion of the patients may have a substantial clinical benefit of the treatment, with durable transfusion independency. Recent safety concerns have been raised, since an unexpectedly high fraction of patients treated with lenalidomide have undergone clonal evolution, with acquisition of complex karyotypes or transformation to AML (Gohring et al. 2010; Jadersten et al. 2011). Therefore, it is advised that even low-risk del(5q) MDS patients treated with lenalidomide are followed closely for signs of disease progression, in particular if they are of transplantable age (Jadersten and Karsan 2011).

5.5.2 Mechanisms of Action

Lenalidomide inhibits numerous cytokines, including IL-6 and TNF-α, and activates T cells and NK cells (Bartlett et al. 2004). After successful treatment with lenalidomide, the stromal defect described above is reversed (Ximeri et al. 2010). This improved function of the stroma may be a direct effect of the treatment or an indirect via suppression of the malignant MDS clone. Other functions ascribed to lenalidomide include inhibition of the cell cycle-regulating phosphatases CDC25C and PP2Acα (Wei et al. 2009). Both these genes are located centromeric to the 5q32–33 CDR but are deleted in most del(5q) MDS patients (Fig. 5.3), potentially contributing to the increased sensitivity of del(5q) cells to lenalidomide. The tumor suppressor gene SPARC was one of four genes significantly upregulated by lenalidomide in vitro and the only one located within the 5q32–33 CDR (Pellagatti et al. 2007). In addition, emerging data indicates that lenalidomide increases miR-143 and miR-145 expression in CD34+ del(5q) progenitors, thus counteracting the haploinsufficiency. This induction may be associated with subsequent clinical response to treatment (Venner et al. 2010). It remains to be resolved which mechanisms are most important for the effects observed in patients with del(5q) MDS.

5.6 Future Directions

Important parts of the pathogenesis of del(5q) MDS have been unraveled; however, it remains to be demonstrated if they are sufficient to induce the disease or if cooperating genetic events are required. Whole genome sequencing is a powerful tool that can address this question. Development of improved mouse models of the disease is also needed to refine our understanding of the disease biology. Efforts to develop targeted therapy are ongoing, exploring approaches such

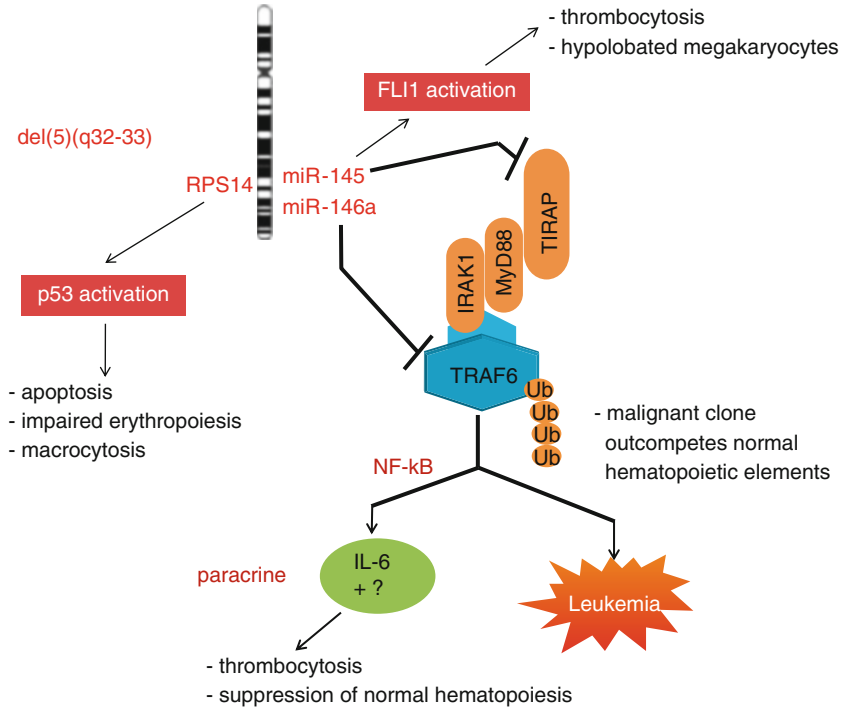

Fig. 5.3 Overview of the biology of MDS with del(5q)

as inhibition of p53 to improve the expansion of del(5q) erythroblasts to alleviate the anemia and inhibition of PP2A to inhibit the malignant clone itself. Deep sequencing of key prognostic genes such as TP53 is likely to improve initial risk stratification and will enable genetic monitoring of emerging adverse subclones in the marrow during the course of the disease. This will be important for optimizing the timing of intensified therapy or allogeneic stem cell transplantation.

References

Barlow JL, Drynan LF, Hewett DR, Holmes LR, Lorenzo-Abalde S, Lane AL, Jolin HE, Pannell R, Middleton AJ, Wong SH, Warren AJ, Wainscoat JS, Boultwood J, McKenzie AN (2010) A p53-dependent mechanism underlies macrocytic anemia in a mouse model of human 5q− syndrome. Nat Med 16(1):59–66

Bartlett JB, Dredge K, Dalgleish AG (2004) The evolution of thalidomide and its IMiD derivatives as anticancer agents. Nat Rev Cancer 4(4):314–322

Blau O, Hofmann WK, Baldus CD, Thiel G, Serbent V, Schumann E, Thiel E, Blau IW (2007) Chromosomal aberrations in bone marrow mesenchymal stroma cells from patients with myelodysplastic syndrome and acute myeloblastic leukemia. Exp Hematol 35(2):221–229

Boultwood J, Fidler C, Strickson AJ, Watkins F, Gama S, Kearney L, Tosi S, Kasprzyk A, Cheng JF, Jaju RJ, Wainscoat JS (2002) Narrowing and genomic annotation of the commonly deleted region of the 5q− syndrome. Blood 99(12):4638–4641

Chen TH, Kambal A, Krysiak K, Walshauser MA, Raju G, Tibbitts JF, Walter MJ (2011) Knockdown of Hspa9, a del(5q31.2) gene, results in a decrease in hematopoietic progenitors in mice. Blood 117(5):1530–1539

Cheng K, Sportoletti P, Ito K, Clohessy JG, Teruya-Feldstein J, Kutok JL, Pandolfi PP (2010) The cytoplasmic NPM mutant induces myeloproliferation in a transgenic mouse model. Blood 115(16):3341–3345

Draptchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, Ball S, Tchernia G, Klar J, Matsson H, Tentler D, Mohandas N, Carlsson B, Dahl N (1999) The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. Nat Genet 21(2):169–175

Dutt S, Narla A, Lin K, Mullally A, Abayasekara N, Megerdichian C, Wilson FH, Currie T, Khanna-Gupta A, Berliner N, Kutok JL, Ebert BL (2011) Haploinsufficiency for ribosomal protein genes causes selective activation of p53 in human erythroid progenitor cells. Blood 117(9):2567–2576

Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N, Raza A, Root DE, Attar E, Ellis SR, Golub TR (2008)

Identification of RPS14 as a 5q− syndrome gene by RNA interference screen. Nature 451(7176):335–339

Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, La Starza R, Diverio D, Colombo E, Santucci A, Bigerna B, Pacini R, Pucciarini A, Liso A, Vignetti M, Fazi P, Meani N, Pettirossi V, Saglio G, Mandelli F, Lo-Coco F, Pelicci PG, Martelli MF (2005) Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med 352(3):254–266

Falini B, Nicoletti I, Martelli MF, Mecucci C (2007) Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc + AML): biologic and clinical features. Blood 109(3):874–885

Fidler C, Watkins F, Bowen DT, Littlewood TJ, Wainscoat JS, Boultwood J (2004) NRAS, FLT3 and TP53 mutations in patients with myelodysplastic syndrome and a del(5q). Haematologica 89(7):865–866

Framson PE, Sage EH (2004) SPARC and tumor growth: where the seed meets the soil? J Cell Biochem 92(4):679–690

Garzon R, Calin GA, Croce CM (2009) MicroRNAs in cancer. Annu Rev Med 60:167–179

Gersuk GM, Beckham C, Loken MR, Kiener P, Anderson JE, Farrand A, Troutt AB, Ledbetter JA, Deeg HJ (1998) A role for tumour necrosis factor-alpha, Fas and Fas-Ligand in marrow failure associated with myelodysplastic syndrome. Br J Haematol 103(1): 176–188

Giagounidis AA, Germing U, Haase S, Hildebrandt B, Schlegelberger B, Schoch C, Wilkens L, Heinsch M, Willems H, Aivado M, Aul C (2004) Clinical, morphological, cytogenetic, and prognostic features of patients with myelodysplastic syndromes and del(5q) including band q31. Leukemia 18(1):113–119

Giagounidis AA, Germing U, Aul C (2006) Biological and prognostic significance of chromosome 5q deletions in myeloid malignancies. Clin Cancer Res 12(1):5–10

Gohring G, Giagounidis A, Busche G, Kreipe HH, Zimmermann M, Hellstrom-Lindberg E, Aul C, Schlegelberger B (2010) Patients with del(5q) MDS who fail to achieve sustained erythroid or cytogenetic remission after treatment with lenalidomide have an increased risk for clonal evolution and AML progression. Ann Hematol 89(4):365–374

Gohring G, Giagounidis A, Busche G, Hofmann W, Kreipe HH, Fenaux P, Hellstrom-Lindberg E, Schlegelberger B (2011) Cytogenetic follow-up by karyotyping and fluorescence in situ hybridization: implications for monitoring patients with myelodysplastic syndrome and deletion 5q treated with lenalidomide. Haematologica 96(2):319–322

Grisendi S, Bernardi R, Rossi M, Cheng K, Khandker L, Manova K, Pandolfi PP (2005) Role of nucleophosmin in embryonic development and tumorigenesis. Nature 437(7055):147–153

Horrigan SK, Arbieva ZH, Xie HY, Kravarusic J, Fulton NC, Naik H, Le TT, Westbrook CA (2000) Delineation of a minimal interval and identification of 9 candidates for a tumor suppressor gene in malignant myeloid disorders on 5q31. Blood 95(7):2372–2377

Jadersten M, Karsan A (2011) Clonal evolution in myelodysplastic syndromes with isolated del(5q): the importance of genetic monitoring. Haematologica 96(2):177–180

Jadersten M, Saft L, Smith A, Kulasekararaj A, Pomplun S, Gohring G, Hedlund A, Hast R, Schlegelberger B, Porwit A, Hellstrom-Lindberg E, Mufti GJ (2011) TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. J Clin Oncol 29(15):1971–1979

Jaffe E, Harris HN, Stein H et al (eds) (2001) WHO classification of tumours: pathology and genetics of haematopoietic and lymphoid tissues. IARC Press, Lyon

Jaju RJ, Jones M, Boultwood J, Kelly S, Mason DY, Wainscoat JS, Kearney L (2000) Combined immunophenotyping and FISH identifies the involvement of B-cells in 5q− syndrome. Genes Chromosomes Cancer 29(3):276–280

Joslin JM, Fernald AA, Tennant TR, Davis EM, Kogan SC, Anastasi J, Crispino JD, Le Beau MM (2007) Haploinsufficiency of EGR1, a candidate gene in the del(5q), leads to the development of myeloid disorders. Blood 110(2):719–726

Kiladjian JJ, Bourgeois E, Lobe I, Braun T, Visentin G, Bourhis JH, Fenaux P, Chouaib S, Caignard A (2006) Cytolytic function and survival of natural killer cells are severely altered in myelodysplastic syndromes. Leukemia 20(3):463–470

Kumar MS, Narla A, Nonami A, Mullally A, Dimitrova N, Ball B, McAuley JR, Poveromo L, Kutok JL, Galili N, Raza A, Attar E, Gilliland DG, Jacks T, Ebert BL (2011) Coordinate loss of a microRNA and protein-coding gene cooperate in the pathogenesis of 5q− syndrome. Blood 118(17):4666–4673

La Starza R, Matteucci C, Gorello P, Brandimarte L, Pierini V, Crescenzi B, Nofrini V, Rosati R, Gottardi E, Saglio G, Santucci A, Berchicci L, Arcioni F, Falini B, Martelli MF, Sambani C, Aventin A, Mecucci C (2010) NPM1 deletion is associated with gross chromosomal rearrangements in leukemia. PLoS One 5(9):e12855

Lane SW, Sykes SM, Al-Shahrour F, Shterental S, Paktinat M, Lo Celso C, Jesneck JL, Ebert BL, Williams DA, Gilliland DG (2010) The Apc(min) mouse has altered hematopoietic stem cell function and provides a model for MPD/MDS. Blood 115(17):3489–3497

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 (2006) Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 355(14):1456–1465

Liu TX, Becker MW, Jelinek J, Wu WS, Deng M, Mikhalkevich N, Hsu K, Bloomfield CD, Stone RM, DeAngelo DJ, Galinsky IA, Issa JP, Clarke MF, Look AT (2007) Chromosome 5q deletion and epigenetic

suppression of the gene encoding alpha-catenin (CTNNA1) in myeloid cell transformation. Nat Med 13(1):78–83

Lopez-Villar O, Garcia JL, Sanchez-Guijo FM, Robledo C, Villaron EM, Hernandez-Campo P, Lopez-Holgado N, Diez-Campelo M, Barbado MV, Perez-Simon JA, Hernandez-Rivas JM, San-Miguel JF, del Canizo MC (2009) Both expanded and uncultured mesenchymal stem cells from MDS patients are genomically abnormal, showing a specific genetic profile for the 5q− syndrome. Leukemia 23(4):664–672

Mallo M, Cervera J, Schanz J, Such E, Garcia-Manero G, Luno E, Steidl C, Espinet B, Vallespi T, Germing U, Blum S, Ohyashiki K, Grau J, Pfeilstocker M, Hernandez JM, Noesslinger T, Giagounidis A, Aul C, Calasanz MJ, Martin ML, Valent P, Collado R, Haferlach C, Fonatsch C, Lubbert M, Stauder R, Hildebrandt B, Krieger O, Pedro C, Arenillas L, Sanz MA, Valencia A, Florensa L, Sanz GF, Haase D, Sole F (2011) Impact of adjunct cytogenetic abnormalities for prognostic stratification in patients with myelodysplastic syndrome and deletion 5q. Leukemia 25(1):110–120

Narla A, Ebert BL (2010) Ribosomopathies: human disorders of ribosome dysfunction. Blood 115(16):3196–3205

Nilsson L, Astrand-Grundstrom I, Arvidsson I, Jacobsson B, Hellstrom-Lindberg E, Hast R, Jacobsen SE (2000) Isolation and characterization of hematopoietic progenitor/stem cells in 5q-deleted myelodysplastic syndromes: evidence for involvement at the hematopoietic stem cell level. Blood 96(6):2012–2021

Nilsson L, Eden P, Olsson E, Mansson R, Astrand-Grundstrom I, Strombeck B, Theilgaard-Monch K, Anderson K, Hast R, Hellstrom-Lindberg E, Samuelsson J, Bergh G, Nerlov C, Johansson B, Sigvardsson M, Borg A, Jacobsen SE (2007) The molecular signature of MDS stem cells supports a stem-cell origin of 5q myelodysplastic syndromes. Blood 110(8):3005–3014

Padua RA, Guinn BA, Al-Sabah AI, Smith M, Taylor C, Pettersson T, Ridge S, Carter G, White D, Oscier D, Chevret S, West R (1998) RAS, FMS and p53 mutations and poor clinical outcome in myelodysplasias: a 10-year follow-up. Leukemia 12(6):887–892

Pedersen-Bjergaard J, Andersen MK, Andersen MT, Christiansen DH (2008) Genetics of therapy-related myelodysplasia and acute myeloid leukemia. Leukemia 22(2):240–248

Pellagatti A, Jadersten M, Forsblom AM, Cattan H, Christensson B, Emanuelsson EK, Merup M, Nilsson L, Samuelsson J, Sander B, Wainscoat JS, Boultwood J, Hellstrom-Lindberg E (2007) 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 104(27):11406–11411

Pellagatti A, Hellstrom-Lindberg E, Giagounidis A, Perry J, Malcovati L, Della Porta MG, Jadersten M, Killick S, Fidler C, Cazzola M, Wainscoat JS, Boultwood J (2008) Haploinsufficiency of RPS14 in 5q− syndrome is associated with deregulation of ribosomal- and translation-related genes. Br J Haematol 142(1):57–64

Pellagatti A, Cazzola M, Giagounidis A, Perry J, Malcovati L, Della Porta MG, Jadersten M, Killick S, Vyas P, Hellstrom-Lindberg E, Wainscoat JS, Boultwood J (2011) Marked down-regulation of nucleophosmin-1 is associated with advanced del(5q) myelodysplastic syndrome. Br J Haematol 155(2):272–274

Ramakrishnan A, Awaya N, Bryant E, Torok-Storb B (2006) The stromal component of the marrow microenvironment is not derived from the malignant clone in MDS. Blood 108(2):772–773

Soenen-Cornu V, Tourino C, Bonnet ML, Guillier M, Flamant S, Kotb R, Bernheim A, Bourhis JH, Preudhomme C, Fenaux P, Turhan AG (2005) Mesenchymal cells generated from patients with myelodysplastic syndromes are devoid of chromosomal clonal markers and support short- and long-term hematopoiesis in vitro. Oncogene 24(15):2441–2448

Sportoletti P, Grisendi S, Majid SM, Cheng K, Clohessy JG, Viale A, Teruya-Feldstein J, Pandolfi PP (2008) Npm1 is a haploinsufficient suppressor of myeloid and lymphoid malignancies in the mouse. Blood 111(7):3859–3862

Starczynowski DT, Kuchenbauer F, Argiropoulos B, Sung S, Morin R, Muranyi A, Hirst M, Hogge D, Marra M, Wells RA, Buckstein R, Lam W, Humphries RK, Karsan A (2010) Identification of miR-145 and miR-146a as mediators of the 5q− syndrome phenotype. Nat Med 16(1):49–58

Starczynowski DT, Morin R, McPherson A, Lam J, Chari R, Wegrzyn J, Kuchenbauer F, Hirst M, Tohyama K, Humphries RK, Lam WL, Marra M, Karsan A (2011) Genome-wide identification of human microRNAs located in leukemia-associated genomic alterations. Blood 117(2):595–607

Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (2008) WHO classification of tumours of haematopoietic and lymphoid tissues. IARC Press, Lyon

Tehranchi R, Woll PS, Anderson K, Buza-Vidas N, Mizukami T, Mead AJ, Astrand-Grundstrom I, Strombeck B, Horvat A, Ferry H, Dhanda RS, Hast R, Ryden T, Vyas P, Gohring G, Schlegelberger B, Johansson B, Hellstrom-Lindberg E, List A, Nilsson L, Jacobsen SE (2010) Persistent malignant stem cells in del(5q) myelodysplasia in remission. N Engl J Med 363(11):1025–1037

Van den Berghe H, Cassiman JJ, David G, Fryns JP, Michaux JL, Sokal G (1974) Distinct haematological disorder with deletion of long arm of no. 5 chromosome. Nature 251(5474):437–438

Venner CP, List AF, Neville TJ, Deeg HJ, Caceres G, Scott BL, Sokol L, Sung S, Karsan A (2010) Induction of micro RNA-143 and 145 in pre-treatment CD34+ cells from patients with myelodysplastic syndrome (MDS) after in vitro exposure to lenalidomide correlates with clinical response in patients harboring the del5q abnormality. Blood 116(21 Suppl):A123 Suppl

Wang J, Fernald AA, Anastasi J, Le Beau MM, Qian Z (2010) Haploinsufficiency of Apc leads to ineffective hematopoiesis. Blood 115(17):3481–3488

Wei S, Chen X, Rocha K, Epling-Burnette PK, Djeu JY, Liu Q, Byrd J, Sokol L, Lawrence N, Pireddu R, Dewald G, Williams A, Maciejewski J, List A (2009) A critical role for phosphatase haplodeficiency in the selective suppression of deletion 5q MDS by lenalidomide. Proc Natl Acad Sci U S A 106(31): 12974–12979

Ximeri M, Galanopoulos A, Klaus M, Parcharidou A, Giannikou K, Psyllaki M, Symeonidis A, Pappa V, Kartasis Z, Liapi D, Hatzimichael E, Kokoris S, Korkolopoulou P, Sambani C, Pontikoglou C, Papadaki HA (2010) Effect of lenalidomide therapy on hematopoiesis of patients with myelodysplastic syndrome associated with chromosome 5q deletion. Haematologica 95(3):406–414

Zhao N, Stoffel A, Wang PW, Eisenbart JD, Espinosa R 3rd, Larson RA, Le Beau MM (1997) 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 94(13):6948–6953

A Personalized Molecular Pathogenesis of MDS

6

Gustavo Rivero and Steven D. Gore

6.1 Introduction

Myelodysplastic syndromes (MDS) are heterogeneous clonal neoplasms characterized by progressive morphologic, functional, and proliferative defects of hematopoietic precursors and high propensity for progression to less differentiated myeloid leukemias (Galili et al. 2007; Mufti et al. 2008). It is estimated that MDS incidence is 3.4 cases per 100,000 annually (Rollison et al. 2008). In the United States, more than 10,000 people develop MDS annually (Galili et al. 2007). In 2002, the World Health Organization (WHO) proposed a revised version of the French-American-British (FAB) system to which cytogenetic abnormalities were incorporated; this system was further revised in 2008 (Vardiman et al. 2009). During 2008, the newly revised WHO classification system included MDS within the chronic myeloid malignancies category (Vardiman et al. 2009). In contrast to de novo AML, the indolent course leading to leukemic progression and evidence of significant dysplasia among differentiating cells suggest different pathogenesis. Bone marrows from patients with low-risk MDS biology display gene expression profiles characterized by deregulated pathways involving chemokine, apoptosis, and Wnt signaling (Fig. 6.1). In higher-risk MDS, deregulation of DNA damage response and checkpoint pathways is frequently identified (Theilgaard-Monch et al. 2011).

Most cases of MDS manifest in patients older than 60 years (Schanz et al. 2012). Microdissecting the contribution of age-associated bone marrow composition as predisposing factor for MDS will be of paramount importance. Decreased lymphogenesis and myeloid-biased lineage commitment which occur during aging (Sudo et al. 2000; Rossi et al. 2005) may present attractive pathogenic substrates for the development of MDS (Rossi et al. 2005; Ergen and Goodell 2010).

The presence of recurrent cytogenetic abnormalities and mutated cancer genes in MDS has generated substantial insight into MDS pathogenesis. Approximately 50 % of MDS patients have a normal karyotype using comprehensive metaphase cytogenetic analysis. In this normal cytogenetic subset, occult chromosomal abnormalities detected by more sensitive technologies, such as single-nucleotide polymorphism arrays, have greatly redefined the molecular complexity of this entity (Look 2005; Bejar et al. 2011a).

In recent years, the role of the cancer epigenome in the vulnerability for acquisition of

G. Rivero, MD
Department of Internal Medicine,
The Section of Hematology/Oncology,
Baylor College of Medicine,
1 Baylor Plaza,
Houston, TX 77030, USA

S.D. Gore, MD (✉)
Department of Pediatrics,
The Sidney Kimmel Comprehensive
Cancer Center at Johns Hopkins,
Division of Hematologic Malignancies,
Johns Hopkins School of Medicine,
1650 Orleans St., CRBI-288,
Baltimore, MD 21231, USA
e-mail: gorest@jhmi.edu

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_6,

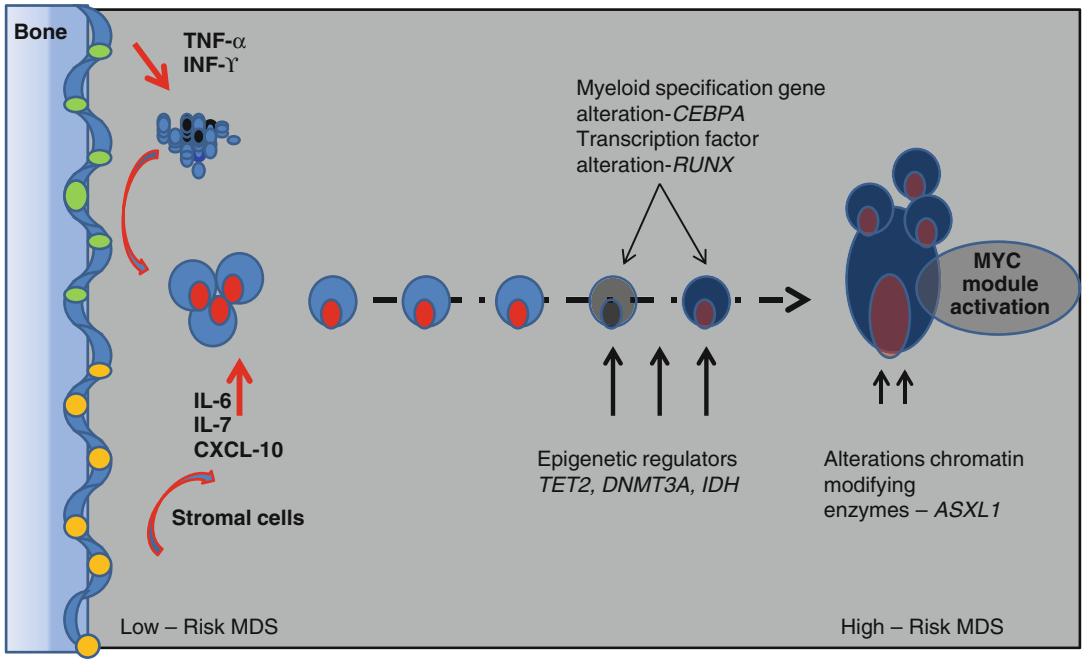

Fig. 6.1 MDS pathogenesis. An abnormal cytokine milieu with overexpressed INF-ϒ, TNF-α, IL-6, IL-7, and CXCL-10, along with immune dysregulation have been implicated in the pathogenesis of low-risk MDS (Theilgaard-Monch et al. 2011). In high-risk subgroups, impaired differentiation results from decreased expression of myeloid specification genes such as *CEBPA*. Disease evolution is characterized by increased proliferation from unrestrained oncogene expression

malignant phenotype, via inheritable somatically acquired alterations, has been recognized (Baylin 2008). Collectively, these have contributed to better understand the molecular backbone of this disease. *TET*2 (Langemeijer et al. 2009a, b), *JAK2* (Gurevich et al. 2011), *ASXL* (Mittelman et al. 2010), *CBL*, *FLT3*, *and TP53* mutations, along with *RPS14 and SPARC* gene abnormalities, constitute part of the biological pathways contributing to the development and maintenance of the disease (McDermott et al. 2011). These mutations are associated with specific disease phenotypes and could assist in tailoring targeted therapy (Fig. 6.2). In spite of the significant progress in the development of targeted therapy in the treatment of MDS, the diverse spectrum and heterogeneity of the disease warrant deeper understanding of the disease pathogenesis. In this chapter, we will emphasize and review the biological and clinical implication of cytogenetic and molecular abnormalities in MDS.

6.2 Karyotypic Abnormalities in MDS

6.2.1 Chromosome 5 Deletions

Within the vast heterogeneity of clinical manifestations of MDS, the functional consequences of chromosome 5q deletion have been the most studied phenotypic expressions of the disease. As a single chromosomal abnormality, interstitial deletion of the long arm of chromosome 5 occurs in about 10–15 % of MDS patients representing the most common cytogenetic abnormality (Sole et al. 2000). The WHO-defined distinctive 5q minus syndrome is characterized by macrocytic anemia, a normal or increased platelet count, erythroid hypoplasia with micromegakaryocytes in bone marrow, and low risk of progression to AML (Boultwood et al. 2010a; Van den Berghe et al. 1974, 1985; Nimer and Golde 1987). Prognostically, (del) 5q clusters within the better

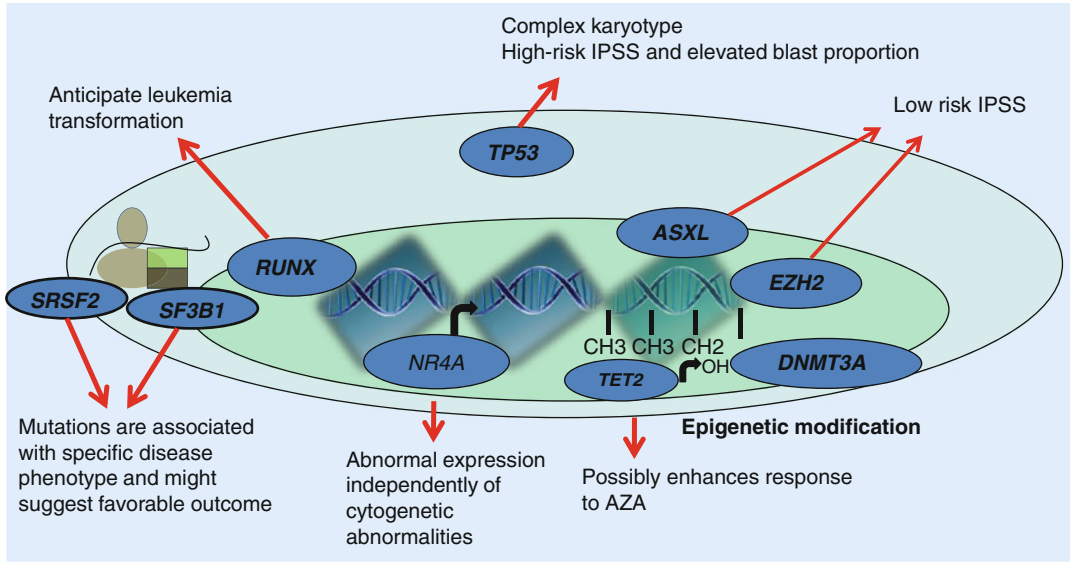

Fig. 6.2 Mutations in MDS genome. Increasing number of mutations are recognized by technologies such as whole-exome and genome sequencing. The repertoire of mutations includes genes that (1) modify chromatin (*ASXL*, *EZH2*, *DNMT3A*, *TET2*), (2) affect oncogene function (*RUNX*), (3) impair tumor suppressor genes (*TP53*), and (4) affect spliceosome machinery. Some specific mutations such as *TP53* and *RUNX* have important role in prognostication. Spliceosome mutations derived in disease-specific phenotype such as RARS. Further studies to clarify the role of chromatin modifying mutations after MDS-directed therapy are needed. To date, no single mutation has been found in all MDS types; however, *NR4A* abnormal expression could be seen independently of cytogenetic abnormalities

risk cytogenetic group and is associated with improved overall survival (OS) and AML-free survival of 57.8 and 202 months, respectively (Schanz et al. 2012). To date, two common deleted regions (CDR) have been described in MDS, suggesting the presence of critical genomic regions containing important tumor suppressor genes (TSG). In advanced MDS and AML, a proximal CDR located at 5q31, which includes genes such as *CDC25c*, *EGR1*, *SPARC*, and alpha catenin (*CTNNA1*), is commonly deleted (Padron et al. 2011a, b). Boultwood et al. described a distally deleted CDR, which is associated with the 5q− syndrome extending between 5q32 and 5q33 that contains *RPS14* (Boultwood et al. 2010a). *RPS14* is an essential 40S ribosomal protein, and allelic haploinsufficiency of this gene recapitulates phenotypic expression of 5q− syndrome in vitro (Boultwood et al. 2010a; Barlow et al. 2010). In addition to the two CDRs, several genes including numerous cytokine genes such as interleukins 3, 4, 5, 9, 13, and 17 β and granulocyte-monocyte colony-stimulating factor are located on 5q (Boultwood et al. 2010a; Ebert 2009).

The unique sensitivity of 5q (del) to the thalidomide analogue lenalidomide (LND) was tested in MDS-001, MDS-002, and the pivotal multi-institutional MDS-003 clinical trials, which led to the FDA approval of the medication (Pellagatti et al. 2007). In the MDS-003 trial, which enrolled low-risk MDS patients with (del) 5q, with or without additional cytogenetic abnormalities, LND induced transfusion independence and cytogenetic responses in 77 and 49 % of patients (List et al. 2006; Bejar et al. 2011b). Mechanistically, in 5q (del), ineffective erythropoiesis is associated with haploinsufficiency of *RPS14*, an important ribosomal processing gene. The functional consequence of deletion or mutations of genes encoding ribosomal proteins (RP) results in impairment of ribosomal assembly, subsequent nucleolar stress, and sequestration of

the human homolog of the E3 ubiquitin ligase mouse double minute 2 protein (MDM2), a critical inhibitor of p53. This results in p53 stabilization, apparently requisite for the disease phenotype (Lohrum et al. 2003; Fumagalli et al. 2009; Ebert et al. 2008). LND inhibits the activity of the PP2Acα phosphatase whose gene is located on 5q and whose gene dosage is thus reduced in patients with (del) 5q. This inhibition of the phosphatase leads to increased MDM2 phosphorylation and stabilization with subsequent inhibition of p53, presumably leading to phenotype reversal. In addition to LND inhibition of PP2Ac, this agent inhibits the G2/M checkpoint regulator Cdc25C (Komrokji and List 2011) and upregulates *SPARC*.

6.2.2 Chromosome 7 Deletions

Chromosome 7 aberrations represent about 20 % of cytogenetically abnormal MDS (Cordoba et al. 2012). Within this context, entire loss (monosomy 7), partial deletion of the long arm (7q del), and translocations involving chromosome 7 have been described (Haase et al. 2007). Less advanced disease with lower proportion of blasts may present in patients with isolated 7q del (Cordoba et al. 2012). Within this subset, isolated deletion of the long arm of chromosome 7 has been recently linked to MDS with hypoplastic feature (hMDS) (Jerez et al. 2012). At the genomic level, inactivating mutations in *EZH2* play an important pathogenic mechanism in establishing disease phenotype (Ernst et al. 2010). The impact of these abnormalities on MDS clinical outcome has facilitated implementation of risk-adapted interventions in high-risk biology group with intrinsic propensity for bad outcome. As recently proposed by Schanz et al., double chromosomic abnormalities (defined as two distinct clonal MDS-related acquired karyotypic abnormalities found within one cell) with and without −7/del (7q) were associated with OS of 5.8 months vs. 15.8 months, respectively (Schanz et al. 2012). As a sole abnormality, data suggests that 7q− could be prognostically better than monosomy 7 (Cordoba et al. 2012; Haase et al. 2007). Critical large gene losses may be associated with inferior outcome in MDS with monosomy 7; 7q- and der (1:7); however, overall and leukemia-free survival analysis, adjusted for evidence of excess blasts or multilineage dysplasia, showed no meaningful difference across the three chromosomal abnormalities (Hussain et al. 2012).

6.2.3 Chromosome 20

20q (del) is a recurrent MDS cytogenetic abnormality and occurs in about 1.7 % of patients (Schanz et al. 2012). Mild dysplasia and high incidence of thrombocytopenia are characteristic features of the disease (Mullier et al. 2012; Braun et al. 2011). Supporting this observation, refractory cytopenia with multilineage dysplasia has been reported in 11.3 % of 62 patients with 20q (del) MDS (Braun et al. 2011). Isolated 20 q (del) has been associated with low risk of progression to AML and better prognosis (Greenberg et al. 1997). A large international database analysis showed that 20q (del) as a single anomaly clustered with normal cytogenetic del (5q) and del (12p) in a good predictive survival subgroup with a median OS of 48.6 months (Schanz et al. 2012). Prognostically, this has been confirmed by (Greenberg et al. 2012) the new Revised International Prognostic Score System (IPSS-R) (Greenberg et al. 2012). The predicted survival achieved by 20q (del) was only surpassed by a very good subgroup with OS of 60.8 months that included loss of Y chromosome and del (11q) (Schanz et al. 2012; Greenberg et al. 2012).

6.2.4 Chromosome 17

Del (17p) has been primarily observed in therapy-related AML and MDS. Particularly in MDS, with an overall incidence of this abnormality of about 0.2–3.7 % (Schanz et al. 2012; Jasek et al. 2010), deletions involving the short arm of chromosome 17 may be associated with complex karyotype involving −5/5q del and/or −7/7q del (Jasek et al. 2010). 17p13.1 contains *TP 53*, which is thought to induce disease phenotype

characterized by high-risk biology and high propensity for leukemia transformation (Bejar et al. 2011b). In patient with complex karyotype harboring 5 q and 7 q cytogenetic abnormalities without del (17p), cryptic copy number-loss of heterozygosity (CN-LOH) 17p lesions can be found by SNP-A (Jasek et al. 2010).

6.2.5 Chromosome 3 Abnormalities

Inv 3 (q21q26) and t(3:3)(q21q26) have been described in de novo and therapy-associated MDS and AML. In MDS, these abnormalities represent about 1 % of recurrent cytogenetic aberrations (Secker-Walker et al. 1995). Given their particular disease phenotype characterized by significant megakaryocytic dysplasia and frequent occurrence of thrombocytosis, these abnormalities are clustered as a distinct entity in the WHO 2008 classification (Vardiman et al. 2009; Tefferi and Vardiman 2008). Most of the patients with de novo inv 3(q21q26) and t(3:3)(q21q26) MDS present with morphological feature consistent with refractory cytopenia multilineage dysplasia (Cui et al. 2011). The Medical Research Council of the United Kingdom (MRC) has recently described a very discernible unfavorable outcome in AML, in which 10-year OS was 3 % and relapse rate 89 % (Grimwade et al. 2010). At the genomic level, MDS and AML with 3q26 rearrangement overexpress the proto-oncogene *EVI1* (Morishita et al. 1988). Abnormal expression of *EVI1* in mice recapitulates MDS phenotype, and in vitro overexpression has been associated with impaired myeloid differentiation (Buonamici et al. 2004). Recruitment of transcriptional and epigenetic regulators by EVI1, encompassing CTBP, HDAC (histone deacetylase), and DNMT (DNA methyltransferase), suggests a functional role in regulation of gene expression (Chakraborty et al. 2001; Senyuk et al. 2011; Vinatzer et al. 2001). Of interest, high expression of EVI1 has been found to be an important pathogenic participant and adversely impact outcome in MDS with chromosomal abnormalities other than those involving the EVI1 locus (Russell et al. 1994; Groschel et al. 2010).

6.2.6 Trisomy 8

In MDS, the estimated incidence of trisomy 8 is about 4.2 % (Schanz et al. 2012). Most patients present with severe pancytopenia associated with hypoplastic bone marrow features (Maciejewski et al. 2002; Sloand et al. 2002). Some investigators suggest that this cytogenetically defined subset demonstrates particular sensitivity to immunosuppressive therapy. In vitro, expanded V_{β} CD8+ cytotoxic T cells repress cellular growth of aneuploid cells to a greater extent than diploid cells (Sloand et al. 2007). Severe cytopenias can result from cellular suppression of normal bystander cells mediated by a deregulated cytokine milieu as a result of immune-directed cytotoxicity (Sato et al. 1995; Selleri et al. 1995). Similar to other recurrent cytogenetic abnormalities, the predicted survival of trisomy 8 has been recently described by Schanz et al., with a reported OS of 23.8 months. Along with del (7q), +19, and i(17p), trisomy 8 clustered in an intermediate prognostic subgroup with a median OS and time to AML transformation of 26 months and 78 months (Schanz et al. 2012).

6.2.7 Trisomy 11

The association of trisomy 11 as a sole cytogenetic abnormality or in the context of noncomplex cytogenetics in MDS is rare (Schanz et al. 2012; Collado et al. 1999; Yamamoto et al. 1997). In a large cytogenetic patient cohort including 22,403 adults older than 18 years, trisomy 11 was found in 19 patients representing an incidence of 0.08 %. From these 19 patients, 14 and 5 patients were AML and MDS, respectively (Caramazza et al. 2010). As a sole abnormality, a higher proportion of patients with RAEB-2 and RAEB-1 are seen (Wang et al. 2010a), supporting the observation that this specific abnormality might contribute to high-risk biology at disease presentation. Despite the low incidence of trisomy 11, there is general agreement that this chromosomal abnormality confers poor prognosis in MDS (Schanz et al. 2012). Within this context, OS and time to AML transformation were 11 months for both variables (Schanz et al. 2012; Wang et al. 2010a).

6.2.8 Complex (Three or More Abnormalities)

The updated cytogenetic scoring system proposed in the IPSS-R recommends stratification of complex karyotype MDS into two groups: three abnormalities and greater than three abnormalities (Platzbecker et al. 2012). With an overall incidence of MDS with complex karyotype of about 10–18 % (Schanz et al. 2012; Sole et al. 2005), patients with three or more than three cytogenetic abnormalities account for 2.1 and 7 %, respectively (Schanz et al. 2012). The predictive value of increasing number of chromosomal abnormalities has been confirmed by analysis of large MDS cytogenetic databases (Schanz et al. 2012). In this study, OS was 15. 6 months exactly with three abnormalities vs. 5.7 months for those patients with more than three abnormalities (P=<0.01) (Schanz et al. 2012). In patients with complex karyotype, the presence of aberrations that included chromosomes 5 and/or 7 represented a subset with adverse prognosis within all cytogenetic categories (Schanz et al. 2012; Sole et al. 2005). Intriguingly, the interaction of different chromosomal abnormalities in patients with complex karyotype seems nonrandom and in line with a multistep sequential process in malignant transformation. This interaction has been recently proposed in patients who sequential acquire *TP53* mutations and complex cytogenetics with deletion of chromosome 5, 7, and 17 (Jasek et al. 2010).

6.2.9 Cytogenetic Evolution

MDS commonly progresses, especially in patients with high-risk biology (Bejar et al. 2011b). Cytogenetic evolution (CE), defined as the acquisition of new chromosomal abnormalities during the clinical course of the disease, occurs in about 30 % of patients with MDS (Malcovati et al. 2007; Bernasconi et al. 2010). At the time of AML transition, cytogenetic shifts could correlate with immunophenotypic variations and, more importantly, intrinsic biological characteristics of the malignancy. Chromosome instability (CIN) has been shown to play a role in malignant transformation and tumor progression in animal models (Heilig et al. 2010). Such changes frequently signal a change in course of the disease towards more aggressive behavior (Wang et al. 2010b). Rising numerical chromosome instability (CIN) predicts evolution of MDS to AML (Heilig et al. 2010). For example, numerical CIN level increased in one patient from 13.5 to 51.2 % within 1 month and was followed by progression to AML 4 months later (Heilig et al. 2010). Bernasconi et al. found that in patient with CE, the 2- and 5-year OS were 40 and 10 % vs. 93 and 70 % in patients without CE (Bernasconi et al. 2010). Unraveling the mechanisms responsible for genomic instability, which result in propensity for CE, would likely yield substantial insight into leukemogenesis-driving forces in MDS. Aberrant telomere biology with altered chromosome protective function (Mittelman et al. 2010) and unleashed retrotransposons may impact genomic architecture in MDS patients (Schneider et al. 2009; Estecio et al. 2010; Boissinot et al. 2004).

6.3 Molecular Alterations Associated with MDS

6.3.1 Ten-Eleven Translocation-2 (TET2)

As many as 30 % of MDS patients display a diverse spectrum of abnormalities of *TET2* gene involving deletions, insertions, and nonsense or missense mutations throughout the nine coding exons of the 150 kB gene resulting in truncated translation (Langemeijer et al. 2009a, b). Mutations of *TET2* are also seen in myeloproliferative neoplasm (MPN) (10 %), secondary AML (25 %), and chronic myelomonocytic leukemia (CMML, 40 %) (Langemeijer et al. 2009b; Mullighan 2009; Delhommeau et al. 2009; Bernard et al. 2009). *TET2* has the classical features of an α-ketoglutarate(KG)-Fe (II) dioxygenase and requires α-KG in order to mediate 5-methylcytosine hydroxylation (Tahiliani et al.

2009; Ito et al. 2010). *TET2* affects epigenetic modulation of gene expression by defective conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), which facilitates passive demethylation by excluding maintenance DNA methyltransferases (Tahiliani et al. 2009). Passive demethylation would result in gene reexpression in the functional *TET2* state but would maintain/increase the methylated state in environments with a *TET2* loss of function. Two recent studies have investigated the impact of hypomethylating dose of azanucleosides in the treatment of high-risk MDS with conflicting results regarding prediction of clinical response in *TET2*-mutated MDS context (Itzykson et al. 2011; Voso et al. 2011). Results from both studies are unique with intriguing results and several observations requiring future clarification. Although both studies evaluated high-risk MDS patients, an important distinction from the Italian study was the incorporation of CMML patients within the analysis and shorter MDS duration as compared to French study (median, 2 months vs. 13 months, respectively) (Itzykson et al. 2011; Voso et al. 2011). It has been suggested that superior survival has been observed in MDS-derived AML patient with longer MDS duration (Lubbert et al. 2012).

6.3.2 RUNX1

The *RUNX1* gene is located at chromosomal band 21q22; its protein product heterodimerizes with core-binding factor-β; the complex binds to specific DNA sequences and serves as a transcription factor (Ito 2004). *RUNX1* can function as an activator or repressor of target gene expression. At the genomic level, *RUNX1* acts as regulator of hematopoiesis by regulating hematopoietic growth factor genes such as GM-CSF, MPO, and IL3. Additionally, *RUNX1* regulates hematopoietic genes involved in surface receptor (*TCRA*, *TCRB*, *M-CSF*) and signaling molecules such as *CDKN1A*, *BLK*, *and BCL2* (Ito 2004; Michaud et al. 2003). Recently, *RUNX1* mutations have been found to be among the most frequent molecular alterations in MDS (Dicker et al. 2010). Overall, *RUNX1* mutations have been reported at high frequency of 24 % at MDS stage (Flach et al. 2011). Along with *TP53*, *EZH2*, ETV6, and *ASXL1* predicted poor outcome in patients with MDS independently from prognostic risk factors (Bejar et al. 2011b). *RUNX1* mutations may be acquired during leukemia progression from earlier MDS stages (Fig. 6.2). *RUNX1* mutations have been reported to be significantly increased in secondary leukemia evolving from myelodysplasia (Dicker et al. 2010).

6.3.3 DNMT3A

DNA methyltransferases (DNMTs) promote conversion of cytosine to 5-methylcytosine. Previously, *DNMT3A* and *DNMT3B* have been recognized to incorporate methyl groups to unmodified DNA, whereas *DNMT1* primarily functions as DNA methylation maintenance enzyme after cell division (Shah and Licht 2011). Recently, somatic *DNMT3A* mutations have been described in hematopoietic malignancies with an incidence of 22 % in de novo AML (Ley et al. 2010). *DNMT3A* mutation was found to be especially prevalent in AML patients with monocytic features (Yan et al. 2011). In MDS, Walter et al. described 13 *DNMT3A* somatic mutations in 12 patients (8 % of cases). Interestingly, *DNMT3A* mutation occurred across different risk categories according to IPSS and independent of FAB and karyotypic subtype (Walter et al. 2011). OS and event-free survival (EFS) were inferior in 12 patients with *DNMT3A* mutation vs. 138 patients without a mutation (long rank $P = 0.005$ for OS and 0.009 for EFS, respectively) (Walter et al. 2011). In the same study, 58 % of patients with and 28 % of patients without the mutation progressed to AML (Walter et al. 2011). In elderly patients with mutated *DNMT3A* AML, decitabine treatment was associated with an OS of 15.2 months vs. 11.0 months for patients with *DNMT3A* wild type. Despite the lack of statistically significant differences in this small study, further exploration of the relations between DNMT3a mutations and azanucleosides sensitivity seems warranted (Metzeler et al. 2012).

6.3.4 ASXL1

The human genome contains three ASX homologues. The role of *ASXL1* is not entirely well understood; however, there is evidence that it could be part of DNA and/or histone-modifying complexes (Boultwood et al. 2010b). In drosophila, *ASX* encodes enhancers of trithorax and polycomb (ETP) which regulate histone methylation at target loci to induce active or repressive chromatin configurations (Thol et al. 2011). Recently, mutation of *ASXL1* has been reported in 62/300 patients (21 %) with myelodysplastic syndrome (Boultwood et al. 2010b). Interestingly, and in accordance with the assumption of a continuum from dysplasia to AML, *ASX* mutation was found in 6, 31, and 25 % of patients with refractory anemia (RA), refractory anemia with excess of blast 1 or 2 (RAEB), and AML, respectively. Mutations were found, primarily, in patients with normal karyotype and del (7q/monosomy 7) (Boultwood et al. 2010b). In evaluating the impact of *ASXL1* exon 12 mutation in patients with MDS, Thol et al. found that beside being a frequent molecular aberration (20.8 % of patients), *ASXL1* mutation conferred an inferior outcome in term of overall survival (OS) when compared to those patients with wild-type *ASXL1* (HR, 1.74; 95 % CI, 1.08–2.82; $P=0.024$) (Thol et al. 2011). In the same study, patients with frameshift mutations had a shorter progression time to AML (Thol et al. 2011).

6.3.5 EZH2

In MDS and AML patients harboring 7q chromosomal abnormalities, SNP-A has allowed identification areas of acquired uniparental disomy (aUPD), and somatic frameshift, nonsense and missense *EZH2* mutations in patients with MDS, MDS/MPN, and MPN (Ernst et al. 2010). Overall, mutational frequency of about 6 % has been described in MDS, especially in patients with complex karyotypes (Nikoloski et al. 2010, 2012). In contrast to this, Bejar et al. found that *EZH2* could be also observed in patients with low-risk disease biology (Fig 6.2) (Bejar et al. 2011b). Along with *ASXL1*, *TET2*, SETD2, *JARID1C*, and *UTX*, *EZH2* represents a novel mutation with chromatin remodeling and epigenetic regulatory properties. *EZH2* is a catalytic component of PRC2 that trimethylates histone H3 at lysine 27(H3K27), leading to transcriptional suppression of its target genes (Tanaka et al. 2012). In mice, deletion of *Ezh2* severely compromised progression of AML by inducing differentiation of AML cells. Collectively, these findings suggest that *Ezh2* can modulate differentiation programs in leukemia stem cells (Tanaka et al. 2012).

6.3.6 TP53

The impact of *TP53* mutations on OS and overall risk of progression in MDS has been widely recognized (Jadersten et al. 2011; (Bejar et al. 2011b). *TP53* mutations are commonly found in therapy-related MDS, particularly in cases with chromosomal abnormalities involving 17p and complex karyotype (Side et al. 2004). In patients with favorable cytogenetics, such as those harboring del (5q), acquisition of TP53 mutation at early stage of the disease is associated with increased propensity for leukemia transformation (Fig. 6.2) (Bejar et al. 2012). In these cases, the 5-year cumulative incidence of leukemia development was 77 % vs. 24 % for mutated vs. unmutated *TP53* (Jadersten et al. 2011). Deregulation of *TP53* in hematological malignancies can occur as a result of mutation of missense and nonsense mutations in the *TP53* gene. Recently, Bejar et al. reported that, in MDS, 8/33 *TP53* mutant samples (24.2 %) had abnormalities of chromosome 17 and intermediate-2 or high risk according to IPSS (Bejar et al. 2011b). In regard to response to hypomethylating agents such as 5-azacytidine, similar response rates were observed within mutated and wild-type *TP53*; however, for those patients with the mutation, median duration of response was only 4 months (Kulasekararaj et al. 2013).

6.3.7 NR4A

NR4A subfamily of nuclear receptors such as NR4A1 and NR4A3 (also known as Nur77 and

Nor1) function as transcription factors for a variety of cellular processes (Mullican et al. 2007). Recently, it has been shown that they coregulate myeloid homeostasis. Coneely et al. showed a Nur 77 and Nor 1 gene dose-effect correlation in the expression of essential myeloid transcription factors such as *Egr1*, *JunB*, and *Plk2* (Ramirez-Herrick et al. 2011). Mice with reduced *NR4A* gene dosage developed MDS/MPN phenotype, while a complete germline abrogation of the genes leads to rapid development of AML (Ramirez-Herrick et al. 2011; Mullican et al. 2007). Similarly, in human genome-wide comparison studies, downregulation of *NR4A* genes occurs in about 40–80 % of MDS CD34+ progenitor cells (Pellagatti et al. 2006).

6.3.8 Spliceosome Mutations

Increasing evidence documents the involvement of mutations of RNA splicing machinery in MDS pathogenesis (Visconte et al. 2011). Within these, *SF3B1*, *SRSF2*, and *U2AF1* exhibit somatic and recurrent mutational patterns and parallel disease phenotype (Yoshida et al. 2011; Malcovati et al. 2011). Specifically, *SF3B1* has been found by high-throughput next-generation sequencing (HT-NGS) to be mutated in patients with RARS and high platelet count and refractory cytopenia with multilineage dysplasia with ≥15 % ring sideroblast in 19/23 (82.6 %) and 38/50 (76 %) patients, respectively (Visconte et al. 2011; Yoshida et al. 2011). In this specific MDS subset, *SF3B1*-mutated patients presented with higher white cell and platelet count. Additionally, *SF3B1* mutations in MDS with RS are associated with longer OS and leukemia-free survival. An apparent association of *SF3B1* mutations with good prognosis has been linked to differences in interpretation of WHO morphologic risk categorization and morphology (Patnaik et al. 2011) resulting in loss of prognostic significance when RARS and RCMD were analyzed separately (Patnaik et al. 2011). While larger studies are awaited, it seems interesting that cytogenetics allows discrimination of the combined RCMD and RCMD-RS (ring sideroblast >15 %) into subgroups with inferior clinical outcome (multivariate analysis, good vs. intermediate/poor karyotypes; $P = <0.001$) (Bacher et al. 2012). Thus, some of the survival benefit observed in *SF3B1*-mutated RCMD patients Sf3B1 may derive from a decreased incidence of high-risk karyotype in this group (Damm et al. 2012).

Recurrent *SRSF2* mutations occurred in a frequency of about 14.6 % within 233 studied MDS patients. The association with high-grade MDS and mutually exclusive patterns from *SF3B1* mutations, particularly described in low-risk subsets, might suggest late biological sequential acquisition resulting in predisposition to AML transformation (Mian et al. 2013). The association of *SRSF2* mutation with *RUNX1*, *ASXL1*, and *IDH-2* mutations has been previously established (Wu et al. 2012). Acquisition of mutations in this splicing factor gene has been associated with inferior OS (Wu et al. 2012).

6.4 Conclusion and Future Directions

Significant advances in the diagnosis and management of MDS have derived from deeper understanding of the cytogenetic and molecular milieu underpinning the pathogenesis of the disease. Several genetic alterations are of crucial importance for disease risk stratification and implementation of MDS-directed therapy. Increasing interest in the critical role of mutations in disease phenotype, survival outcome, and, possibly, responses to treatment is expected to increase as access to these analyses becomes generally available. To date, especially in low-risk MDS, mutations such as *EZH2* have identified biological subgroups with more aggressive behavior that could benefit from more intensive interventions. Promising understanding of MDS mechanism of disease initiation and potential pattern of treatment response have been achieved with new novel diagnostic methodologies such as single-nucleotide polymorphism array (SNP-A), DNA, and exome sequencing. This will allow a better characterization of the links between molecular pathogenesis and improved therapeutic strategies in the era of personalized medicine.

References

Bacher U, Kern W, Alpermann T, Schnittger S, Haferlach C, Haferlach T (2012) Prognoses of MDS subtypes RARS, RCMD and RCMD-RS are comparable but cytogenetics separates a subgroup with inferior clinical course. Leuk Res 36(7):826–831

Barlow JL, Drynan LF, Trim NL, Erber WN, Warren AJ, McKenzie AN (2010) New insights into 5q− syndrome as a ribosomopathy. Cell Cycle 9(21):4286–4293

Baylin SB (2008) Stem cells, cancer, and epigenetics. In: StemBook. Stephen B. Baylin, Cambridge

Bejar R, Levine R, Ebert BL (2011a) Unraveling the molecular pathophysiology of myelodysplastic syndromes. J Clin Oncol 29(5):504–515

Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G et al (2011b) Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 364(26):2496–2506

Bejar R, Stevenson KE, Caughey BA, Abdel-Wahab O, Steensma DP, Galili N et al (2012) Validation of a prognostic model and the impact of mutations in patients with lower-risk myelodysplastic syndromes. J Clin Oncol 30(27):3376–3382

Bernard OA, Delhommeau F, Fontenay M, Vainchenker W (2009) Mutations in TET2 in myeloid cancers. Med Sci (Paris) 25(10):785–788

Bernasconi P, Klersy C, Boni M, Cavigliano PM, Giardini I, Rocca B et al (2010) Does cytogenetic evolution have any prognostic relevance in myelodysplastic syndromes? A study on 153 patients from a single institution. Ann Hematol 89(6):545–551

Boissinot S, Entezam A, Young L, Munson PJ, Furano AV (2004) The insertional history of an active family of L1 retrotransposons in humans. Genome Res 14(7): 1221–1231

Boultwood J, Pellagatti A, McKenzie AN, Wainscoat JS (2010a) Advances in the 5q− syndrome. Blood 116(26):5803–5811

Boultwood J, Perry J, Pellagatti A, Fernandez-Mercado M, Fernandez-Santamaria C, Calasanz MJ et al (2010b) Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. Leukemia 24(5):1062–1065

Braun T, de Botton S, Taksin AL, Park S, Beyne-Rauzy O, Coiteux V et al (2011) Characteristics and outcome of myelodysplastic syndromes (MDS) with isolated 20q deletion: a report on 62 cases. Leuk Res 35(7):863–867

Buonamici S, Li D, Chi Y, Zhao R, Wang X, Brace L et al (2004) EVI1 induces myelodysplastic syndrome in mice. J Clin Invest 114(5):713–719

Caramazza D, Ketterling RP, Knudson RA, Hanson CA, Siragusa S, Pardanani A et al (2010) Trisomy 11: prevalence among 22,403 unique patient cytogenetic studies and clinical correlates. Leukemia 24(5):1092–1094

Chakraborty S, Senyuk V, Sitailo S, Chi Y, Nucifora G (2001) Interaction of EVI1 with cAMP-responsive element-binding protein-binding protein (CBP) and p300/CBP-associated factor (P/CAF) results in reversible acetylation of EVI1 and in co-localization in nuclear speckles. J Biol Chem 276(48):44936–44943

Collado R, Badia L, Garcia S, Sanchez H, Prieto F, Carbonell F (1999) Chromosome 11 abnormalities in myelodysplastic syndromes. Cancer Genet Cytogenet 114(1):58–61

Cordoba I, Gonzalez-Porras JR, Nomdedeu B, Luno E, de Paz R, Such E et al (2012) Better prognosis for patients with del(7q) than for patients with monosomy 7 in myelodysplastic syndrome. Cancer 118(1):127–133

Cui W, Sun J, Cotta CV, Medeiros LJ, Lin P (2011) Myelodysplastic syndrome with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) has a high risk for progression to acute myeloid leukemia. Am J Clin Pathol 136(2): 282–288

Damm F, Kosmider O, Gelsi-Boyer V, Renneville A, Carbuccia N, Hidalgo-Curtis C et al (2012) Mutations affecting mRNA splicing define distinct clinical phenotypes and correlate with patient outcome in myelodysplastic syndromes. Blood 119(14):3211–3218

Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Masse A et al (2009) Mutation in TET2 in myeloid cancers. N Engl J Med 360(22):2289–2301

Dicker F, Haferlach C, Sundermann J, Wendland N, Weiss T, Kern W et al (2010) Mutation analysis for RUNX1, MLL-PTD, FLT3-ITD, NPM1 and NRAS in 269 patients with MDS or secondary AML. Leukemia 24(8):1528–1532

Ebert BL (2009) Deletion 5q in myelodysplastic syndrome: a paradigm for the study of hemizygous deletions in cancer. Leukemia 23(7):1252–1256

Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N et al (2008) Identification of RPS14 as a 5q− syndrome gene by RNA interference screen. Nature 451(7176): 335–339

Ergen AV, Goodell MA (2010) Mechanisms of hematopoietic stem cell aging. Exp Gerontol 45(4):286–290

Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV et al (2010) Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet 42(8):722–726

Estecio MR, Gallegos J, Vallot C, Castoro RJ, Chung W, Maegawa S et al (2010) Genome architecture marked by retrotransposons modulates predisposition to DNA methylation in cancer. Genome Res 20(10):1369–1382

Flach J, Dicker F, Schnittger S, Schindela S, Kohlmann A, Haferlach T et al (2011) An accumulation of cytogenetic and molecular genetic events characterizes the progression from MDS to secondary AML: an analysis of 38 paired samples analyzed by cytogenetics, molecular mutation analysis and SNP microarray profiling. Leukemia 25(4):713–718

Fumagalli S, Di Cara A, Neb-Gulati A, Natt F, Schwemberger S, Hall J et al (2009) Absence of nucleolar disruption after impairment of 40S ribosome biogenesis reveals an rpL11-translation-dependent mechanism of p53 induction. Nat Cell Biol 11(4): 501–508

Galili N, Cerny J, Raza A (2007) Current treatment options: impact of cytogenetics on the course of myelodysplasia. Curr Treat Options Oncol 8(2):117–128

Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G et al (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89(6):2079–2088

Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Sole F et al (2012) Revised international prognostic scoring system for myelodysplastic syndromes. Blood 120(12):2454–2465

Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH et al (2010) Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood 116(3):354–365

Groschel S, Lugthart S, Schlenk RF, Valk PJ, Eiwen K, Goudswaard C et al (2010) High EVI1 expression predicts outcome in younger adult patients with acute myeloid leukemia and is associated with distinct cytogenetic abnormalities. J Clin Oncol 28(12):2101–2107

Gurevich I, Luthra R, Konoplev SN, Yin CC, Medeiros LJ, Lin P (2011) Refractory anemia with ring sideroblasts associated with marked thrombocytosis: a mixed group exhibiting a spectrum of morphologic findings. Am J Clin Pathol 135(3):398–403

Haase D, Germing U, Schanz J, Pfeilstocker M, Nosslinger T, Hildebrandt B et al (2007) New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. Blood 110(13):4385–4395

Heilig CE, Loffler H, Mahlknecht U, Janssen JW, Ho AD, Jauch A et al (2010) Chromosomal instability correlates with poor outcome in patients with myelodysplastic syndromes irrespectively of the cytogenetic risk group. J Cell Mol Med 14(4):895–902

Hussain FT, Nguyen EP, Raza S, Knudson R, Pardanani A, Hanson CA et al (2012) Sole abnormalities of chromosome 7 in myeloid malignancies: spectrum, histopathologic correlates, and prognostic implications. Am J Hematol 87(7):684–686

Ito Y (2004) Oncogenic potential of the RUNX gene family: 'overview'. Oncogene 23(24):4198–4208

Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y (2010) Role of tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 466(7310):1129–1133

Itzykson R, Kosmider O, Cluzeau T, Mansat-De Mas V, Dreyfus F, Beyne-Rauzy O et al (2011) Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia 25(7):1147–1152

Jadersten M, Saft L, Smith A, Kulasekararaj A, Pomplun S, Gohring G et al (2011) TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. J Clin Oncol 29(15):1971–1979

Jasek M, Gondek LP, Bejanyan N, Tiu R, Huh J, Theil KS et al (2010) TP53 mutations in myeloid malignancies are either homozygous or hemizygous due to copy number-neutral loss of heterozygosity or deletion of 17p. Leukemia 24(1):216–219

Jerez A, Sugimoto Y, Makishima H, Verma A, Jankowska AM, Przychodzen B et al (2012) Loss of heterozygosity in 7q myeloid disorders: clinical associations and genomic pathogenesis. Blood 119(25):6109–6117

Komrokji RS, List AF (2011) Role of lenalidomide in the treatment of myelodysplastic syndromes. Semin Oncol 38(5):648–657

Kulasekararaj AG, Smith AE, Mian SA, Mohamedali AM, Krishnamurthy P, Lea NC et al (2013) TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. Br J Haematol 160(5):660–672

Langemeijer SM, Aslanyan MG, Jansen JH (2009a) TET proteins in malignant hematopoiesis. Cell Cycle 8(24):4044–4048

Langemeijer SM, Kuiper RP, Berends M, Knops R, Aslanyan MG, Massop M et al (2009b) Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet 41(7):838–842

Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE et al (2010) DNMT3A mutations in acute myeloid leukemia. N Engl J Med 363(25):2424–2433

List A, Dewald G, Bennett J, Giagounidis A, Raza A, Feldman E et al (2006) Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 355(14):1456–1465

Lohrum MA, Ludwig RL, Kubbutat MH, Hanlon M, Vousden KH (2003) Regulation of HDM2 activity by the ribosomal protein L11. Cancer Cell 3(6):577–587

Look AT (2005) Molecular pathogenesis of MDS. Hematology Am Soc Hematol Educ Program 156–160

Lubbert M, Ruter BH, Claus R, Schmoor C, Schmid M, Germing U et al (2012) A multicenter phase II trial of decitabine as first-line treatment for older patients with acute myeloid leukemia judged unfit for induction chemotherapy. Haematologica 97(3):393–401

Maciejewski JP, Risitano A, Sloand EM, Nunez O, Young NS (2002) Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia. Blood 99(9):3129–3135

Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R et al (2007) Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 25(23):3503–3510

Malcovati L, Papaemmanuil E, Bowen DT, Boultwood J, Della Porta MG, Pascutto C et al (2011) Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood 118(24):6239–6246

McDermott U, Downing JR, Stratton MR (2011) Genomics and the continuum of cancer care. N Engl J Med 364(4):340–350

Metzeler KH, Walker A, Geyer S, Garzon R, Klisovic RB, Bloomfield CD et al (2012) DNMT3A mutations and response to the hypomethylating agent decitabine in acute myeloid leukemia. Leukemia 26(5):1106–1107

Mian SA, Smith AE, Kulasekararaj AG, Kizilors A, Mohamedali AM, Lea NC, et al (2013) Spliceosome mutations exhibit specific associations with epigenetic

modifiers and proto-oncogenes mutated in myelodysplastic syndrome. Haematologica [Epub]
Michaud J, Scott HS, Escher R (2003) AML1 interconnected pathways of leukemogenesis. Cancer Invest 21(1):105–136
Mittelman M, Oster HS, Hoffman M, Neumann D (2010) The lower risk MDS patient at risk of rapid progression. Leuk Res 34(12):1551–1555
Morishita K, Parker DS, Mucenski ML, Jenkins NA, Copeland NG, Ihle JN (1988) Retroviral activation of a novel gene encoding a zinc finger protein in IL-3-dependent myeloid leukemia cell lines. Cell 54(6):831–840
Mufti GJ, Bennett JM, Goasguen J, Bain BJ, Baumann I, Brunning R et al (2008) Diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts. Haematologica 93(11):1712–1717
Mullican SE, Zhang S, Konopleva M, Ruvolo V, Andreeff M, Milbrandt J et al (2007) Abrogation of nuclear receptors Nr4a3 and Nr4a1 leads to development of acute myeloid leukemia. Nat Med 13(6):730–735
Mullier F, Daliphard S, Garand R, Dekeyser M, Cornet Y, Luquet I et al (2012) Morphology, cytogenetics, and survival in myelodysplasia with del(20q) or ider(20q): a multicenter study. Ann Hematol 91(2):203–213
Mullighan CG (2009) TET2 mutations in myelodysplasia and myeloid malignancies. Nat Genet 41(7):766–767
Nikoloski G, Langemeijer SM, Kuiper RP, Knops R, Massop M, Tonnissen ER et al (2010) Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet 42(8):665–667
Nikoloski G, van der Reijden BA, Jansen JH (2012) Mutations in epigenetic regulators in myelodysplastic syndromes. Int J Hematol 95(1):8–16
Nimer SD, Golde DW (1987) The 5q− abnormality. Blood 70(6):1705–1712
Padron E, Komrokji R, List AF (2011a) Biology and treatment of the 5q− syndrome. Expert Rev Hematol 4(1):61–69
Padron E, Komrokji R, List AF (2011b) The 5q− syndrome: biology and treatment. Curr Treat Options Oncol 12(4):354–368
Patnaik MM, Lasho TL, Hodnefield JM, Knudson RA, Ketterling RP, Garcia-Manero G et al (2011) SF3B1 mutations are prevalent in myelodysplastic syndromes with ring sideroblasts but do not hold independent prognostic value. Blood 119(2):569–572
Pellagatti A, Cazzola M, Giagounidis AA, Malcovati L, Porta MG, Killick S et al (2006) Gene expression profiles of CD34+ cells in myelodysplastic syndromes: involvement of interferon-stimulated genes and correlation to FAB subtype and karyotype. Blood 108(1):337–345
Pellagatti A, Jadersten M, Forsblom AM, Cattan H, Christensson B, Emanuelsson EK et al (2007) Lenalidomide inhibits the malignant clone and upregulates the SPARC gene mapping to the commonly deleted region in 5q− syndrome patients. Proc Natl Acad Sci U S A 104(27):11406–11411
Platzbecker U, Santini V, Mufti GJ, Haferlach C, Maciejewski JP, Park S et al (2012) Update on developments in the diagnosis and prognostic evaluation of patients with myelodysplastic syndromes (MDS): consensus statements and report from an expert workshop. Leuk Res 36(3):264–270
Ramirez-Herrick AM, Mullican SE, Sheehan AM, Conneely OM (2011) Reduced NR4A gene dosage leads to mixed myelodysplastic/myeloproliferative neoplasms in mice. Blood 117(9):2681–2690
Rollison DE, Howlader N, Smith MT, Strom SS, Merritt WD, Ries LA et al (2008) Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001–2004, using data from the NAACCR and SEER programs. Blood 112(1):45–52
Rossi DJ, Bryder D, Zahn JM, Ahlenius H, Sonu R, Wagers AJ et al (2005) Cell intrinsic alterations underlie hematopoietic stem cell aging. Proc Natl Acad Sci U S A 102(26):9194–9199
Russell M, List A, Greenberg P, Woodward S, Glinsmann B, Parganas E et al (1994) Expression of EVI1 in myelodysplastic syndromes and other hematologic malignancies without 3q26 translocations. Blood 84(4):1243–1248
Sato T, Selleri C, Young NS, Maciejewski JP (1995) Hematopoietic inhibition by interferon-gamma is partially mediated through interferon regulatory factor-1. Blood 86(9):3373–3380
Schanz J, Tuchler H, Sole F, Mallo M, Luno E, Cervera J et al (2012) 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 30(8):820–829
Schneider AM, Duffield AS, Symer DE, Burns KH (2009) Roles of retrotransposons in benign and malignant hematologic disease. Cellscience 6(2):121–145
Secker-Walker LM, Mehta A, Bain B (1995) Abnormalities of 3q21 and 3q26 in myeloid malignancy: a United Kingdom Cancer Cytogenetic Group Study. Br J Haematol 91(2):490–501
Selleri C, Sato T, Anderson S, Young NS, Maciejewski JP (1995) Interferon-gamma and tumor necrosis factor-alpha suppress both early and late stages of hematopoiesis and induce programmed cell death. J Cell Physiol 165(3):538–546
Senyuk V, Premanand K, Xu P, Qian Z, Nucifora G (2011) The oncoprotein EVI1 and the DNA methyltransferase Dnmt3 co-operate in binding and de novo methylation of target DNA. PLoS One 6(6):e20793
Shah MY, Licht JD (2011) DNMT3A mutations in acute myeloid leukemia. Nat Genet 43(4):289–290
Side LE, Curtiss NP, Teel K, Kratz C, Wang PW, Larson RA et al (2004) RAS, FLT3, and TP53 mutations in therapy-related myeloid malignancies with abnormalities of chromosomes 5 and 7. Genes Chromosomes Cancer 39(3):217–223

Sloand EM, Kim S, Fuhrer M, Risitano AM, Nakamura R, Maciejewski JP et al (2002) Fas-mediated apoptosis is important in regulating cell replication and death in trisomy 8 hematopoietic cells but not in cells with other cytogenetic abnormalities. Blood 100(13):4427–4432

Sloand EM, Pfannes L, Chen G, Shah S, Solomou EE, Barrett J et al (2007) CD34 cells from patients with trisomy 8 myelodysplastic syndrome (MDS) express early apoptotic markers but avoid programmed cell death by up-regulation of antiapoptotic proteins. Blood 109(6):2399–2405

Sole F, Espinet B, Sanz GF, Cervera J, Calasanz MJ, Luno E et al (2000) Incidence, characterization and prognostic significance of chromosomal abnormalities in 640 patients with primary myelodysplastic syndromes. Grupo cooperativo espanol de citogenetica hematologica. Br J Haematol 108(2):346–356

Sole F, Luno E, Sanzo C, Espinet B, Sanz GF, Cervera J et al (2005) Identification of novel cytogenetic markers with prognostic significance in a series of 968 patients with primary myelodysplastic syndromes. Haematologica 90(9):1168–1178

Sudo K, Ema H, Morita Y, Nakauchi H (2000) Age-associated characteristics of murine hematopoietic stem cells. J Exp Med 192(9):1273–1280

Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y et al (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 324(5929):930–935

Tanaka S, Miyagi S, Sashida G, Chiba T, Yuan J, Mochizuki-Kashio M et al (2012) Ezh2 augments leukemogenecity by reinforcing differentiation blockage in acute myeloid leukemia. Blood 120(5):1107–1117

Tefferi A, Vardiman JW (2008) Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. Leukemia 22(1):14–22

Theilgaard-Monch K, Boultwood J, Ferrari S, Giannopoulos K, Hernandez-Rivas JM, Kohlmann A et al (2011) Gene expression profiling in MDS and AML: potential and future avenues. Leukemia 25(6):909–920

Thol F, Friesen I, Damm F, Yun H, Weissinger EM, Krauter J et al (2011) Prognostic significance of ASXL1 mutations in patients with myelodysplastic syndromes. J Clin Oncol 29(18):2499–2506

Van den Berghe H, Cassiman JJ, David G, Fryns JP, Michaux JL, Sokal G (1974) Distinct haematological disorder with deletion of long arm of no. 5 chromosome. Nature 251(5474):437–438

Van den Berghe H, Vermaelen K, Mecucci C, Barbieri D, Tricot G (1985) The 5q-anomaly. Cancer Genet Cytogenet 17(3):189–255

Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A et al (2009) The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 114(5):937–951

Vinatzer U, Taplick J, Seiser C, Fonatsch C, Wieser R (2001) The leukaemia-associated transcription factors EVI-1 and MDS1/EVI1 repress transcription and interact with histone deacetylase. Br J Haematol 114(3):566–573

Visconte V, Makishima H, Jankowska A, Szpurka H, Traina F, Jerez A et al (2011) SF3B1, a splicing factor is frequently mutated in refractory anemia with ring sideroblasts. Leukemia 26(3):542–545

Voso MT, Fabiani E, Piciocchi A, Matteucci C, Brandimarte L, Finelli C et al (2011) Role of BCL2L10 methylation and TET2 mutations in higher risk myelodysplastic syndromes treated with 5-azacytidine. Leukemia 25(12):1910–1913

Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan M et al (2011) Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. Leukemia 25(7):1153–1158

Wang SA, Jabbar K, Lu G, Chen SS, Galili N, Vega F et al (2010a) Trisomy 11 in myelodysplastic syndromes defines a unique group of disease with aggressive clinicopathologic features. Leukemia 24(4):740–747

Wang H, Wang XQ, Xu XP, Lin GW (2010b) Cytogenetic evolution correlates with poor prognosis in myelodysplastic syndrome. Cancer Genet Cytogenet 196(2):159–166

Wu SJ, Kuo YY, Hou HA, Li LY, Tseng MH, Huang CF et al (2012) The clinical implication of SRSF2 mutation in patients with myelodysplastic syndrome and its stability during disease evolution. Blood 120(15):3106–3111

Yamamoto K, Hamaguchi H, Nagata K, Kobayashi M, Taniwaki M (1997) Tandem duplication of the MLL gene in myelodysplastic syndrome-derived overt leukemia with trisomy 11. Am J Hematol 55(1):41–45

Yan XJ, Xu J, Gu ZH, Pan CM, Lu G, Shen Y et al (2011) Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet 43(4):309–315

Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R et al (2011) Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 478(7367):64–69

Myelodysplastic/Myeloproliferative Neoplasms

7

Manojkumar Bupathi, Ramon V. Tiu, and Jaroslaw P. Maciejewski

Myeloid malignancies exemplified by acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPNs) are all characterized by abnormal proliferation of stem cells. AML is characterized by proliferation of myeloid blasts that ultimately perturb normal bone marrow (BM) function and suppress hematopoiesis. The hallmarks of MDS are cytopenias (anemia, leukopenia, or thrombocytopenia), impaired differentiation in one or more of myeloid cell lines, and ineffective hematopoiesis (Tiu et al. 2011a). MPNs manifest with proliferation of one or more cell lines in the BM with accompanying BM fibrosis and extramedullary hematopoiesis. When features of both MDS and MPN coexist in the same patient, the disease is called MDS/MPN overlap neoplasms. The recognition that some MDS patients have overlapping MPN features led to the coining of the term MDS/MPN overlap. This group was first described in 1997 at the clinical advisory meeting of the World Health Organization (WHO) (Harris et al. 1999) and later adapted in the 2001 WHO classification (Jaffe et al. 2001). As in the case of MDS and MPNs, MDS/MPN patients are also at risk for AML evolution. Within this overlapping class, four different disease entities were classified: Juvenile myelomonocytic leukemia (JMML), chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia (*BCR-ABL1* negative) (aCML), and MDS/MPN-unclassifiable (MDS/MPN-U), which also included the provisional disease category, refractory anemia with ring sideroblast associated with marked thrombocytosis (RARS-T). Of note, each of these disease entities has a defined natural history, influenced by a variety of factors such as BM blast counts, presence of concomitant diseases (e.g., systemic mastocytosis with associated clonal hematologic non-mast cell lineage disease [SM-AHNMD]), and different cytogenetic and epigenetic/molecular profile which may explain the clinicopathologic diversity of these diseases.

M. Bupathi
Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, 9500 Euclid Avenue R40, Cleveland, OH 44195, USA

R.V. Tiu
Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, 9500 Euclid Avenue R40, Cleveland, OH 44195, USA

Department of Hematologic Oncology and Blood Disorders, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

J.P. Maciejewski, MD, PhD (✉)
Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, 9500 Euclid Avenue R40, Cleveland, OH 44195, USA

Department of Hematologic Oncology and Blood Disorders, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

Experimental Hematology and Hematopoiesis Section, Taussig Cancer Center/R40, 9500 Euclid Ave., Cleveland, OH 44195, USA
e-mail: maciejj@ccf.org

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_7,

7.1 Juvenile Myelomonocytic Leukemia

Juvenile myelomonocytic leukemia (JMML) is a myeloid malignancy of the pluripotent stem cells that primarily occurs in childhood (Busque et al. 1995; Cooper et al. 2000). It was originally described by French researchers in the late 1960s. After substantial considerations, the term JMML was adopted by the international JMML association (Hasle et al. 1999).

7.1.1 Epidemiology

JMML is an aggressive disease characterized by the proliferation of myelomonocytic cells and has an incidence of about 1.2 cases per million accounting for about 1–2 % of all childhood leukemias per year in the United States (Chan et al. 2009). Most cases are diagnosed between the ages of 1 and 2 months after birth till 6 years old with a median age of 2 years (Emanuel 2008).

7.1.2 Clinical Manifestation

JMML is a rare disease with patients typically presenting with fevers, hepatosplenomegaly, failure to thrive, irritability, dry cough, tachypnea, lymphadenopathy (LAP), skin rash, pallor, and elevated white blood cell counts (WBC) (Loh 2010). Leukemic infiltration can cause tonsillar enlargement, hepatosplenomegaly, diarrhea, skin rash, and LAP. Dermatologic findings like cafe au lait spots can be seen in association with neurofibromatosis type 1 (NF1) disease (Raygada et al. 2010).

Monocytosis in childhood when accompanied by these associated symptoms can be a diagnostic challenge since they are nonspecific and could indicate an underlying infectious process like those seen in patients infected with Epstein-Barr virus, cytomegalovirus, human herpesvirus-6, histoplasmosis, and toxoplasmosis (Yoshida et al. 2012).

7.1.3 Histomorphology, Cytogenetics, and Molecular Profile

As JMML is the childhood counterpart of chronic myelomonocytic leukemia (CMML), monocytosis and even presence of considerable numbers of macrophages is a typical feature of the disease. Basophilia and eosinophilia are infrequently seen. Anemia is common and usually normocytic although macrocytic forms (particularly in patients with monosomy 7) and microcytic ones (as observed in patients with concurrent iron deficiency anemia and/or hemoglobinopathies) may be detected. Thrombocytopenia is also frequent. There is no pathognomonic cytogenetic abnormality in JMML, although monosomy 7 and abnormalities in chromosome 8 have been identified. The diagnostic criteria for JMML based on the 2008 WHO classification include persistent peripheral blood (PB) monocytosis in the setting of PB and BM blasts not reaching or exceeding 20 %, absence of chromosomal findings diagnostic of *BCR-ABL*-positive chronic myeloid leukemia (CML) in a patient with additional hematologic and pathogenic features like elevated hemoglobin F, PB immature granulocytes, leukocytosis, clonal cytogenetic abnormality, and increased GM-CSF sensitivity of myeloid progenitors in vitro (Table 7.1). The hallmark of JMML is the hypersensitivity of the myeloid cells to granulocyte-macrophage colony-stimulating factor (GM-CSF) with normal response to IL-3 and G-CSF. This hypersensitivity leads to activation of the RAS-mediated signaling pathway and eventually MAP kinase (MAPK) (Emanuel 2008). Children with certain congenital disorders are at a higher risk of developing JMML, for example, NF1 and Noonan syndrome (NS) (Loh 2010). NS is characterized by facial dysmorphism, short stature, webbed neck, and cardiac abnormalities. Children with this disease also have a self-resolving myeloproliferative disorder in infancy which is similar to JMML. Both NF and NS are diseases that occur due to a mutation in a gene, called *neurofibromin* or *neurofibromatosis-related protein* (*NF1)* which is involved in the RAS pathway (Emanuel 2008).

Table 7.1 Diagnostic features for CMML, JMML, aCML, and MDS/MPN-U

Disease group	Criteria
CMML	Peripheral blood monocyte counts of $>1 \times 10^9$/L Absence of the Philadelphia chromosome or *BCR-ABL1* Absence of *PDGFRA/PDGFRB* rearrangements <20 % peripheral and bone marrow of blasts or equivalents cells (myeloblasts, monoblasts, and promonocytes) Myeloid dysplasia in one or more lineages
JMML	Peripheral blood monocyte counts of $>1 \times 10^9$ <20 % blasts and/or promonocytes in the peripheral blood and nucleated bone marrow cells Negative for Philadelphia chromosome or *BCR-ABL1* In conjunction with two or more of the following criteria: Increased Hemoglobin F levels for age Leukocytosis ($>10 \times 10^9$/L) Presence of peripheral blood circulating immature granulocytes Presence of chromosomal abnormality (including monosomy 7) In vitro sensitivity of myeloid precursors to GM-CSF
aCML	Persistent WBCs $>13 \times 10^9$/L Negative for Philadelphia chromosome translocation or *BCR-ABL1* Absence of *PDGFRA/PDGFRB* rearrangement Hypercellular bone marrow with proliferation in granulocytic precursors and granulocytic dysplasia Monocytes <10 % Peripheral and bone marrow blasts <20 % Basophil counts <2 % of WBCs
MDS/MPN-U	Peripheral and bone marrow blasts <20 % Findings consistent with clinical, para-clinical, and morphological features of MDS subtypes Findings consistent with clinical, para-clinical, and morphological features of MPN Negativity for Philadelphia chromosome translocation or *BCR-ABL1* Absence of *PDGFRA/PDGFRB* rearrangement Absence of chromosomal abnormalities (del(5q), t(3;3)(q21;q26), inv(3)(q21q26) No previous history of treating with cytotoxic and/or growth factor modalities

CMML chronic myelomonocytic leukemia, *JMML* juvenile myelomonocytic leukemia, *aCML* atypical chronic myeloid leukemia, *MDS/MPN-U* myelodysplastic/myeloproliferative neoplasms – unclassifiable, *WBCs* white blood cells, *PDGFRA/PDGFRB* platelet-derived growth factor receptor A and B, *GM-CSF* granulocyte-macrophage colony-stimulating factor

Fundamentally, occurrence of any mutation in this gene perturbs the function of the protein leading to increased activity of the RAS pathway. About 15 % of patients with JMML have a mutation in *NF1*, and about 30 % of these patients can develop leukemia (Loh 2010, 2011; Park et al. 2012). Of note, 70–85 % of cases of JMML have mutation of genes in the RAS pathway. *PTPN11* is considered the second most important gene involving in JMML pathophysiology and mostly detected in NS. *PTPN11* encodes a tyrosine phosphatase called SHP2 which modulates the RAS/MAPK pathway (Loh 2010, 2011). Mutations in *PTPN11* cause overstimulation of this pathway leading to the development of distinct clinicopathologic features similar to what is seen in patients with MPNs (Sugimoto et al. 2010). Overall, 35 % of patients with JMML carry *PTPN11* as a somatic mutation. *CBL,* a ubiquitin ligase involved in the regulation of phosphotyrosine kinase (TRK)-mediated signals, is also mutated in JMML leading to an increase activity of the RAS pathway (Flotho et al. 1999). *ASXL1*, a chromatin binding protein, is another gene reported to play a role in the pathophysiology of JMML.

7.1.4 Treatment

7.1.4.1 Hematopoietic Cell Transplant

Similar to other myeloid malignancies, allogeneic hematopoietic cell transplant (HCT) is a potential curative treatment for patients with JMML. However, there is controversy regarding how soon patients should be transplanted, and despite best efforts, 30–40 % of these patients will continue to relapse usually within the first year after transplantation (Locatelli et al. 2005). Attempts in using donor lymphocyte infusions to salvage relapse cases after transplant have also not been successful (Yoshimi et al. 2005). A large study involving 100 patients with JMML was conducted by the European Working Group on Childhood MDS (EWOG/MDS) and European Group for Blood and Marrow Transplantation. The patients received a combination of busulfan, cyclophosphamide, and melphalan as conditioning regimen and primarily received bone marrow as the source of stem cells ($N=74$). The median age of the patients at diagnosis is 1.4 years, and the median age at the time of transplant is 2.5 years. The vast majority have normal karyotype ($N=66$) while abnormal karyotypes were seen in 33 patients (−7 [$N=20$], +8 [$N=9$], other abnormalities [$N=4$]). It showed that 50 % of patients with JMML treated with allogeneic HCT are cured. The 5-year event-free survival (EFS) rate is 55 % for HLA-identical donors and 49 % for matched unrelated donors. Disease relapse remains common usually within a median of 6 months after transplantation and predicted by older age (age >4 years old). Chemotherapeutic approaches particularly in those that entail giving high doses of chemotherapy pre-HCT to reduce relapse rates have not been effective. However, second transplantation can be successful in some patients (Locatelli et al. 2005). Approaches that can help maximize graft versus leukemia (GVL) effect to help decrease the incidence of relapse are currently being evaluated. Approaches taken include immediate reduction in immunosuppressants to maximize GVL effects.

7.1.4.2 Chemotherapy/Pharmacotherapy

The primary goal of therapy for JMML patients especially for those who are symptomatic and have high-risk disease features is to undergo allogeneic HCT. Asymptomatic patients are observed until a suitable donor becomes available, while those with symptoms and have high-risk disease features are generally treated with different chemotherapeutic regimens including low-dose cytarabine given at 40 mg/m^2 IV daily for 5 days and a combination of 6-mercaptopurine with or without cis-retinoic acid. Sometimes, high-dose cytarabine with fludarabine can be considered (Loh 2011). The RAS pathway is integral in the pathogenesis of JMML. Attempts to target this pathway have also been made. This is primarily done through the use of farnesyl transferase inhibitors like tipifarnib which have resulted in some clinical responses primarily in the reduction of WBC counts and organomegaly but did no results in improvement of event-free survival (EFS) (Castleberry et al. 2011).

7.1.4.3 Prognostic Parameters

Several studies have shown that different factors can contribute to the outcome of patients with JMML. "Self-resolving" JMML has been described as a phenomenon in the literature (Hasle et al. 2004). Specifically, Matusda et al. were able to show three children with *N/K RAS* mutations which had a mild clinical course with spontaneous disease regression (Matsuda et al. 2010). So far, there is no correlation between presence of *N/K RAS* mutations and longer overall survival (OS) in the absence of HSCT. There are specific parameters associated with poor prognosis and better outcome after HSCT. Such parameters are older age at diagnosis (>2 years), increased hemoglobin F for age, male sex, presence of hepatosplenomegaly, and/or platelet count $<33\times10^9$/ L at presentation (Passmore et al. 1995). Locatelli et al. showed that presence of these factors consistently correlated with lower EFS as well as OS after HCT (Locatelli et al. 2005).

Moreover, some reports showed that mutations of certain genes may also correlate with OS. However, this remains controversial. Initially, Locatelli demonstrated that mutational status of *NF1*, *PTPN11*, and/or *RAS* were not statistically significant as independent risk factors for survival.

Nonetheless, Yoshida et al. were able to show that mutations in *PTPN11* were associated with older age at diagnosis, increased hemoglobin F, reduced OS, and tendency to relapse following transplantation (Yoshida et al. 2012).

Targeted therapy for the downregulation of the mutated genes seemed to be an encouraging approach for the treatment of this disease. Targeting the RAS pathway is being explored. However, since the RAS pathway plays a significant role in many diverse physiologic processes, there are some concerns regarding the potential consequences of the inhibition of RAS proteins.

7.2 Chronic Myelomonocytic Leukemia

Chronic myelomonocytic leukemia (CMML) is the most common disease entity in the MDS/MPN category. It is characterized by PB monocytosis $>1\times10^9$/L, absence of *BCR-ABL1* fusion gene, and morphologic dysplasia. It was first introduced as a disease entity in 1982 by the French-American-British (FAB) Classification and subsequently included in the MDS/MPD category in the 2001 WHO classification (Bennett et al. 1982). In 2008, the WHO revised the criteria for CMML (Table 7.1). Based upon this classification, CMML-1 has <5% blasts/blast equivalent (promonocytes) in the PB and <10% blasts/blast equivalent (promonocytes) in the BM, while CMML-2 has blasts/blast equivalent (promonocytes) in the range of 5–19% in the PB and >10% blasts/blast equivalent (promonocytes) in the BM or the presence of Auer rods regardless of the percentage of blasts. Similar to what is expected to other subtypes of MDS and even MDS/MPN, higher levels of BM blasts as observed in CMML-2 represent a more aggressive subtype and associated with a shorter OS with higher incidence of progression to AML (Germing et al. 1998).

7.2.1 Epidemiology

CMML is more commonly seen in males compared to females (ratio, 1.5:3 and 1:1) with a median age of presentation of around 65–75 years. Although there is no reliable incidental data for this disease based on the fact that it was classified in some literatures as CML and in the others as MDS, it is estimated that the annual incidence is about 12.8 new cases per 100,000 individuals annually.

The median survival for CMML is about 3 years. The precise etiology of CMML is not known; however, it has been associated with chemical and environmental exposures such as smoking, solvents, and/or agricultural chemicals (Williamson et al. 1994; Germing et al. 2000).

7.2.2 Clinical Manifestations

Patients with CMML can present with various clinical features mimicking both MDS and MPNs. However, most patients may be asymptomatic at the time of diagnosis. A routine blood test can show abnormalities such as anemia; neutropenia; leukocytosis, specifically monocytosis; and thrombocytopenia.

Of note, since monocytosis can occur in other physiologic (after receding of neutropenia, pregnancy, and post-splenectomy) or non-hematologic/pathologic conditions such as infections (rickettsial infections, tuberculosis, syphilis) and connective tissue diseases (systemic lupus erythematosus and rheumatoid arthritis), a careful history and adequate workup are substantial to exclude any other possibility.

Furthermore, it can present with nonspecific clinical features, such as bruising, headaches, infections, night sweating, and pallor. Tissue infiltration in spleen, skin, liver, or lymph nodes by monocytes can also be observed which eventually cause lymphadenopathies and hepatosplenomegaly. In CMML, splenomegaly is found in 25–70% of the patients. Rarely, CMML can present itself with chronic non-bloody diarrhea due to colonic involvement or even cardiac tamponade because of leukemic infiltration. Reactive monocytosis, chronic myelogenous leukemia (CML), and atypical chronic myelogenous leukemia (aCML) are important differential diagnoses to keep in mind when considering CMML (Bradford et al. 1993; Onida et al. 2002).

7.2.3 Histomorphology, Cytogenetics, and Molecular Profile

The diagnosis of a patient with CMML relies mostly on morphologic clues found in PB and BM. In the PB, presence of monocytosis ($>1\times10^9$/L) with anemia is a hallmark of the disease. Most cases of CMML have normal or low WBC counts. However, in the proliferative type, WBC can be $80–100\times10^9$/L due to neutrophilia or monocytosis. Basophils and eosinophils may be elevated, and if the total is $\geq1.5\times10^9$/L, it would suggest the possibility of CMML with eosinophilia or the presence of abnormal rearrangements of *PDGFRA* and *PDGRFB* genes (Swerdlow et al. 2008). As noted, blasts and promonocytes can be detected in BM or PB. The blast composition includes promonocytes, monoblasts, and myeloblasts (Table 7.1). Generally, patients at time of diagnosis have varying degrees of thrombocytopenia and anemia.

The BM of patients with CMML is typically hypercellular with prominent granulocytic hyperplasia with or without monocytic proliferation. Dysplastic changes can be seen in any of the three hematopoietic cell lines (erythroid, granulocytic, and megakaryocytic). In addition, reticulin fibrosis can be positive in about 30 % of patients, while nodules composed of mature plasmacytoid dendritic cells can occur in the core biopsy in about 20 % of patients (Swerdlow et al. 2008).

In MDS, detection of chromosomal abnormalities is important for diagnostic, therapeutic, and prognostic reasons. Traditionally, metaphase cytogenetics (MC) is typically used to identify underlying chromosomal abnormalities; however, these lesions are only present in about 50 % of patients (Tiu et al. 2011b). More recently, single nucleotide polymorphism array (SNP-A), a high-resolution karyotyping method, has been able to detect unbalanced DNA defects including somatic uniparental disomy (UPD). In CMML, SNP-A can increase the detection rate of cytogenetic abnormalities by up to 60 % (Jankowska et al. 2011). Cytogenetic abnormalities seen in CMML are variable; some may have monosomy 7, del7q, +8, −X, −Y, and del12p or even balanced translocations (Onida et al. 2002; Jankowska et al. 2011). Rarely, some CMML patients may have concomitant eosinophilia and ultimately found to have *PDGFRB* rearrangements by MC or FISH. The 2008 WHO classification has designated a separate disease category for patients with CMML morphology with concomitant *PDGFRB* rearrangements called myeloid neoplasms associated with eosinophilia and abnormalities of *PDGFRB* (Swerdlow et al. 2008).

The exact pathogenesis of CMML is not known. Until recently, the most common genetic mutations detected in CMML were in *NRAS* (4 %) and *KRAS* (7 %) genes. Despite the presence of MPN features in CMML, the *JAK2V617F* is found in only a low frequency of patients (1 %). More recently, specifically *CBL* (14 %), *TET2* (49 %), *ASXL1* (43 %), *EZH2* (6 %), *DNMT3A* (10 %), *UTX* (8 %), and *IDH1/2* (4 %) were found in varying frequencies in CMML and have been linked to disease pathogenesis. Fusion proteins involving the tyrosine kinase family can be seen in CMML, specifically platelet-derived growth factor receptor beta (PDGFRB). The presence of *PDGFRB* rearrangements indicates that myeloblast/monocyte proliferation is mediated by the phosphotyrosine kinase pathway. More recently, mutations involving the spliceosomal machinery have also been found in patients with CMML, specifically *SRSF2* (36 %) (Gelsi-Boyer et al. 2008; Kuo et al. 2009). *CBL* gene has also been found in CMML. *CBL* is an E3 ubiquitin protein ligase that is involved in cell signaling and protein rearrangements by negatively regulating tyrosine kinase enzymatic pathway. Mutations of *CBL* lead to a prolonged activation of tyrosine kinase that causes proliferation in hematopoietic progenitor cells. Clinically, patients with a *CBL* mutation tend to have a monocyte/monoblast proliferation and splenomegaly. However, there is no difference in OS between mutant and wild-type patients (Gelsi-Boyer et al. 2008; Makishima et al. 2011). Ten-Eleven Translocation 2 (*TET2*) is a gene located on chromosome 4 that has a biological function in converting 5-methylcytosine to 5-hydroxymethylcytosine. Methylation of cytosine residues controls important epigenetic modification and gene expression. The incidence of *TET2* mutations ranges from 10 to 25 % in various myeloid diseases (Delhommeau et al.

Table 7.2 Distribution of molecular mutations based on MDS/MPN subtypes

	CMML (%)	JMML (%)	aCML (%)	MDS/MPN-U	RARS-T (%)
CBL	5–22	7–10	–	–	–
RAS	11–27	20	–	–	–
RUNX1	9–37	–	–	–	–
JAK2(V617F)	2–13	–	~1–5	–	60
TET2	20–51	–	–	–	9–26
ASXL1	27–58	4	–	–	10
UTX	8–9	–	–	–	–
EZH2	5.5–13	–	–	–	–
IDH1/2	1–10	–	–	–	–
SRSF2	36	–	–	–	–
PTPN11	–	30	–	–	–
MPL	–	–	–	–	30
SF3B1	4.5	–	–	–	72
NF-1	–	30	–	–	–
DNMT3A	10	–	–	–	17
LNK	–	–	–	–	5

Muramatsu et al. (2012), Perez et al. (2010), Szpurka et al. (2010), Jankowska et al. (2011), Makishima et al. (2010), Visconte et al. (2012a), Papaemmanuil (2011), Yoshida et al. (2011), Reiter et al. (2009)

2009). Mutations of *TET2* cause a loss of function which eventually leads to hypermethylation of upstream promoters of genes operating in cell proliferation. Kosmider et al. noted that patients who carry *TET2* mutations have better 5 years OS and 3 years leukemia event-free survival (Kosmider et al. 2009). Of note, these patients have been treated with hypomethylating agents with 20 % response rate. In contrast, CMML patients with *TET2* mutations have an increased risk for AML transformation compared to wild-type cases, albeit there is no difference in OS. Overall, monocytosis and higher number of immature dysplastic granulocytes were seen in patients with CMML who harbor this mutation (Makishima et al. 2011; Kosmider et al. 2009).

ASXL1 and *EZH2* are genes that are associated with the class II polycomb gene complex. *ASXL1* is located in chromosome 20. *ASXL1* mutations are seen in about 43 % of patients with CMML. It is unknown whether *ASXL1* is associated with an early or secondary event since in murine models, a mutation in this gene does not lead to a myeloid malignancy phenotype. Patients with this mutation have a higher WBC, higher PB/BM monocytosis, and lower hemoglobin levels. *ASXL1* is a poor prognostic factor in patients with CMML. *EZH2* is located in chromosome 7 and is important in the trimethylation of H3K27 (Makishima et al. 2010). This genetic mutation is also found in other myeloid malignancies. The presence of this genetic mutation confers worse outcomes in patients (Jankowska et al. 2011; Makishima et al. 2010). Multiple other molecular defects can be seen in patients with CMML including those involving genes like DNA (cytosine-5)-methyltransferase 3 alpha (*DNMT3A*) and *UTX*. *DNMT3A* probably causes changes in the DNA-binding groove or interacts with other essential intranuclear elements. *UTX* mutations are usually present at the C-terminus and N-terminus of the protein. A mutation in this gene causes haploinsufficiency probably affecting differentiation of progenitor cells (Jankowska et al. 2011). A summary of all the genes mutated in CMML is provided in Table 7.2.

7.2.4 Treatment

There are a number of different therapeutic approaches available for patients with CMML. However, it is difficult to assess the response to treatments and outcomes in these patients based on the fact that most of the studies usually

incorporate patients with MDS and other types of MDS/MPN overlap neoplasms. In this book chapter, we cited studies that either had a cohort more than ten patients or achieved conclusive results.

7.2.4.1 Group 1: High-Intensity Chemotherapy (HIC)

There are several potential agents encompassing this category that can be used for treatment such as cytarabine + mitoxantrone, topotecan + cytarabine, and 9-nitro-camptothecin (9-NC). There was a phase II cooperative group study which looked at using the combination of intermediate-dose cytarabine and mitoxantrone with G-CSF in patients with MDS and AML, and it also included two patients with CMML. Unfortunately, this study was terminated prematurely because it did not reach the primary objective that was three complete remissions and also due to the occurrence of unexpected hepatotoxicity (hyperbilirubinemia) (Bennett et al. 2001). There are two topoisomerase-based regimens that are currently used, topotecan and 9-nitro-camptothecin (9-NC). Topotecan acts mainly as a topoisomerase I inhibitor, and it is an orally bioavailable chemotherapeutic agent. MD Anderson Cancer Center (MDACC) conducted a study using the combination of topotecan and high-dose cytarabine both given daily for 5 days. This particular study enrolled 59 patients with MDS and 27 with CMML. In addition, the majority of patients were previously untreated (66 %). The median follow-up time was 7 months. Complete remission was observed in 44 % of patients with CMML. The median duration of response was 8½ months, and OS was 11 months. Mucositis, diarrhea, fever, and infections were the toxicities encountered. Mortality rate from induction chemotherapy was 7 % (Beran et al. 1999). 9-NC, a novel topoisomerase I inhibitor, has been tested in a cohort of 44 patients (MDS, $N = 12$, and CMML, $N = 32$). A daily oral dose of 2 mg/m^2 was given for 5 days/week every 4–6 weeks. The overall response rate (ORR) was 41 % (Complete Response = 11 %, Partial Response = 16 %, Hematological Improvement = 14 %) which was similar for MDS (41 %) and CMML (40 %) patients. The 2-year survival rate was 28 % for CMML and 17 % for MDS (Quintas-Cardama et al. 2006).

7.2.4.2 Group 2: Low-Intensity Chemotherapy (LIC)

Low-Dose Cytarabine

Typically, cytarabine (Ara-C) has been used in the treatment of myeloid neoplasms, particularly AML. Several studies conducted on a few number of patients revealed that low-dose Ara-C in combination with G-CSF or alone can be used as a therapeutic approach in CMML (Economopoulos et al. 1992; Gerhartz et al. 1994).

Hypomethylating Agents

Although CMML patients have been enrolled in MDS studies where patients were treated with hypomethylating agents such as azacitidine or decitabine, they usually represent a small number, and therefore only few studies focused specifically on efficacy of these treatment modalities in CMML.

Aribi et al. in 2007 used decitabine in 19 CMML patients. The median age of the patients was 66 years (range = 44–82 years). There were three different dosing schedules of decitabine, including (1) 20 mg/m^2 IV daily for days 1–5, (2) 20 mg/m^2 SC daily for days 1–5, or (3) 10 mg/m^2 IV for days 1–10. Each treatment schedule was repeated every 28 days. Overall, CR was observed in 58 % of patients and HI in 11 %. Reduction in size of spleen was reported in 75 % of patients, and the median OS of the patients was 19 months (Aribi et al. 2007).

Patients with CMML treated with decitabine in three major trials (one phase III and 2 phase II) were included in a pooled analytical report. This report included 31 patients with a median age of 71 years (range = 53–81 years). The treatment regimen was decitabine 15 mg/m^2 three times per each day for days 1–3, and each cycle was repeated every 6 weeks. The ORR was 26 % (CR = 10 %; PR = 16 %). HI and stable disease (SD) were reported in 19 and 32 % of patients, respectively. Cytogenetic improvements were noted in 3/8 patients with chromosomal abnormalities documented before starting therapy. The median number of decitabine cycles received was four, and median OS was 15 months. Toxicity profiles included were nausea/vomiting (42 %), pneumonia (21 %), epistaxis (11 %), and diarrhea (11 %) which were also previously reported

in other studies using this therapeutic schedule. A study conducted in 38 CMML patients treated with azacitidine given at a dose of 75 mg/m^2/day for 7 days or 100 mg/m^2/day for 5 days every 4 weeks reported 39 % (14/36), of which 11 % were CR, 3 % were PR, and 25 % were HI. The median age of the cohort was 71 years old and included MDS/CMML (30 %) and MPD/CMML (70 %). Fifty-six percent of patients were pretreated with hydroxyurea (HU), G-CSF, and erythropoiesis-stimulating agents. In two patients, these regimens resulted in resolution of skin rash which developed as a consequence of myeloid cell infiltration. The median OS was 12 months. Responders to azacitidine had longer OS compared to nonresponders (15 vs. 9 months; $p = .04$), but no difference in OS was noted between CMML-1 and CMML-2 patients (12 vs. 11 months; $p = .03$). Side effects were comparable to what has been previously reported and included irritation at injection site, fatigue, anorexia, and skin rash (Costa et al. 2011).

Histone Deacetylase Inhibitors

In addition to high-/low-dose chemotherapy and hypomethylating agents, histone deacetylase inhibitors, specifically valproic acid, have been used for the treatment of CMML. This agent can inhibit proliferation of leukemic cells and induce cellular differentiation. To enhance the response of valproic acid, a study investigated the combination of valproic acid together with two other differentiating agents, 13-cis-retinoic acid and 1,25-dihydroxyvitamin D3, in 19 patients with either CMML ($N = 4$) or MDS ($N = 15$). The median age of the cohort was 73 years. All patients included in this study received supportive therapies. Sixteen percent of patients achieved an HI. Based upon these results, it was concluded that the combination of these agents had minimal activity (Siitonen et al. 2007).

7.2.4.3 Group 3: Supportive Therapies

In 1996, hydroxyurea (HU) was compared to etoposide in 105 patients with advanced CMML where the median age was 71 years. Oral doses of HU were 1,000 g/day, and etoposide was 150 mg/week (doubled in cases of visceral involvement and escalated in nonresponders). Initial analysis demonstrated that HU was superior over etoposide and resulted in early discontinuation of the study. After a median follow-up of 11 months, responders were seen in 60 % of patients treated with HU and 36 % in the etoposide group ($p = .02$). However, the median time to response was shorter in the HU versus etoposide-treated group (2 vs. 3.5 months; $p = .003$). Median survival was 20 months in HU versus 9 months in the etoposide arm (Wattel et al. 1996).

7.2.4.4 Group 4: Tyrosine Kinase Inhibitors

A rare subtype of CMML is characterized by the presence of t(5;12) which is responsive to imatinib and other tyrosine kinase inhibitors. This subtype is now a separate disease category called myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1* (Gotlib 2012).

7.2.4.5 Group 5: Hematopoietic Cell Transplantation (HCT)

The largest published data looking at the feasibility of allogeneic HCT in CMML included 85 patients with CMML from the Fred Hutchinson Cancer Center. The median age of patients was 52 years (range = 1–69 years). The median follow-up was 19 years. They reported a relapse or progression rate of 26 % at 6 months after transplantation. Predictors of relapse in multivariate analyses included female patients who received stem cells from female donors and poor score with the MD Anderson Prognostic Scoring System. The non-relapse mortality rate (NRM) was 34 %. Predictors of NRM included poor-risk cytogenetics and HSCT comorbidity index >2. The predictors of overall mortality by multivariable analyses were poor-risk cytogenetics HSCT-CI >2 and pretransplant hematocrit levels. Relapse-free survival (RFS) at 10 years was estimated at 40 %, and predictors of poor RFS are (1) poor-risk cytogenetics, (2) HSCT-CI >2, and (3) age (Eissa et al. 2011). A study conducted by the EBMT included 50 patients with CMML. The median age of the patients was 44 years old (range = 19–61). The median follow-up was 40 months. The patients underwent myeloablative conditioning. The stem cell sources were primarily BM in 40, PBSC in 9,

and both in 1 patient. Match related donor was available for 43 patients while match unrelated donor for the remainder. The 5-year survival outcome for disease-free survival (DFS) was 18 %, OS was 21 %, and relapse rate (RR) was 49 %. Factors predictive of better DFS were early transplant, male donor, unmanipulated grafts, and occurrence of acute GVHD (Kroger et al. 2002).

7.2.5 Prognosis

The determinants of prognosis in CMML are diverse. The most commonly used prognostic scoring system in MDS and MDS-related disorder is the International Prognostic Scoring System (IPSS). It used the original French-American-British (FAB) Classification for MDS which included CMML patients. Similarly, earlier forms of prognostic scoring systems including the modified Bournemouth (Mufti et al. 1985), the Spanish Scoring system (Sanz et al. 1989), the Dusseldorf system (Aul et al. 1992), and the Lille classification incorporated a subset of CMML patients (Morel et al. 1993). However, it is clear that MDS/MPN overlap neoplasms like CMML have distinct biologic and clinical differences compared to their pure MDS counterpart. Therefore, prognostic scoring systems that incorporate CMML only as a subset of disease may not adequately reflect the true outcomes of patients with CMML. The only prognostic scoring system devised specifically for CMML is the MD Anderson Cancer Center (MDACC) prognostic scoring system for CMML. The factors that were identified as prognostic included (1) Hgb <12 g/dl; (2) absolute lymphocyte count $>2.5\times10^9$/L; (3) the presence of immature myeloid cells (IMCs) which include PB myeloblasts, promyelocytes, myelocytes, and metamyelocytes; and (4) BM blasts ≥10 %. Based on the four factors, patients can have low-risk (score = 0–1, median OS = 24 months), intermediate-1-risk (score = 2; median OS = 15 months), intermediate-2-risk (score = 3, OS = 8 months), and high-risk (score = 4, OS = 5 months) disease (Onida et al. 2002). Cytogenetics is one of the most powerful predictors of outcomes in myeloid malignancies, and CMML is not an exception. One of the largest studies investigating the prognostic role of cytogenetics in CMML included 414 patients from various institutions in Spain. Patients with abnormal karyotype by MC had worse OS and higher risk of AML progression. The three cytogenetic risk groupings were low risk (normal karyotype or sole –Y), intermediate risk (other abnormalities that are not in the low- or high-risk categories), and high risk (+8, chromosome 7 abnormalities, complex karyotype). OS at 5 years for patients in the low-, intermediate-, and high-risk cytogenetic categories was 35, 26, and 4 %, respectively ($p < .001$) (Such et al. 2011).

Using SNP-A karyotyping, previously cryptic chromosomal defects specifically microdeletions, microduplications, and acquired UPD are detected in myeloid malignancies including CMML (Gondek et al. 2008). SNP-A lesions can lead to worse OS (16 vs. 21 months; $p = .04$) (Jankowska et al. 2011). The presence of certain molecular mutations, specifically *DNMT3A* and *CBL* mutations, can also lead to poor OS (Jankowska et al. 2011).

7.3 Atypical Chronic Myeloid Leukemia

7.3.1 Epidemiology

Atypical chronic myeloid leukemia (aCML) is a rare subtype of MDS/MPN overlap neoplasms. The exact incidence and prevalence of the disease is actually unknown and further complicated by difficulty in distinguishing this disease from CMML. Males and females in some series are equally affected. In one study, a slight female predominance was found (females, 57 % > males, 43 %) (Breccia et al. 2006). The cause is not known.

7.3.2 Clinical Manifestations

Clinically, patients with aCML may present with pancytopenia, isolated anemia, or thrombocytopenia with or without leukocytosis. Organomegaly,

specifically hepatosplenomegaly, may be seen (Swerdlow et al. 2008).

7.3.3 Histomorphology, Cytogenetics, and Molecular Profile

Granulocyte dysplasia is a prominent feature seen in patients with aCML. The diagnosis of aCML is based on PB leukocytosis, absence of Philadelphia chromosome or the *BCL-ABL* gene, absence of the PDGFRA/ PDGFRB gene rearrangement, <20 % blasts in the PB or BM, basophilia of <2 % of WBC, neutrophil precursors ≥10 % of leukocytes, minimal absolute monocytosis (<10 % of leukocytes), and hypercellular BM with granulocytic proliferation and dysplasia (Table 7.1) (Swerdlow et al. 2008). In the PB, leukocytosis is common, and neutrophilic precursors include 10–20 % of the total WBC count. Neutrophil changes can also be seen in the PB smear with clumped chromatin, nuclear segmentation, cytoplasmic granularity, and acquired Pelger-Huet abnormality. PB blasts may be present in <5 % of leukocytes. Moderate anemia and thrombocytopenia may be present with or without accompanying dysplasia. Dysgranulopoiesis is a distinctive morphologic feature that can be seen. BM is usually hypercellular and often presents with an increase in neutrophils and neutrophilic precursors. The increased percentage of neutrophils and their precursors can exhibit exaggerated clumping of nuclear chromatin in aCML and can lead to a condition called "syndrome of abnormal chromatin clumping." Dysplasia is seen in megakaryocytes and erythrocytes (50 %). Megakaryocytes can be small mega-, micro-, hypo-/non-lobulated. Increased reticulin fibrosis may be seen in some cases. Cytochemical staining has limited utility in the diagnosis of aCML since there are no specific stains for diagnosing this disease. However, certain cytochemical stains are useful in assessing monocyte percentage and discriminating the disease from CMML (Swerdlow et al. 2008). Cytogenetic defects, trisomy 8 and del20q, are seen in patients with aCML. Lesions involving chromosomes 5, 11, 12, 13, 14, 17, 19, and 21 have also been identified in aCML (Hernandez et al. 2000; Kurzrock et al. 2001). The exact molecular pathogenesis is not known in aCML. In order to elucidate this, several investigators screened genetic mutations identified in other myeloid malignancies. *TET2*, *ASXL1*, *EZH2*, *IDH1/2*, *DNMT3A*, *SF3B1*, and *SRSF2* were unreported. However, *NRAS* and *KRAS* mutations are commonly found in 30 % of cases (Swerdlow et al. 2008). *JAK2V617F* mutations are also sporadically seen in aCML. Recently, one patient with aCML was recently identified to be mutated for *U2AF1*. However, this needs to be further evaluated (Makishima et al. 2012). So far, no other recurrent mutations have been consistently identified in aCML. Immunophenotyping and IHC stains are mainly useful for the exclusion of other diseases. However, there are no specific immunophenotypic features for the diagnosis of aCML, and IHC stains have limited utility in the diagnosis of aCML.

7.3.4 Treatment

Several treatment modalities are available for aCML, such as hydroxyurea, immunotherapy (interferon), and HCT. Each of the treatment modalities has different impact on the disease. Hydroxyurea, also used in CMML, has been used successfully in aCML. There is a high response rate (80 %); however, the response rates are usually short-lived (median duration of 2–4 months) (Kurzrock et al. 2001). In addition, immunotherapy such as interferon-α (IFN-α) has also been used in the treatment of aCML. Responses with this agent vary depending on whether it was given as PEG-IFN or IFN-α. When IFN-α was given at a dose range of 3×10^6 to 6×10^6 units/day, there was only a single responder out of seven patients (14 %). However, this patient did have a durable long-term response lasting 100+ months (Kurzrock et al. 2001). Moreover, PEG-IFN-α2b has also been tested in a phase II setting in aCML. Patients were given PEG-IFN-α2b at a starting dose of 3 μg/kg/week and were adjusted accordingly based on tolerability. In five aCML patients, CR was achieved in 40 % (2/5) 3 months after initiating

therapy. However, only two patients were able to continue therapy for a median of 37 months because of toxicities from the therapy (Jabbour et al. 2007). Similar to other overlap syndromes, HSCT is the only curative option in patients with aCML. In a retrospective study involving nine aCML patients, there were various donor stem cell sources, including four from HLA-identical siblings, four from HLA-compatible unrelated donors, and one from a twin brother. Various treatments were used prior to transplant, including TBI + cyclophosphamide (Cy) ($N=5$), busulfan (Bu) + Cy ($N=2$), TBI + Cy + alemtuzumab ($N=1$), and Bu + fludarabine (Flu) + ATG ($n=1$). A median follow-up of 55 months post transplant indicated that the patient who obtained his stem cell source from his twin brother relapsed 19 months from his original transplant. However, he was successfully salvaged using stem cells from the original donor. There was one patient who died of complications related to cerebral toxoplasmosis 9 months from the transplant. However, the remaining patients were in CR at last follow-up. Excluding the patient who died, acute GVHD (grades II–IV) occurred in 63 % of patients, while chronic GVHD occurred in all patients (Koldehoff et al. 2004).

7.3.5 Prognosis

Several factors are involved in determining the prognosis for patients with aCML. The median OS for aCML patients is between 11 and 26 months (Onida et al. 2002; Breccia et al. 2006; Kurzrock et al. 2001; Galton 1992; Bennett et al. 1994). Factors influencing morbidity and mortality in aCML patients include anemia, thrombocytopenia, infections, refractory leukocytosis, and hepatosplenomegaly (Kurzrock et al. 2001). Similar to other myeloid malignancies, aCML can also transform to AML in 40 % of cases (Breccia et al. 2006). A prognostic scoring system devised specifically for aCML patients was devised and included 76 patients. Poor predictors of OS included age >65 years, Hgb <10 g/dl, and leukocytosis of $>50\times10^9$/L. A score is generated based on the presence or absence of any one of the three factors and patients may be assigned to any one of the two risk groups, low-risk (score of 0–1; median OS = 38 months) and high-risk disease (score of 2–3; median OS = 9 months) (Onida et al. 2002). Breccia et al. also investigated the prognostic variables that impact survival in aCML and found that age >65 years, high WBC count ($>50\times10^9$/L), and the presence of immature circulating precursors are poor predictors of OS, while the presence of monocytosis (<3 >8 % with monocytes $<1\times10^9$/L), organomegaly (liver/spleen), increased BM blasts (>5 %), presence of marked dyserythropoiesis, and need for transfusions are predictors of leukemic transformation in aCML by multivariate analyses (Breccia et al. 2006).

7.4 Myelodysplastic/Myeloproliferative Neoplasms: Unclassifiable (MDS/MPN-U)

7.4.1 Epidemiology

MDS/MPN-U is defined as an entity which encompasses diseases characteristics of both myelodysplastic (MDS) and myeloproliferative syndromes (MPN) but do not meet the criteria for CMML, JMML, or aCML. MDS/ MPN-U is characterized by anomalies in myeloid cells, specifically dysplasia or increased proliferation. Moreover, laboratory evaluation often shows anemia, dimorphic red cells, leukocytosis, and thrombocytosis. Genetically, MDS/MPN-U does not have any specific cytogenetic or molecular findings even though *JAK2V617F* mutation has been associated.

7.4.2 Clinical Manifestations

Clinically, the primary manifestation of the MDS/MPN-U is anemia. Anemia can be normocytic or macrocytic and is almost always present. WBC count can be either low or mildly elevated with variable heterogeneity in the differential. Usually, abnormalities in WBC or platelet counts and morphology can occur. PB blasts may be present but are usually <20 %. The BM aspirate can show

dyserythropoiesis, dysgranulopoiesis, or dysmegakaryopoiesis. Neutropenia and thrombocytopenia can occur. Hepatosplenomegaly can occur due to secondary erythropoiesis. Cytogenetic abnormalities can be present but are nonspecific.

7.4.3 Histomorphology, Cytogenetics, and Molecular Profile

According to the 2008 WHO, refractory anemia with ring sideroblast associated with thrombocytosis (RARS-T) is a provisional entity under the MDS/MPN-U category. The exact incidence of RARS-T is not known. Patients with RARS-T usually have de novo disease with hybrid features of RARS (MDS subtype) and essential thrombocythemia (ET) (MPN subtype) (Swerdlow et al. 2008). The clinical outcome has been investigated in a retrospective study where patients with RARS-T showed an OS of 71 months similar to the ones of RARS but lower than patients with ET. Criteria to classify RARS-T include the presence of the following features: refractory anemia associated with erythroid lineage, ringed sideroblast ≥15 %, <5 % blasts in the BM, platelet count $\geq 450 \times 10^9$/L or PB leukocytosis with/without splenomegaly, and presence of large atypical megakaryocytes similar to those observed in *BCR/ABL1*-negative MPNs. Cytogenetic analysis can be helpful in excluding others. From the molecular point of view, until a year ago, the two most frequently mutated genes in RARS-T were *JAK2*V617F (60 %) and *MPL* (23 %) frequently found in MPN. Advancements in molecular technologies like SNP-A and whole exome/genome amplification have led to better understanding of the molecular pathogenesis of this disorder. We now know that a novel class of mutations involving genes of the spliceosomal machinery, specifically *SF3B1*, has been discovered. *SF3B1* is mutated in approximately 72 % of patients with RARS-T (Visconte et al. 2012a; Papaemmanuil et al. 2011; Yoshida et al. 2011). Functional data suggests that *SF3B1* haploinsufficiency can lead to ring sideroblast formation in RARS and RARS-T. Clinical data also suggest that patients with RARS-T and concomitant *SF3B1* mutations have better OS compared to patients who do not have the mutations (Visconte et al. 2012b).

7.4.4 Treatment/Prognosis

In regard to the treatment, none is specific for MDS/MPN-U. Usually, treatment strategies are based on the ones used for patients with MDS or CMML. Supportive care (erythropoietin and G/GM-CSF) can help in the improvement of anemia and neutropenia. Allogeneic HCT remains the only possible curative option. In the future, it might be possible that targeted therapies possibly against the recently discovered *SF3B1* mutations may be developed. Moreover, given the good prognostic effects of the presence of *SF3B1* mutations on OS, it may be useful in refining future prognostic scoring schemes. Currently, no specific prognostic scoring system is available for patients with MDS-unclassified or MDS/MPN-U.

References

Aribi A, Borthakur G, Ravandi F, Shan J, Davisson J, Cortes J et al (2007) Activity of decitabine, a hypomethylating agent, in chronic myelomonocytic leukemia. Cancer 109(4):713–717

Aul C, Gattermann N, Heyll A, Germing U, Derigs G, Schneider W (1992) Primary myelodysplastic syndromes: analysis of prognostic factors in 235 patients and proposals for an improved scoring system. Leukemia 6(1):52–59

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR et al (1982) Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51(2):189–199

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick H et al (1994) The chronic myeloid leukaemias: guidelines for distinguishing chronic granulocytic, atypical chronic myeloid, and chronic myelomonocytic leukaemia. Proposals by the French-American-British Cooperative Leukaemia Group. Br J Haematol 87(4):746–754

Bennett JM, Young MS, Liesveld JL, Paietta E, Miller KB, Lazarus HM et al (2001) Phase II study of combination human recombinant GM-CSF with intermediate-dose cytarabine and mitoxantrone chemotherapy in patients with high-risk myelodysplastic syndromes (RAEB, RAEBT, and CMML): an Eastern Cooperative Oncology Group Study. Am J Hematol 66(1):23–27

Beran M, Estey E, O'Brien S, Cortes J, Koller CA, Giles FJ et al (1999) Topotecan and cytarabine is an active combination regimen in myelodysplastic syndromes and chronic myelomonocytic leukemia. J Clin Oncol 17(9):2819–2830

Bradford CR, Smith SR, Wallis JP (1993) Pericardial extramedullary haemopoiesis in chronic myelomonocytic leukaemia. J Clin Pathol 46(7):674–675

Breccia M, Biondo F, Latagliata R, Carmosino I, Mandelli F, Alimena G (2006) Identification of risk factors in atypical chronic myeloid leukemia. Haematologica 91(11):1566–1568

Busque L, Gilliland DG, Prchal JT, Sieff CA, Weinstein HJ, Sokol JM et al (1995) Clonality in juvenile chronic myelogenous leukemia. Blood 85(1):21–30

Castleberry RP, Loh ML, Jayaprakash N et al (2011) Phase II Window Study of the Farnesyltransferase Inhibitor R115777 (Zarnestra(R)) in Untreated Juvenile Myelomonocytic Leukemia (JMML): A Children's Oncology Group Study. Blood (ASH Annu Meet Abstr) 106:2587

Chan RJ, Cooper T, Kratz CP, Weiss B, Loh ML (2009) Juvenile myelomonocytic leukemia: a report from the 2nd International JMML Symposium. Leuk Res 33(3):355–362

Cooper LJ, Shannon KM, Loken MR, Weaver M, Stephens K, Sievers EL (2000) Evidence that juvenile myelomonocytic leukemia can arise from a pluripotential stem cell. Blood 96(6):2310–2313

Costa R, Abdulhaq H, Haq B, Shadduck RK, Latsko J, Zenati M et al (2011) Activity of azacitidine in chronic myelomonocytic leukemia. Cancer 117(12):2690–2696

Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Masse A et al (2009) Mutation in TET2 in myeloid cancers. N Engl J Med 360(22):2289–2301

Economopoulos T, Papageorgiou E, Stathakis N, Asprou N, Karmas P, Dervenoulas J et al (1992) Treatment of myelodysplastic syndromes with human granulocytic-macrophage colony stimulating factor (GM-CSF) or GM-CSF combined with low-dose cytosine arabinoside. Eur J Haematol 49(3):138–142

Eissa H, Gooley TA, Sorror ML, Nguyen F, Scott BL, Doney K et al (2011) Allogeneic hematopoietic cell transplantation for chronic myelomonocytic leukemia: relapse-free survival is determined by karyotype and comorbidities. Biol Blood Marrow Transplant 17(6): 908–915

Emanuel PD (2008) Juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia. Leukemia 22(7):1335–1342

Flotho C, Valcamonica S, Mach-Pascual S, Schmahl G, Corral L, Ritterbach J et al (1999) RAS mutations and clonality analysis in children with juvenile myelomonocytic leukemia (JMML). Leukemia 13(1):32–37

Galton DA (1992) Haematological differences between chronic granulocytic leukaemia, atypical chronic myeloid leukaemia, and chronic myelomonocytic leukaemia. Leuk Lymphoma 7(5–6):343–350

Gelsi-Boyer V, Trouplin V, Adelaide J, Aceto N, Remy V, Pinson S et al (2008) Genome profiling of chronic myelomonocytic leukemia: frequent alterations of RAS and RUNX1 genes. BMC Cancer 8:299

Gerhartz HH, Marcus R, Delmer A, Zwierzina H, Suciu S, Dardenne M et al (1994) A randomized phase II study of low-dose cytosine arabinoside (LD-AraC) plus granulocyte-macrophage colony-stimulating factor (rhGM-CSF) in myelodysplastic syndromes (MDS) with a high risk of developing leukemia. EORTC Leukemia Cooperative Group. Leukemia 8(1):16–23

Germing U, Gattermann N, Minning H, Heyll A, Aul C (1998) Problems in the classification of CMML – dysplastic versus proliferative type. Leuk Res 22(10): 871–878

Germing U, Gattermann N, Strupp C, Aivado M, Aul C (2000) Validation of the WHO proposals for a new classification of primary myelodysplastic syndromes: a retrospective analysis of 1600 patients. Leuk Res 24(12):983–992

Gondek LP, Tiu R, O'Keefe CL, Sekeres MA, Theil KS, Maciejewski JP (2008) Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS, MDS/MPD, and MDS-derived AML. Blood 111(3): 1534–1542

Gotlib J (2012) World Health Organization-defined eosinophilic disorders: 2012 update on diagnosis, risk stratification, and management. Am J Hematol 87(9): 903–914

Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J et al (1999) 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 17(12):3835–3849

Hasle H, Arico M, Basso G, Biondi A, Cantu Rajnoldi A, Creutzig U et al (1999) Myelodysplastic syndrome, juvenile myelomonocytic leukemia, and acute myeloid leukemia associated with complete or partial monosomy 7. European Working Group on MDS in Childhood (EWOG-MDS). Leukemia 13(3):376–385

Hasle H, Baumann I, Bergstrasser E, Fenu S, Fischer A, Kardos G et al (2004) The International Prognostic Scoring System (IPSS) for childhood myelodysplastic syndrome (MDS) and juvenile myelomonocytic leukemia (JMML). Leukemia 18(12):2008–2014

Hernandez JM, del Canizo MC, Cuneo A, Garcia JL, Gutierrez NC, Gonzalez M et al (2000) Clinical, hematological and cytogenetic characteristics of atypical chronic myeloid leukemia. Ann Oncol 11(4): 441–444

Jabbour E, Kantarjian H, Cortes J, Thomas D, Garcia-Manero G, Ferrajoli A et al (2007) PEG-IFN-alpha-2b therapy in BCR-ABL-negative myeloproliferative disorders: final result of a phase 2 study. Cancer 110(9):2012–2018

Jaffe EL, Harris NL, Stein HJ, Vardiman JW (2001) Pathology and genetics: tumours of haematopoietic and lymphoid tissues (WHO classification of tumours). IRAC Press, Lyon

Jankowska AM, Makishima H, Tiu RV, Szpurka H, Huang Y, Traina F et al (2011) Mutational spectrum analysis

of chronic myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX, EZH2, and DNMT3A. Blood 118(14):3932–3941

Koldehoff M, Beelen DW, Trenschel R, Steckel NK, Peceny R, Ditschkowski M et al (2004) Outcome of hematopoietic stem cell transplantation in patients with atypical chronic myeloid leukemia. Bone Marrow Transplant 34(12):1047–1050

Kosmider O, Gelsi-Boyer V, Ciudad M, Racoeur C, Jooste V, Vey N et al (2009) TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia. Haematologica 94(12):1676–1681

Kroger N, Zabelina T, Guardiola P, Runde V, Sierra J, Van Biezen A et al (2002) Allogeneic stem cell transplantation of adult chronic myelomonocytic leukaemia. A report on behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Br J Haematol 118(1):67–73

Kuo MC, Liang DC, Huang CF, Shih YS, Wu JH, Lin TL et al (2009) RUNX1 mutations are frequent in chronic myelomonocytic leukemia and mutations at the C-terminal region might predict acute myeloid leukemia transformation. Leukemia 23(8):1426–1431

Kurzrock R, Bueso-Ramos CE, Kantarjian H, Freireich E, Tucker SL, Siciliano M et al (2001) BCR rearrangement-negative chronic myelogenous leukemia revisited. J Clin Oncol 19(11):2915–2926

Locatelli F, Nollke P, Zecca M, Korthof E, Lanino E, Peters C et al (2005) Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. Blood 105(1):410–419

Loh ML (2010) Childhood myelodysplastic syndrome: focus on the approach to diagnosis and treatment of juvenile myelomonocytic leukemia. Hematology Am Soc Hematol Educ Program 2010:357–362

Loh ML (2011) Recent advances in the pathogenesis and treatment of juvenile myelomonocytic leukaemia. Br J Haematol 152(6):677–687

Makishima H, Jankowska AM, Tiu RV, Szpurka H, Sugimoto Y, Hu Z et al (2010) Novel homo- and hemizygous mutations in EZH2 in myeloid malignancies. Leukemia 24(10):1799–1804

Makishima H, Jankowska AM, McDevitt MA, O'Keefe C, Dujardin S, Cazzolli H et al (2011) CBL, CBLB, TET2, ASXL1, and IDH1/2 mutations and additional chromosomal aberrations constitute molecular events in chronic myelogenous leukemia. Blood 117(21):e198–e206

Makishima H, Visconte V, Sakaguchi H, Jankowska AM, Abu Kar S, Jerez A et al (2012) Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. Blood 119(14):3203–3210

Matsuda K, Sakashita K, Taira C, Tanaka-Yanagisawa M, Yanagisawa R, Shiohara M et al (2010) Quantitative assessment of PTPN11 or RAS mutations at the neonatal period and during the clinical course in patients with juvenile myelomonocytic leukaemia. Br J Haematol 148(4):593–599

Morel P, Hebbar M, Lai JL, Duhamel A, Preudhomme C, Wattel E et al (1993) Cytogenetic analysis has strong independent prognostic value in de novo myelodysplastic syndromes and can be incorporated in a new scoring system: a report on 408 cases. Leukemia 7(9):1315–1323

Mufti GJ, Stevens JR, Oscier DG, Hamblin TJ, Machin D (1985) Myelodysplastic syndromes: a scoring system with prognostic significance. Br J Haematol 59(3): 425–433

Muramatsu H, Makishima H, Maciejewski JP (2012) Chronic myelomonocytic leukemia and atypical chronic myeloid leukemia: novel pathogenetic lesions. Semin Oncol 39(1):67–73

Onida F, Kantarjian HM, Smith TL, Ball G, Keating MJ, Estey EH et al (2002) Prognostic factors and scoring systems in chronic myelomonocytic leukemia: a retrospective analysis of 213 patients. Blood 99(3): 840–849

Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D et al (2011) Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med 365(15):1384–1395

Park HD, Lee SH, Sung KW, Koo HH, Jung NG, Cho B et al (2012) Gene mutations in the Ras pathway and the prognostic implication in Korean patients with juvenile myelomonocytic leukemia. Ann Hematol 91(4):511–517

Passmore SJ, Hann IM, Stiller CA, Ramani P, Swansbury GJ, Gibbons B et al (1995) Pediatric myelodysplasia: a study of 68 children and a new prognostic scoring system. Blood 85(7):1742–1750

Perez B, Kosmider O, Cassinat B, Renneville A, Lachenaud J, Kaltenbach S et al (2010) Genetic typing of CBL, ASXL1, RUNX1, TET2 and JAK2 in juvenile myelomonocytic leukaemia reveals a genetic profile distinct from chronic myelomonocytic leukaemia. Br J Haematol 151(5):460–468

Quintas-Cardama A, Kantarjian H, O'Brien S, Jabbour E, Giles F, Ravandi F et al (2006) Activity of 9-nitrocamptothecin, an oral topoisomerase I inhibitor, in myelodysplastic syndrome and chronic myelomonocytic leukemia. Cancer 107(7):1525–1529

Raygada M, Arthur DC, Wayne AS, Rennert OM, Toretsky JA, Stratakis CA (2010) Juvenile xanthogranuloma in a child with previously unsuspected neurofibromatosis type 1 and juvenile myelomonocytic leukemia. Pediatr Blood Cancer 54(1):173–175

Reiter A, Invernizzi R, Cross NC, Cazzola M (2009) Molecular basis of myelodysplastic/myeloproliferative neoplasms. Haematologica 94(12):1634–1638

Sanz GF, Sanz MA, Vallespi T, Canizo MC, Torrabadella M, Garcia S et al (1989) Two regression models and a scoring system for predicting survival and planning treatment in myelodysplastic syndromes: a multivariate analysis of prognostic factors in 370 patients. Blood 74(1):395–408

Siitonen T, Timonen T, Juvonen E, Terava V, Kutila A, Honkanen T et al (2007) Valproic acid combined with 13-cis retinoic acid and 1,25-dihydroxyvitamin D3 in the treatment of patients with myelodysplastic syndromes. Haematologica 92(8):1119–1122

Such E, Cervera J, Costa D, Sole F, Vallespi T, Luno E et al (2011) Cytogenetic risk stratification in chronic myelomonocytic leukemia. Haematologica 96(3):375–383

Sugimoto Y, Muramatsu H, Makishima H, Prince C, Jankowska AM, Yoshida N et al (2010) Spectrum of molecular defects in juvenile myelomonocytic leukaemia includes ASXL1 mutations. Br J Haematol 150(1):83–87

Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (eds) (2008) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. International Agency for Research on Cancer, Lyon

Szpurka H, Jankowska AM, Makishima H, Bodo J, Bejanyan N, Hsi ED et al (2010) Spectrum of mutations in RARS-T patients includes TET2 and ASXL1 mutations. Leuk Res 34(8):969–973

Tiu RV, Visconte V, Traina F, Schwandt A, Maciejewski JP (2011a) Updates in cytogenetics and molecular markers in MDS. Curr Hematol Malig Rep 6(2):126–135

Tiu RV, Gondek LP, O'Keefe CL, Elson P, Huh J, Mohamedali A et al (2011b) Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies. Blood 117(17):4552–4560

Visconte V, Makishima H, Jankowska A, Szpurka H, Traina F, Jerez A et al (2012a) SF3B1, a splicing factor is frequently mutated in refractory anemia with ring sideroblasts. Leukemia 26(3):542–545

Visconte V, Rogers HJ, Singh J, Barnard J, Bupathi M, Traina F et al (2012b) SF3B1 haploinsufficiency leads to formation of ring sideroblasts in myelodysplastic syndromes. Blood 120(16):3173–3186

Wattel E, Guerci A, Hecquet B, Economopoulos T, Copplestone A, Mahe B et al (1996) A randomized trial of hydroxyurea versus VP16 in adult chronic myelomonocytic leukemia. Groupe Francais des Myelodysplasies and European CMML Group. Blood 88(7):2480–2487

Williamson PJ, Kruger AR, Reynolds PJ, Hamblin TJ, Oscier DG (1994) Establishing the incidence of myelodysplastic syndrome. Br J Haematol 87(4): 743–745

Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R et al (2011) Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 478(7367):64–69

Yoshida N, Doisaki S, Kojima S (2012) Current management of juvenile myelomonocytic leukemia and the impact of RAS mutations. Paediatr Drugs 14(3): 157–163

Yoshimi A, Bader P, Matthes-Martin S, Stary J, Sedlacek P, Duffner U et al (2005) Donor leukocyte infusion after hematopoietic stem cell transplantation in patients with juvenile myelomonocytic leukemia. Leukemia 19(6):971–977

Classification and Staging of Myelodysplastic Syndromes

8

Torsten Haferlach and Ulrike Bacher

8.1 Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal hematologic disorders that are usually diagnosed based on findings in peripheral blood and especially the bone marrow. MDS is characterized by ineffective hematopoiesis, showing dysplastic features in at least one hematopoietic lineage in the bone marrow. Considering the introduction of more differentiated therapeutic options, e.g., due to demethylating agents, lenalidomide, or allogeneic stem cell transplantation for high-risk cases, valid and easily reproducible classification systems became of major importance in the care of patients with MDS. With the use of standard classification systems, e.g., formerly the French-American-British (FAB) classification (Bennett et al. 1982), the International Prognostic Scoring System (IPSS) (Greenberg et al. 1997) or the Revised IPSS (IPSS-R) (Greenberg et al. 2012), the WPSS (Malcovati et al. 2007), or the new World Health Organization (WHO) proposal (Brunning et al. 2008), staging and classification of MDS are readily achieved. The IPSS and IPSS-R are often combined with FAB or WHO morphological criteria to provide the most complete clinical picture and the most accurate prognostic assessment possible (Bennett 2005). However, various problems remain critical in daily routine:

- How to diagnose early stages of MDS and discriminate these from other nonmalignant disorders
- How to measure and reproducibly assess dysplasia, the hallmark of MDS staging and classification
- How to reproducibly determine the proportion of blasts, an essential parameter for MDS diagnosis and classification as well as treatment decisions
- How to include multiparameter flow cytometry in diagnosis and follow-up in MDS
- How to incorporate biological markers, e.g., new aspects of cytogenetics (Haase et al. 2007; Haase 2008) or, very recently, molecular mutations (Papaemmanuil et al. 2011; Yoshida et al. 2011), into prognostic staging and scoring systems
- Determining how different MDS is from AML and if current classification systems propagate artificial distinctions by adhering to rigid definitions

Clearly, even 30 years after the development of the FAB classification of MDS, many questions on classification and staging remain and still have to be addressed in the future.

T. Haferlach, MD (✉) • U. Bacher, MD
MLL Munich Leukemia Laboratory,
Max-Lebsche-Platz 31, 81377 Munich, Germany
e-mail: torsten.haferlach@mll.com,
ulrike.bacher@mll.com

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_8, © Springer-Verlag Berlin Heidelberg 2013

8.2 Diagnostic Procedures Needed for Staging and Classification

During the past 30 years, the diagnosis, classification, and staging of MDS have evolved from relying on cytomorphology alone to a comprehensive setting of different methods that have improved our ability to establish the diagnosis and to arrive at treatment decisions. State-of-the-art staging and classification should be based on a stepwise algorithm that first combines cytomorphology and cytochemistry accompanied by cytogenetics and later may be completed by immunophenotyping and now also molecular genetic methods in a laboratory setting.

Generally, we start with peripheral blood smears and bone marrow cytomorphology, cytochemistry, and iron stains. Metaphase cytogenetics should be obtained in every case in which MDS is suspected. Where needed, the latter should be accompanied by interphase fluorescence in situ hybridization (FISH) or other FISH techniques, e.g., 24-color FISH, to confirm aberrant findings observed in metaphase karyotyping. As the karyotype plays an important role in MDS classification according to IPSS (Greenberg et al. 1997) or IPSS-R (Greenberg et al. 2012), WPSS (Malcovati et al. 2007), and WHO (Brunning et al. 2008), cytogenetic results are mandatory for prognostication and may, in case of missing metaphases, also be addressed using FISH on interphase cells.

8.2.1 Sample Collection and Preanalytic Procedures

Several prerequisites must be fulfilled for reproducible results at diagnosis of MDS:

- Different methods rely on different sources of biologic materials. For example, cytomorphology is impaired by heparin, and good metaphase spreads cannot be expected if EDTA was added to the syringe.
- In all cases with cytopenia or suspected MDS, blood and bone marrow samples should be obtained in parallel.
- A trephine biopsy is recommended, especially in cases with very hypocellular or inaspirable bone marrow ("punctio sicca") and peripheral cytopenia. In these circumstances, peripheral blood should be analyzed (including FISH panel), but smears for cytomorphology can also be produced from trephine cylinders.
- Similarly, for cytogenetics (in case of a "punctio sicca" or a dry tap, respectively), a metaphase analysis can be done after culturing the trephine biopsy in appropriate medium (i.e., NaCl 0.9 % with heparin) and processing the medium (plus trephine cylinder) for karyotyping.

A comprehensive investigation at diagnosis requires 3–5 mL EDTA-anticoagulated bone marrow for morphology, 10 mL peripheral blood in EDTA, 5–10 mL heparinized bone marrow for cytogenetics, and 10–20 mL heparinized peripheral blood. Investigations can be performed with fewer cells; however, one should not jeopardize a comprehensive diagnostic workup by too limited biologic specimen. The material should reach the laboratory within 24 h. It should be shipped at room temperature without adding cool packs or dry ice. With these precautions, a successful investigation is possible in more than 90 % of cases, including metaphase cytogenetics. Multiparameter flow cytometry and molecular investigation, by different PCR assays or next-generation sequencing (NGS), can be performed either on heparinized or on EDTA-anticoagulated sample material. Expression of genes (e.g., *WT1*) may be investigated by real-time PCR or microarrays. This means that a central reference laboratory has to be available on a daily basis for optimal service.

8.2.2 Cytomorphology and Cytochemistry

For cytomorphology and cytochemistry, at least five peripheral blood smears and five bone marrow smears should be available. After they have been air-dried without any further fixation for at least 45 min, Pappenheim or May-Grünwald-Giemsa (MGG) staining (Figs. 8.1 and 8.2) should be performed and accompanied

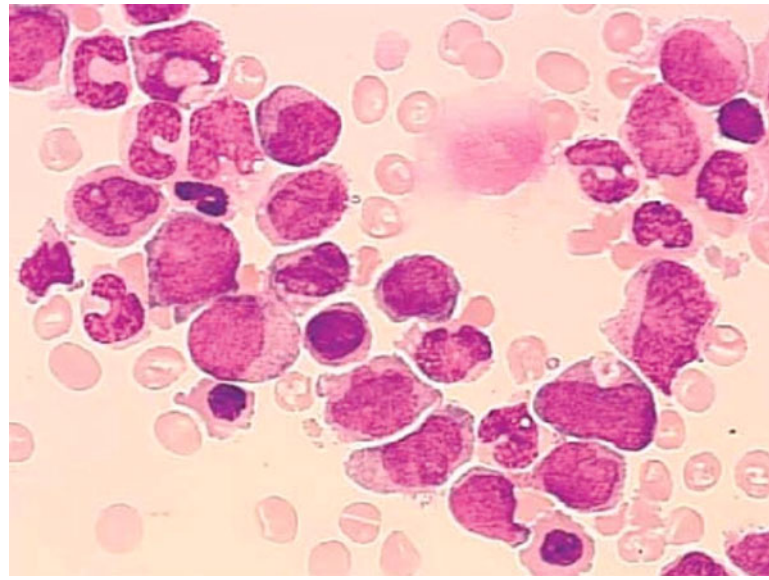

Fig. 8.1 Pappenheim staining (×630) of a bone marrow smear. In some cases, the morphology is difficult and can lead to the diagnosis of MDS RAEB-2, CMML, or even AML FAB M4. This difficulty underscores the need for clear definitions in classification and staging in MDS

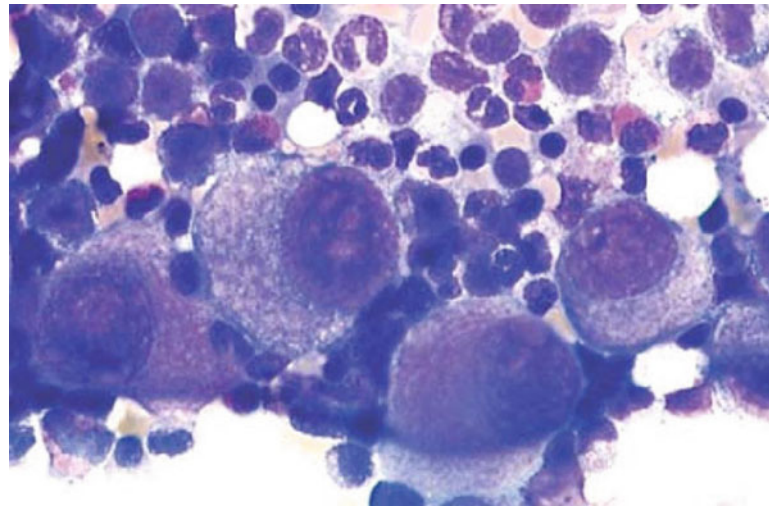

Fig. 8.2 Pappenheim staining (×630) of a bone marrow smear. Typical aspects of MDS with isolated 5q-deletion (or "5q− syndrome," respectively) with megakaryocytes with round, non-segmented nuclei and relatively mature cytoplasm. No blasts are present

by myeloperoxidase (MPO, Fig. 8.3) and nonspecific esterase (NSE, Fig. 8.4) stainings (Löffler et al. 2010). These stains are necessary to satisfy the cytomorphological needs: determination of percentage of blasts, degree of dysplasia in all three cell lineages, and myeloperoxidase deficiency in the MPO-negative and MPO-positive cell compartments. The detection or exclusion of monocytes or monoblasts leading to the diagnosis of CMML is possible by NSE. In addition, an iron stain is mandatory for staging and classification in MDS (Fig. 8.5). Stainings such as periodic acid-Schiff (PAS) reaction, acid phosphatase, or chloroacetate esterase (CE) stains do not add important information today. Exceptions may be special cases, for instance, the demonstration of glycogen in the erythroid lineage (PAS) as a sign of dyserythropoiesis or the use of CE in histological sections where this stain represents the best method for the demonstration of neutrophilic/granulocytic lineage.

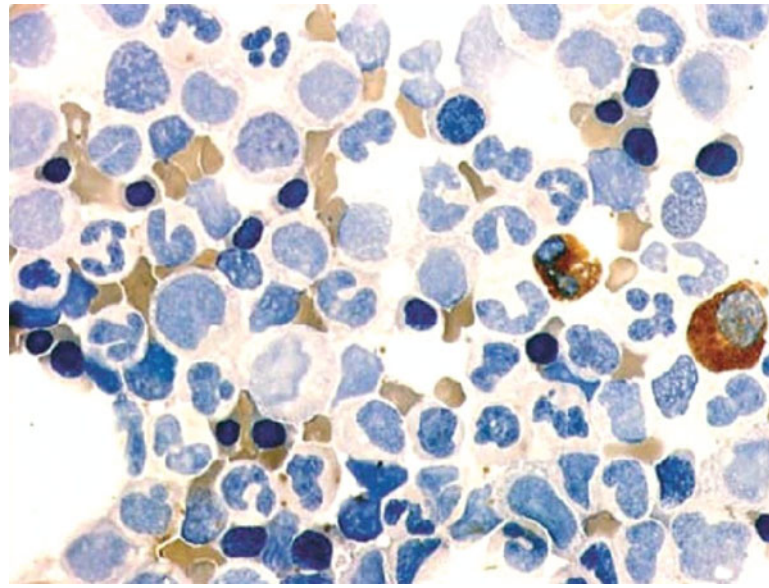

Fig. 8.3 Myeloperoxidase reaction (MPO, ×630) on a bone marrow smear from a patient with RAEB-2. Most polymorphonuclear neutrophils (PMNs) stain negative, demonstrating MPO deficiency as a dysplastic feature in granulopoiesis

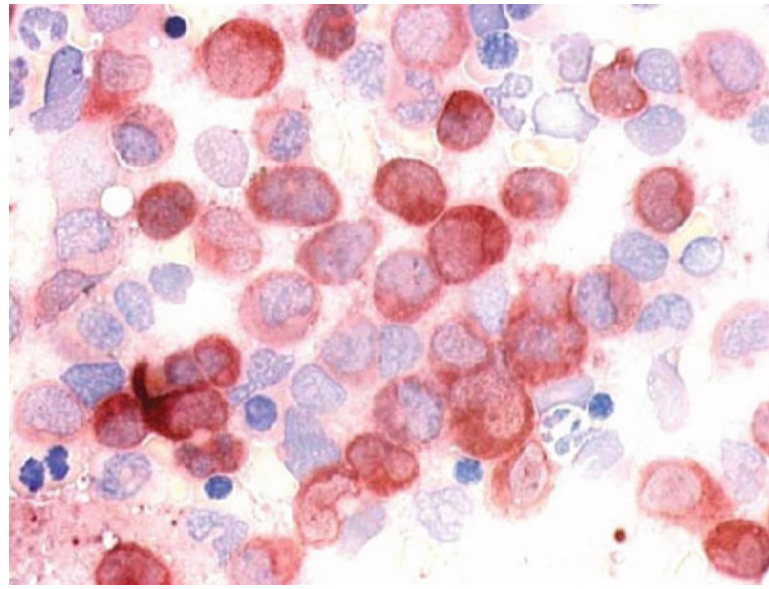

Fig. 8.4 Nonspecific esterase (NSE, ×630) on a bone marrow smear from a patient with CMML. The number of abnormal monocytes is high and would be underestimated if only Pappenheim or May-Grünwald-Giemsa (MGG) stains were used

8.2.3 Cytogenetics

A chromosome banding analysis is absolutely mandatory in the diagnostic approach to suspected or proven MDS. The importance of cytogenetics is addressed in detail in Chap. 6. For fluorescence in situ hybridization (FISH), metaphases as well as interphase nuclei from cytomorphological smears of bone marrow or peripheral blood can be used. Probes for interphase FISH (IP-FISH), whole chromosome painting (WCP-) FISH, 24-color FISH, or comparative genomic hybridization (CGH) are usually hybridized in an overnight procedure and are available for analysis 24 h after the sample had reached the laboratory. Thus, a suspected MDS with isolated 5q-deletion (previously called "5q− syndrome") can generally be proven in 24 h after bone marrow biopsy using interphase FISH.

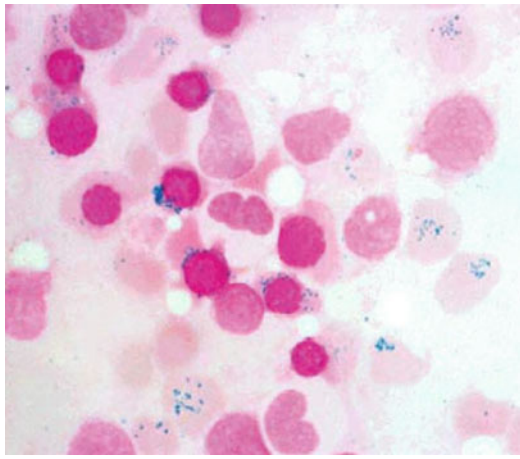

Fig. 8.5 Iron staining (×630) in a case of RARS

8.3 Diagnostic Criteria

The classification of MDS should follow the WHO proposal (2008) (Brunning et al. 2008) and the IPSS-R (Greenberg et al. 2012).

8.3.1 Cytomorphology and Cytochemistry

The first step for the diagnosis of MDS is cytomorphology and cytochemistry plus iron stain. It is quick and cheap, and the results allow drawing up an optimal workflow for other much more labor-intensive and expensive techniques. Cytomorphology also needs to discriminate MDS from AML or other diseases. At least 500 nucleated cells in the bone marrow aspirate and 200 cells in the peripheral blood should be analyzed according to WHO standards (2008). Histopathology may contribute additional aspects, e.g., with regard to cellularity or the degree of myelofibrosis, and should be performed at diagnosis of MDS as well. Some aspects of cytomorphology deserve to be detailed here.

8.3.1.1 Dysplasia in MDS: The WHO Criteria (2008)

According to the WHO, the threshold for MDS is 10 % or more of cells of a given hematopoietic lineage (Brunning et al. 2008) demonstrating one or more dysplastic features as outlined below. In contrast, to define dysplasia in AML, a threshold of 50 % of cells has been chosen (Goasguen et al. 1992).

8.3.1.2 Criteria for Dysgranulopoiesis

Ten percent or more of polymorphonuclear neutrophils (PMNs) show one or more of the following abnormalities:

- Hypo- or agranulation
- Nuclear hyposegmentation (pseudo-Pelger-Huët anomaly) or hypersegmentation
- Myeloperoxidase deficiency

8.3.1.3 Criteria for Dyserythropoiesis

Ten percent or more of erythroid precursors investigated show one of the following abnormalities:

- Karyorrhexis, budding, and internuclear bridging
- Megaloblastoid aspects
- Multinuclearity
- Nuclear fragments
- Ring sideroblasts
- Vacuolization and PAS positivity

8.3.1.4 Criteria for Dysmegakaryopoiesis

Of at least 30 megakaryocytes investigated, 10 % or more show one of the following abnormalities:

- Micromegakaryocytes with hypolobulated nuclei
- Multiple, widely separated nuclei
- Large mononuclear forms

8.3.1.5 Ring Sideroblasts

As the presence of ring sideroblasts defines specific subgroups of MDS according to both FAB (Bennett et al. 1982) and WHO (2008) (Brunning et al. 2008), specific criteria for ring sideroblasts have been established as follows:

- In refractory anemia with ring sideroblasts (RARS), "ring sideroblasts account for 15 % or more of red cell precursors in the bone marrow."
- Ring sideroblasts have five or more iron granules encircling one third or more of the nucleus (Mufti et al. 2011).

8.3.2 Cytogenetics and FISH (See Chap. 6)

Considering the cytogenetic heterogeneity of MDS and considering that the karyotype is one of the strongest prognostic parameters in this entity, chromosome banding analysis and FISH both play a central role for diagnosis and therapeutic decisions in these patients. The results of chromosome banding analysis and FISH should be given according to the ISCN nomenclature (Shaffer et al. 2013).

According to European LeukemiaNet (ELN) guidelines, at least two cultures should be performed for metaphase cultivation, one for 24 h and one for 48 h. Bone marrow should be used for cytogenetics because the cells belonging to the MDS clone are usually not dividing in peripheral blood. Before harvesting, colcemid exposure for 0.5–2 h is recommended. In MDS cases with low cell count, prolonged colcemid exposure increases the success rate. In case of insufficient metaphase harvest, FISH assays including probes for the detection of deletions of 5q or 7q, loss of chromosome 7, trisomy 8, *TP53* deletions, and loss of the Y chromosome (in male patients) should be performed as these abnormalities constitute the most frequent abnormalities in MDS (Haferlach et al. 2007). According to International Working Group (IWG) criteria, 20 analyzable metaphases are required to diagnose or exclude the presence of a cytogenetic abnormality. For the detection of a normal karyotype, 20 metaphases provide optimal diagnostic safety (Cheson et al. 2006). To define the degree of cytogenetic response, 20 metaphases are recommended by the International Working Group (Cheson et al. 2006).

Following the WHO, the detection of the reciprocal translocations such as the t(15;17)/*PML-RARA*, inv(16)/t(16;16)/*CBFB-MYH11*, or t(8;21)/*RUNX1-RUNX1T1* by cytogenetics or molecular genetics classifies a case as AML independently of the percentage of bone marrow myeloblasts (Arber et al. 2008). This is a very important step for a genetically based approach in classification and clearly separates these subentities from MDS. The same is true when discriminating MDS associated with isolated 5q-deletion (formerly called "5q− syndrome") from all other MDS subtypes. MDS associated with isolated 5q-deletion, which is caused by a defect in ribosomal protein function (Ebert et al. 2008), shows, by definition, less than 5 % blasts and has been recognized as a unique entity for several reasons: age ≥60 years, preponderance of female sex, high platelet counts, and a favorable prognosis (Van den Berghe and Michaux 1997). Other cytogenetic findings confer a significant prognostic impact and, thus, are needed to allow a risk-adapted treatment selection, although they are not yet included in diagnostic classification systems in MDS.

8.3.3 Molecular Methods

Each method revealing new insights into the biologic background of MDS may generate disease-specific markers important for the management of the disease: Several new aspects have been added to the characterization of MDS by way of molecular biology. Known mutations in MDS include *RUNX1* (= *AML1*; in 10–15 % of all cases), *NRAS* (around 10 %), and, in lower frequencies, *MLL*-PTD, *FLT3*-ITD, *FLT3*-TKD, *NPM1*, or *CEBPA*. These markers have been shown to increase in frequency with the progression of MDS stages and the transformation to s-AML. In recent years, other mutations were identified to be frequent in MDS, e.g. of the *TET2* gene. Moreover, mutations in genes with a function for the spliceosome, e.g. affecting the *SF3B1* or *U2AF1* genes, were found to show frequent occurrence in MDS (Papaemmanuil et al. 2011; Yoshida et al. 2011). Thereby, mutations of *SF3B1* show a close association to MDS subtypes with an increase of ring sideroblasts. Bejar et al. demonstrated that the occurrence of point mutations in one or more of the genes *TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1* was predictive of poor overall survival in patients with MDS, independently of established risk factors such as IPSS (Bejar et al. 2011). The introduction of next-generation sequencing (NGS) presently allows a rapid expansion of the molecular marker panel in MDS. Measurement of *WT1* gene expression by real-time PCR can be used for follow-up studies. The analysis of gene expression profiles using microarrays showed interesting correlations with MDS stages and allowed a discrimination of MDS cases from AML.

Table 8.1 The FAB classification of myelodysplastic syndromes (Bennett et al. 1982)

Subtype	Blasts in PB (%)	Blasts in BM (%)	Ring sideroblasts (BM) (%)	Monocytes in PB	Auer rods
Refractory anemia (RA)	<1	<5	<15	$<1.0\times10^9$/L	No
Refractory anemia with ring sideroblasts (RARS)	<1	<5	>=15	$<1.0\times10^9$/L	No
Refractory anemia with excess of blasts (RAEB)	<5	5–20	<15/>=15	$<1.0\times10^9$/L	No
Refractory anemia with excess of blasts in transformation (RAEB-t)	≥5	21–30	<15/>=15	$<1.0\times10^9$/L	Possible
Chronic myelomonocytic leukemia (CMML)	<5	Up to 20	<15	$>=1.0\times10^9$/L	No

PB peripheral blood, *BM* bone marrow

8.4 Overview of Classification and Staging Systems

8.4.1 The FAB Classification

The French-American-British (FAB) classification of MDS (1982) (Bennett et al. 1982) has influenced the classification of MDS (Table 8.1) up to today as this classification system represents one cornerstone of the WHO proposal.

8.4.2 The Revised International Prognostic Scoring System (IPSS-R)

The first version of the International Prognostic Scoring System (IPSS) was published in 1997 by Greenberg et al. (1997). This scoring system already allowed clinicians to perform risk stratification for patients with MDS based on the number of peripheral blood cytopenias, the blast count, and the cytogenetic risk group. The IPSS separated patients into four risk groups. Recently, the International Working Group for Prognosis in MDS (IWG-PM) published a revision of the IPSS, the "Revised International Prognostic Scoring System" (IPSS-R) (Greenberg et al. 2012). Based on the evaluation of a large data set of 7,012 patients with different MDS subtypes, the Working Group succeeded to create a novel and more differentiated prognostic scoring system. This novel system finally was discriminating five risk groups. The following parameters were used:

Karyotypes: Patients are separated into five different prognostic categories ("very good"; "good"; "intermediate"; "poor"; "very poor") following the proposal of Schanz et al. (2012).

Bone marrow blast percentages: <=2; >2–<5; 5–10; >10 %.

Peripheral blood values: More differentiated thresholds for peripheral blood values were introduced.

Hemoglobin: >=10; 8–<10; <8 g/dL.

Platelets: >=100; 50–<100; $<50\times10^9$/L.

Neutrophils: >=0.8; $<0.8\times10^9$/L.

The sum of the scoring points (Table 8.2) results in the following prognostic risk categories: "very low risk": <=1.5; "low risk": >1.5–3; "intermediate risk": >3–4.5; "high risk": >4.5–6; "very high risk": >6 scoring points.

Table 8.2 The Revised International Prognostic Scoring System (IPSS-R) for Myelodysplastic Syndromes (Greenberg et al. 2012)

	Scoring points						
Prognostic variable	0	0.5	1	1.5	2	3	4
Cytogenetic category	Very good		Good		Intermediate	Poor	Very poor
Bone marrow blasts (%)	≤2	–	>2–<5	–	5–10	>10	–
Hemoglobin (g/dL)	≥10	–	8–<10	<8	–	–	–
Platelets ($\times 10^9$/L)	≥100	50–<100	<50	–	–	–	–
Neutrophils ($\times 10^9$/L)	≥0.8	<0.8	–	–	–	–	–

8.4.3 The WHO Classification (2008)

The classification proposed by the WHO was published in 2001 (Jaffe et al. 2001) and revised in 2008 (Swerdlow et al. 2008). Details are shown in Table 8.3. This classification system should be used in the staging and classification of MDS. It also can be applied in daily routine but should be accompanied by the IPSS or IPSS-R, respectively, for clinical decision-making and prognostication. This is particularly important since the WHO system considers cytogenetics only for one category, MDS associated with isolated 5q-deletion ("5q− syndrome"). As cytogenetics are very informative for prognostication in MDS (see IPSS and IPSS-R scores), it is mandatory to include the results in staging and classification.

As in the FAB classification, the WHO classification is mostly based on the percentage of myeloblasts in the bone marrow and peripheral blood, the type and degree of dysplasia, and the presence of ring sideroblasts. Some new categories were introduced, which must be validated with regard to their unique biologic and clinical pattern in future studies. Refractory cytopenia with unilineage dysplasia (RCUD) comprises the new subcategories (1) refractory thrombocytopenia (RT) and (2) refractory neutropenia (RN) in addition to the previously established (3) refractory anemia (RA), which had already been part of the FAB classification. Refractory cytopenia with multilineage dysplasia (RCMD) has been created for cases with dysplastic cells in ≥10 % of cells in two or three lineages. Refractory anemia with ring sideroblasts (RARS) was maintained from the previous FAB classification. According to WHO 2008, cases with multilineage dysplasia and an increase of ring sideroblasts to 15 % or more of erythroid precursors (the previous "RCMD-RS" in WHO, 2001) are also classified as RCMD due to the overlapping clinical outcomes of both entities (Germing et al. 2000). Refractory anemia with excess of blasts (RAEB) was subdivided by blast percentages in the RAEB-1 (5–9 % bone marrow and 2–4 % peripheral blasts) and RAEB-2 (10–19 % bone marrow and 5–19 % peripheral blasts) subcategories. Cases with Auer rods categorize a case as RAEB-2 irrespective of a blast threshold below this cytomorphological category. Myelodysplastic syndrome, unclassifiable (MDS-U) lacks findings appropriate for any other MDS category.

An important and major change in comparison to the FAB classification has been the elimination of the former FAB category RAEB in transformation (RAEB-t), as all patients with 20 % myeloblasts or more are to be classified as AML in the WHO system. This step was based on the clinical similarities of RAEB-t and AML cases, although this has not been without controversy.

Furthermore, the WHO classification 2008 incorporated therapy-related myelodysplastic syndrome (t-MDS) together with t-AML into the category of "therapy-related myeloid neoplasms" due to the uniqueness of the clinical syndrome.

Chronic myelomonocytic leukemia (CMML) was excluded from the MDS category by the WHO and was transferred to the category of overlapping myelodysplastic/myeloproliferative neoplasms (see below).

8.4.3.1 Validation of the WHO Classification of MDS

The use of the WHO system in MDS in comparison to FAB and IPSS has been addressed

Table 8.3 The WHO classification of myelodysplastic syndromes, 2008 (Brunning et al. 2008)

Entity	Dysplasia	Blasts PB (%)	Blasts BM (%)	Ring sideroblasts (%)	Cytogenetics
MDS with isolated 5q-deletion	Mostly DysE	<1	<5	<15	Sole 5q-deletion
RA, RN, RT, RCUD	DysE, DysG, DysM	<1	<5	<15	Various
RARS	Mostly DysE	0	<5 %	≥15	Various
RCMD	2–3 lineages	<1	<5	<15/≥15	Various
RAEB-1	1–3 lineages	<5	5–9	<15/≥15	Various
RAEB-2	1–3 lineages	5–19	10–19	<15/≥15	Various
		Auer rods +/–	Auer rods +/–		
MDS-U	1–3 lineages	≤1	<5	<15	Various

PB peripheral blood, *BM* bone marrow, *RA* refractory anemia, *RN* refractory neutropenia, *RT* refractory thrombocytopenia, *RCUD* refractory cytopenia with unilineage dysplasia, *DysE* dysplastic erythropoiesis, *DysG* dysplastic granulopoiesis, *DysM* dysplastic megakaryopoiesis, *RARS* refractory anemia with ring sideroblasts, *RCMD* refractory cytopenia with multilineage dysplasia, *RAEB* refractory anemia with excess of blasts, *MDS-U* MDS, unclassifiable

in several reports. In particular, in MDS cases with less than 5 % bone marrow blasts, i.e., RA, RCMD, and MDS with isolated 5q-deletion ("5q− syndrome"), the WHO classification has been shown to provide significant prognostic information. In a series of 103 patients, the median survival for RCMD cases was 27 months compared with 53 months and more than 102 months in cases with a 5q− syndrome and RA, respectively (Cermak et al. 2003). Importantly, RA and RCMD comprise similarly sized subgroups, making these differences in outcome clinically relevant. Furthermore, it has been reported that, within the subgroups of patients with less than 5 % bone marrow blasts, the number of cell lineages involved has a prognostic impact. In a series of 64 patients, those with unilineage dysplasia had a significantly better survival than patients with multilineage dysplasia (median not reached vs. 29 months) (Howe et al. 2004). In a large series of 1,243 patients with MDS, similar differences were found between RA and RCMD with regard to median survival (69 vs. 33 months) (Germing et al. 2000). While in MDS subgroups with higher blast counts the similarities of the disease to AML may determine the clinical course, these data clearly suggest that in cases with low blast counts, the WHO classification is an improvement over the former classifications and their clinical utility.

8.4.4 Diagnosis of MDS Cases with Less than 5 % of Bone Marrow Blasts

The classification of MDS, especially with less than 5 % bone marrow blasts, is the most difficult task for cytomorphologists in hematology today. Furthermore, the threshold for the definition of dysplasia (only 10 %) is very different from that used in AML (50 %). Thus, the categories "MDS with isolated 5q-deletion" ("5q− syndrome"), RA, RCMD, and RARS are very difficult to diagnose unequivocally according to the WHO classification. In these cases, it is recommended that the diagnosis will be confirmed in a reference laboratory. In many cases with borderline features, a repeated bone marrow examination should be performed after an interval of 2–3 months before the final diagnosis of MDS is made. In all cases, additional methods such as cytogenetics, FISH, molecular mutation screening, or even multiparametric flow cytometry may support these subcategories if they detect specific and unquestionable markers or signs for MDS or can exclude this diagnosis.

Several differential diagnoses have to be considered in cases suspected for MDS: nutritional or toxic factors such as alcohol; drugs, including chemotherapy; arsenic intoxication; exposure to heavy metals; treatment of HIV; or virus infections. Other factors that may mimic MDS can be

the administration of cytokines, parvovirus B19 infection, and especially deficiency in vitamin E, B12, or folic acid. Even congenital dyserythropoietic anemia, hairy cell leukemia, or paroxysmal nocturnal hemoglobinuria (PNH) has been misdiagnosed as MDS. This must lead to the conclusion that the classification of MDS is still difficult, needs highly experienced cytomorphologists and cytogeneticists, and should rely on the combination of several comprehensive techniques. In equivocal cases, at least two different examinations at different time points and by different observers should be performed including a close follow-up of the patient's history.

Recently, two premalignant myeloid conditions, "idiopathic cytopenia of undetermined significance" (ICUS) and "idiopathic bone marrow dysplasia of uncertain significance" (IDUS), were described. ICUS refers to cases with mild dysplasia which do not fulfill the WHO criteria for MDS but in which cytopenias can be severe. In IDUS, the dysplasia is prominent, but cytopenias, if detectable, are mild. Both conditions may progress to an overt MDS but may also progress to another myeloid neoplasm such as AML, a myeloproliferative neoplasm (MPN), or even mastocytosis. In both conditions, a neoplastic clone may already have replaced most or all of normal bone marrow cells when ICUS or IDUS is detected, but evidence to support this possibility is not necessarily available. Thorough hematologic follow-up is recommended for both groups of patients (Valent et al. 2012).

8.4.5 Myelodysplastic/ Myeloproliferative Neoplasms

The WHO introduced a separate category for disorders with overlapping myelodysplastic and myeloproliferative features. This category comprises the entities unclassifiable myelodysplastic/ myeloproliferative neoplasm (MDS/MPN, U), CMML, juvenile myelomonocytic leukemia (JMML), and atypical *BCR-ABL1*-negative CML.

Cases which show at initial presentation clinical, laboratory, and morphological features that overlap both MDS and MPN and cannot be clearly assigned to any of the other entities are called "MDS/MPN, U." Refractory anemia with marked thrombocytosis (RARS-T) is part of the MDS/MPN, U category and is defined by the presence of ring sideroblasts in ≥15 % of the erythroid precursors, a thrombocyte count $\geq 450 \times 10^9$/L, and the proliferation of large atypical megakaryocytes. Prognosis is favorable. There is a high rate of *JAK2*V617F and *SF3B1* mutations, less common are *MPL*W515 mutations.

CMML is defined by persistent monocytosis $>=1.0 \times 10^9$/L in the peripheral blood and fewer than 20 % blasts (promonocytes are considered blast equivalents) in the peripheral blood and bone marrow. CMML is further subdivided into the CMML-1 and CMML-2 subcategories according to the numbers of blasts plus promonocytes in the peripheral blood or bone marrow. Cytogenetic abnormalities are detectable in only 20 % of CMML cases. In recent years, an increasing panel of molecular markers has been detected in patients with CMML (Kohlmann et al. 2010), including mutations of the *TET2*, *CBL*, or *EZH2* genes (Grossmann et al. 2011). Furthermore, mutations of the *SRSF2* gene were identified to be highly frequent in patients with CMML (Meggendorfer et al., Blood, 2012)

8.4.6 Response Criteria in MDS

According to the revised criteria of the International Working Group (IWG), the criteria for complete remission (CR) and partial remission (PR) involve specific improvements in marrow and peripheral blood measurements obtained on two or more successive assessments, and the response parameters in peripheral blood must be maintained for at least 8 weeks. Complete remission requires less than 5 % marrow blasts without evidence of dysplasia and normalization of peripheral blood counts (Hb >=11.0 g/L, neutrophil count $\geq 1.5 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L). A major

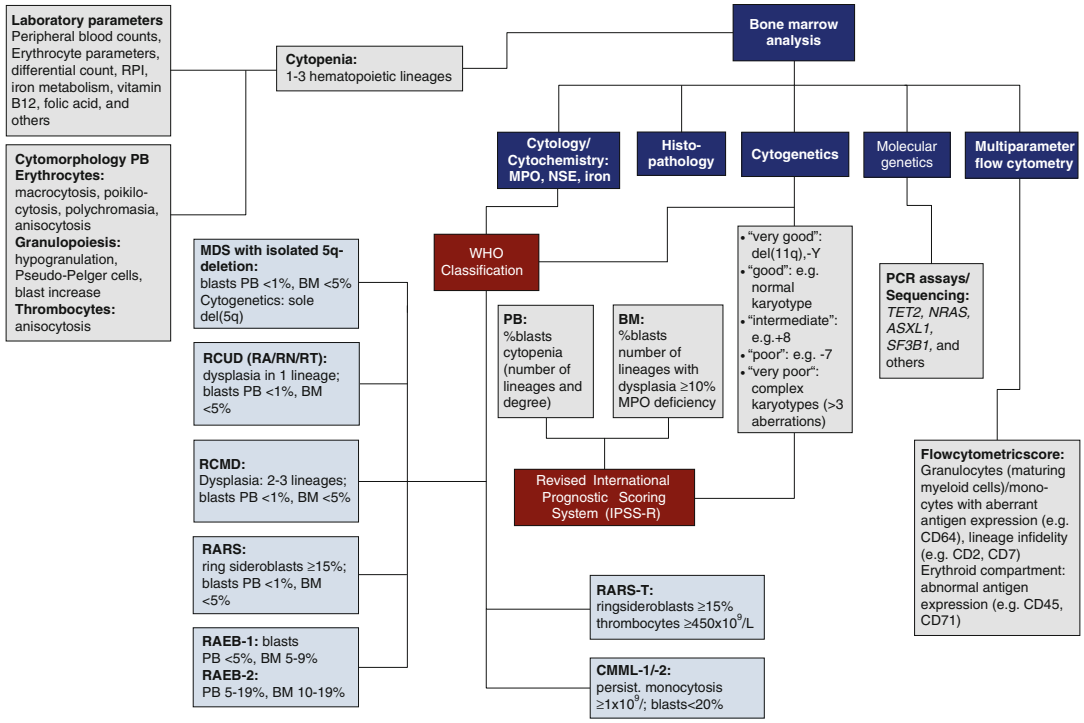

Fig. 8.6 Algorithm of diagnostic procedures and classification systems in myelodysplastic syndromes (*RPI* reticulocyte production index, *PB* peripheral blood, *BM* bone marrow, *MPO* myeloperoxidase, *NSE* nonspecific esterase, *KT* karyotype, *del* deletion, *RCUD* refractory cytopenia with unilineage dysplasia, *RA* refractory anemia, *RN* refractory neutropenia, *RT* refractory thrombocytopenia, *RCMD* refractory cytopenia with multilineage dysplasia, *RARS* refractory anemia with ring sideroblasts, *RAEB* refractory anemia with excess of blasts, *RARS-T* refractory anemia with ring sideroblasts and marked thrombocytosis, *CMML* chronic myelomonocytic leukemia)

cytogenetic response refers to the disappearance of a cytogenetic abnormality; a minor cytogenetic response is 50 % or more reduction of abnormal metaphases. Other than that, the criteria for partial remission, for hematologic improvement, and for treatment failure and relapse were defined (Cheson et al. 2006).

Conclusion

So far established diagnostic staging and classification systems for myelodysplastic syndromes as the WHO classification (2008) (Brunning et al. 2008) and the IPSS or IPSS-R (Greenberg et al. 1997; Greenberg et al. 2012) result from a sophisticated combination of cytomorphology and cytogenetics (Fig. 8.6) in combination with peripheral blood parameters. With the detection of an increasing panel of molecular markers (Bejar et al. 2011) which has been shown to correlate with the stages of MDS (Dicker et al. 2010) and distinct morphological aspects (Papaemmanuil et al. 2011; Yoshida et al. 2011), it has to be anticipated that future classification and risk stratification systems for MDS patients will increasingly include these new markers. Further on, the revision of the IPSS attributes more weight to cytogenetic parameters. Therefore, the diagnosis and classification of myelodysplastic syndromes may undergo important changes already in the near future based mainly on cytogenetics and molecular genetic discoveries.

References

Arber B, Brunning R, Le Beau MM, Falini B (2008) Acute myeloid leukemia with recurrent genetic abnormalities. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (eds) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. IARC Press, Lyon, pp 110–123

Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, Kantarjian H, Raza A, Levine RL, Neuberg D, Ebert BL (2011) Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 364:2496–2506

Bennett JM (2005) A comparative review of classification systems in myelodysplastic syndromes (MDS). Semin Oncol 32:S3–S10

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C (1982) Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51:189–199

Brunning R, Orazi A, Germing U, Le Beau MM, Porwit A, Baumann I, Vardiman JW, Hellström-Lindberg E (2008) Myelodysplastic syndromes/neoplasms, overview. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (eds) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. IARC Press, Lyon, pp 87–93

Cermak J, Michalova K, Brezinova J, Zemanova Z (2003) A prognostic impact of separation of refractory cytopenia with multilineage dysplasia and 5q− syndrome from refractory anemia in primary myelodysplastic syndrome. Leuk Res 27:221–229

Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, Pinto A, Beran M, de Witte TM, Stone RM, Mittelman M, Sanz GF, Gore SD, Schiffer CA, Kantarjian H (2006) Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 108:419–425

Dicker F, Haferlach C, Sundermann J, Wendland N, Weiss T, Kern W, Haferlach T, Schnittger S (2010) Mutation analysis for RUNX1, MLL-PTD, FLT3-ITD, NPM1 and NRAS in 269 patients with MDS or secondary AML. Leukemia 24:1528–1532

Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N, Raza A, Root DE, Attar E, Ellis SR, Golub TR (2008) Identification of RPS14 as a 5q− syndrome gene by RNA interference screen. Nature 451:335–339

Germing U, Gattermann N, Strupp C, Aivado M, Aul C (2000) Validation of the WHO proposals for a new classification of primary myelodysplastic syndromes: a retrospective analysis of 1,600 patients. Leuk Res 24:983–992

Goasguen JE, Matsuo T, Cox C, Bennett JM (1992) Evaluation of the dysmyelopoiesis in 336 patients with de novo acute myeloid leukemia: major importance of dysgranulopoiesis for remission and survival. Leukemia 6:520–525

Greenberg P, Cox C, Le Beau 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 (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89:2079–2088

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, Luebbert M, Maciejewski J, Magalhaes SM, Miyazaki Y, Pfeilstöcker M, Sekeres M, Sperr WR, Stauder R, Tauro S, Valent P, Vallespi T, van de Loosdrecht AA, Germing U, Haase D (2012) Revised international prognostic scoring system for myelodysplastic syndromes. Blood 120:2454–2465

Grossmann V, Kohlmann A, Eder C, Haferlach C, Kern W, Cross NC, Haferlach T, Schnittger S (2011) Molecular profiling of chronic myelomonocytic leukemia reveals diverse mutations in >80 % of patients with TET2 and EZH2 being of high prognostic relevance. Leukemia 25:877–879

Haase D (2008) Cytogenetic features in myelodysplastic syndromes. Ann Hematol 87:515–526

Haase D, Germing U, Schanz J, Pfeilstöcker M, Nösslinger T, Hildebrandt B, Kundgen A, Lübbert M, Kunzmann R, Giagounidis AA, Aul C, Trümper L, Krieger O, Stauder R, Müller TH, Wimazal F, Valent P, Fonatsch C, Steidl C (2007) New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2,124 patients. Blood 110:4385–4395

Haferlach C, Rieder H, Lillington DM, Dastugue N, Hagemeijer A, Harbott J, Stilgenbauer S, Knuutila S, Johansson B, Fonatsch C (2007) Proposals for standardized protocols for cytogenetic analyses of acute leukemias, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myeloproliferative disorders, and myelodysplastic syndromes. Genes Chromosomes Cancer 46:494–499

Howe RB, Porwit-MacDonald A, Wanat R, Tehranchi R, Hellström-Lindberg E (2004) The WHO classification of MDS does make a difference. Blood 103:3265–3270

Jaffe ES, Harris NL, Stein H, Vardiman JW (2001) Pathology and genetics of tumours of haematopoietic and lymphoid tissues, 3rd edn, World Health Organization classification of tumours. IARC Press, Lyon

Kohlmann A, Grossmann V, Klein HU, Schindela S, Weiss T, Kazak B, Dicker F, Schnittger S, Dugas M, Kern W, Haferlach C, Haferlach T (2010) Next-generation sequencing technology reveals a characteristic pattern of molecular mutations in 72.8 % of chronic myelomonocytic leukemia by detecting frequent alterations in TET2, CBL, RAS, and RUNX1. J Clin Oncol 28:3858–3865

Löffler H, Raststetter J, Haferlach T (2010) Atlas of clinical hematology, 6th edn. Springer, Berlin

Malcovati L, Germing U, Kuendgen A, la Porta MG, Pascutto C, Invernizzi R, Giagounidis A, Hildebrandt

B, Bernasconi P, Knipp S, Strupp C, Lazzarino M, Aul C, Cazzola M (2007) Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 25:3503–3510

Meggendorfer M, Roller A, Haferlach T, Eder C, Dicker F, Grossmann V, Kohlmann A, Alpermann T, Yoshida K, Ogawa S, Koeffler HP, Kern W, Haferlach C, Schnittger S (2012) SRSF2 mutations in 275 cases with chronic myelomonocytic leukemia (CMML). Blood 120:3080–3088

Mufti G, Bennett J, Goasguen J, Bain B, Baumann I, Brunning R, Cazzola M, Fenaux P, Germing U, Hellström-Lindberg E, Jinnai I, Manabe A, Matsuda A, Niemeyer C, Sanz G, Tomonaga M, Vallespi T, Yoshimi A (2011) Diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts. Haematologica 93:1712–1717

Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D, Pellagati A, Wainscoat J, Hellström-Lindberg E, Gambacorti-Passerini C, Godfrey A, Rapado I, Cvejic A, Rance R, McGee C, Ellis P, Mudie L, Stephens P, McLaren S, Massie C, Tarpey P, Varela I, Nik-Zainal S, Davies H, Shlien A, Jones D, Raine K, Hinton J, Butler A, Teague J, Baxter E, Score J, Galli A, Della-Porta M, Travaglino E, Groves M, Tauro S, Munshi N, Anderson K, El-Naggar A, Fischer A, Mustonen V, Warren A, Cross N, Green A, Futreal P, Stratton M, Campbell P, and the Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium (2011) Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med 365:1384–1395

Schanz J, Tüchler H, Solé F, Mallo M, Luño E, Cervera J, Granada I, Hildebrandt B, Slovak ML, Ohyashiki K, Steidl C, Fonatsch C, Pfeilstöcker M, Nösslinger T, Valent P, Giagounidis A, Aul C, Lübbert M, Stauder R, Krieger O, Garcia-Manero G, Faderl S, Pierce S, Le Beau MM, Bennett JM, Greenberg P, Germing U, Haase D (2012) 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 30:820–829

Shaffer LG, McGowan-Jordan J, Schmid M (2013). An International System for Human Cytogenetic Nomenclature (2013) Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature. S. Karger, Basel.

Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (2008) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. IARC Press, Lyon

Valent P, Bain BJ, Bennett JM, Wimazal F, Sperr WR, Mufti G, Horny HP (2012) Idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of uncertain significance (IDUS), and their distinction from low risk MDS. Leuk Res 36:1–5

Van den Berghe H, Michaux L (1997) 5q−, twenty-five years later: a synopsis. Cancer Genet Cytogenet 94:1–7

Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, Sato Y, Sato-Otsubo A, Kon A, Nagasaki M, Chalkidis G, Suzuki Y, Shiosaka M, Kawahata R, Yamaguchi T, Otsu M, Obara N, Sakata-Yanagimoto M, Ishiyama K, Mori H, Nolte F, Hofmann W, Miyawaki S, Sugano S, Haferlach C, Koeffler H, Shih L, Haferlach T, Chiba S, Nakauchi H, Miyano S, Ogawa S (2011) Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 478:64–69

Immunophenotyping in Myelodysplastic Syndromes

9

Wolfgang Kern and Arjan A. van de Loosdrecht

9.1 Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal hematological disorders that is diagnosed cytomorphologically. They are characterized by ineffective hematopoiesis resulting in peripheral blood cytopenias and showing by definition in all cases dysplastic features in at least one lineage in the bone marrow, an increased myeloid blast count in the bone marrow or typical chromosomal aberrations. They all share a variable risk for transformation into an acute myeloid leukemia.

MDS have been diagnosed by cytomorphology and cytochemistry according to the FAB classification (Bennett et al. 1982) as well as by the WHO classifications in 2001 and 2008 (Jaffe 2001; Swerdlow et al. 2008). In addition, MDS is classified with regard to prognosis and risk of progression to AML by several scoring systems such as the International Prognostic Scoring System (IPSS) (Greenberg et al. 1997) and its revision (Greenberg et al. 2011), the Bournemouth score (Mufti et al. 1985), and the WHO-based Prognostic Scoring System (WPSS) and its revision (Jaffe 2001; Brunning et al. 2008).

While these diagnostic and prognostic classifications have been proven clinically highly relevant, there still is a need for further optimizing both of them given the large number of cases with borderline findings and inconclusive results. To this end, cytogenetic aberrations have already been introduced into the routine diagnostic work-up of patients with proven or suspected MDS, and the increasing number of molecular genetic mutations is anticipated to even strengthen the diagnostic and prognostic power of genetic analyses in MDS. Being a method more related to cytomorphology by describing the phenotype of different cell populations, immunophenotyping by use of multiparameter flow cytometry has been shown to provide specific data on aberrantly expressed antigens on several hematopoietic cells related to both the presence of MDS and the prognosis of patients with proven MDS (Stetler-Stevenson et al. 2001; Wells et al. 2003; Ogata et al. 2002; van de Loosdrecht et al. 2008; Kern et al. 2010; Westers et al. 2012). It is anticipated that immunophenotyping will be added to the diagnostic work-up of patients with suspected MDS and will thus form a multimodal approach in combination with cytomorphology, cytogenetics, and molecular genetics (van de Loosdrecht and Westers 2011; Ossenkoppele et al. 2011; Ogata 2011).

The current WHO 2008 classification recommendations recognize multiple immunophenotype aberrancies (>3) in maturation patterns

W. Kern, MD (✉)
MLL Munich Leukemia Laboratory,
Max-Lebsche-Platz 31, 81377 Munich, Germany
e-mail: wolfgang.kern@mll.com

A.A. van de Loosdrecht, MD, PhD
Department of Hematology, Cancer Center Amsterdam,
VU University Medical Center, De Boelelaan 1117,
1081 HV Amsterdam, The Netherlands
e-mail: a.vandeloosdrecht@vumc.nl

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_9,

as indicative for MDS; moreover, the significance of increased percentages of progenitor cells and aberrant expression of CD34 and/or CD117 on these cells are acknowledged. However, in order to be included as a diagnostic tool for MDS in the next WHO classification, immunophenotypic criteria have to be significantly broadened, standardized, and validated. This chapter provides an overview on available data supporting the diagnostic and prognostic role of immunophenotyping in MDS.

9.2 Application of Immunophenotyping in MDS

Immunophenotyping by multiparameter flow cytometry can identify abnormal progenitor cells and distinguish them from normal ones, enumerate them, as well as detect abnormalities in the maturing neutrophil and monocytic and erythroid subpopulations, respectively. In order to get conclusive results, diagnostic procedures have to be standardized including sample processing, data acquisition, analysis of data, and interpretation of findings (van de Loosdrecht et al. 2009; Westers et al. 2012). Furthermore, it is essential to compare flow cytometric findings in cases with suspected MDS to normal bone marrow controls as well as to nonmalignant diagnoses sharing single clinical findings of MDS, i.e., isolated cytopenias.

9.2.1 Sample Processing

9.2.1.1 Type of Samples

The preferred cell source is bone marrow although the detection of myeloid blasts in peripheral blood may be suggestive of MDS, particularly if an aberrant antigen expression is present. Heparin as an anticoagulant has the advantage over EDTA of a prolonged cell viability and of less alterations in antigen expression. In order to prevent loss of viability and also change in antigen expression, sample processing should be performed within 24 h (van de Loosdrecht et al. 2009).

9.2.1.2 Red Blood Cell Lysis

Samples should be prepared by an ammonium chloride-based erythrocyte lysis to eliminate the nonnucleated red blood cells. The use of mononuclear cells obtained by density separation in general is not recommended due to potential selective loss of cells.

9.2.1.3 Antibody Staining

Staining of samples by different antibody combinations follows standardized procedures. Antibodies at well-titrated concentrations are added to the cell suspension and incubated at room temperature in the dark. Special care should be taken if tandem-fluorochromes are used, since these fluorochromes may be highly sensitive to light exposure (particularly PE-Cy5 conjugates), and compensation settings frequently vary from batch to batch. Given the importance of the strength of an antigen expression as a diagnostic co-criterion in the evaluation of MDS, careful and precise sample preparation is essential in this context.

9.2.1.4 Instrument Setup and Data Acquisition

Given the magnitude of qualitative analysis and interpretation of acquired data, it is essential to assure a standardized setup of the flow cytometer which is quality checked daily per working day. Standard procedures as recommended by manufacturers and cytometry organizations should be followed.

9.2.2 Design of Antibody Panels and Analysis of Different Cell Populations

Immunophenotyping is capable of digitally separating different cell populations from each other by use of CD45-side scatter (SSC)-gating (Borowitz et al. 1993) (Fig. 9.1) and providing antigen expression data for each cell population separately. The design of different antibody combinations which are added to an antibody panel therefore should be based on known aberrancies in antigen expression occurring simultaneously

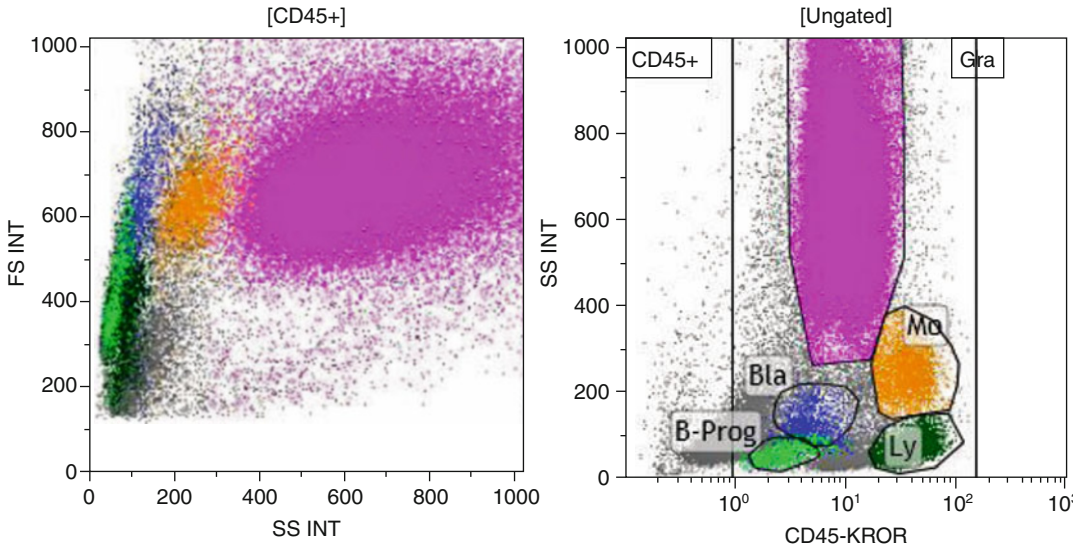

Fig. 9.1 CD45-SSC-gating. Normal BM sample: conventional forward-scatter (FSC)-SSC plot (*left panel*) and CD45-SSC plot (*right panel*). The respective cell populations are identified as follows: granulocytes (*Gra*, *pink*), SSC + CD45(+); monocytes (*Mo*, *orange*), SSC(+)CD45+; lymphocytes (*Ly*, *dark green*), SSC-CD45+; myeloid progenitor cells (*Bla*, *blue*), SSC(+)CD45(+); and B cell progenitors (*B cell Prog*, *light green*) SSC-CD45(+)

in distinct cell populations. A minimal marker set for immunophenotyping analysis of MDS should enable the analysis of abnormal expression of specified antigens and the relation between antigens of relevance in the respective cell populations. Tables 9.1 and 9.2 summarize core markers and minimal requirements, respectively, as recommended by the ELN working group (Westers et al. 2012). In the following sections, relevant antigen expression is described for the respective cell populations.

9.2.2.1 Analysis of the Immature Myeloid and Lymphoid Progenitor Compartments

Definition and Enumeration of Myeloid Progenitor Cells

The basis for the identification of myeloid progenitor cells is the CD45-SSC plot (Fig. 9.1) which is augmented by additional markers helping to differentiate them from B cell precursors, monoblasts, basophils, erythroblasts, and plasmacytoid dendritic cell precursors. Besides the CD45dimSSClow/int findings, negativity for lymphoid markers and expression of HLA-DR, CD117, and CD34 are used in this regard. However, it is important to recognize that even these markers may be expressed aberrantly. For quantification of myeloid progenitor cells, the most commonly used denominator is the total number of nucleated cells (i.e., amongst others, erythroid precursors, progenitor cells, neutrophils, and monocytes). While there in general is a good accordance between the results of this procedure and the cytomorphologically quantified myeloid blasts, differences may arise from different sample specimen, different degree of sample dilution by peripheral blood, and, most importantly, inherent non-congruence of both methods, i.e., in cell populations morphologically classified as blasts but lacking the characteristics of myeloid progenitor cells as described above. Quantification of myeloid progenitor cells in peripheral blood is prognostically relevant and increasingly used clinically; however, standard immunophenotyping on peripheral blood alone is not recommended in MDS in general.

Definition and Enumeration of Lymphoid Progenitor Cells

Progenitor B cells can be identified in the CD45dim/lowSSClow region by their CD34+

Table 9.1 Proposed core markers in the analysis of dysplasia by flow cytometry by the ELNet working group on flow cytometry in MDS

General core markers	Erythroid	Progenitors	Maturing neutrophils	Monocytes
CD45	CD45	CD45	CD45	CD45
	CD71			
	CD235a			
CD34		CD34	CD34	CD34
CD117	CD117	CD117	CD117	CD117
HLA-DR		HLA-DR	HLA-DR	HLA-DR
CD11b		CD11b	CD11b	CD11b
CD13		CD13	CD13	CD13
CD16			CD16	CD16
CD33			CD33	CD33
CD14			CD14	CD14
	CD36			CD36
			CD64	CD64
CD7		CD7		
CD56		CD56	CD56	CD56
CD19		CD19		
		CD5		
				CD2
		CD15	CD15	
			CD10	

Adapted from Westers et al. (2012), used with permission

Table 9.2 Recommended minimal requirements to assess dysplasia by flow cytometry by the ELNet working group on flow cytometry and MDS

Bone marrow subset	Recommended analyses
Erythroid compartment[a]	% of nucleated erythroid cells
	Relation CD71 and CD235a
	Expression of CD71
	Expression of CD36
	Percentage of CD117+ precursors
Immature myeloid and monocytic progenitors	% of cells in nucleated cell fraction[b]
	Expression of CD45
	Expression of CD34
	Expression of CD117
	Expression of HLA-DR
	Expression of CD13 and CD33
	Asynchronous expression of CD11b, CD15
	Expression of CD5, CD7, CD19, CD56[c]
Maturing neutrophils	% of cells as ratio to lymphocytes
	SSC as ratio vs. SSC of lymphocytes
	Relation of CD13 and CD11b
	Relation of CD13 and CD16
	Relation CD15 and CD10
Monocytes	% of cells as ratio to lymphocytes
	Distribution of maturation stages
	Relation of HLA-DR and CD11b
	Relation of CD36 and CD14
	Expression of CD13 and CD33
	Expression of CD56[c]
Progenitor B cells	Enumeration as fraction of total CD34+
	Based on CD45/CD34/SSC in combination with CD10 or CD19

Adapted from Westers et al. (2012), used with permission

[a]Under evaluation

[b]Discrepancies in counts between several definitions indicate aberrancies

[c]Do not overcall, be aware of normal cutoff values (also in stressed marrow)

CD19+ or CD19+ CD10+ phenotype with lack of myeloid antigens and are frequently found decreased in MDS, although the relevance of this finding is not yet known. To circumvent problems regarding hemodilution in the

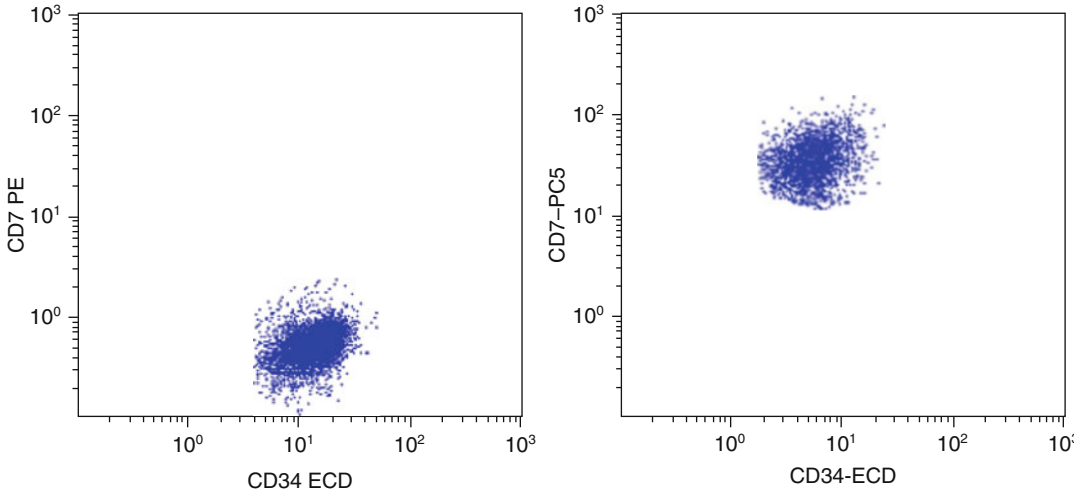

Fig. 9.2 Aberrant antigen expression in myeloid precursor cells. Myeloid progenitors are identified based on dim expression of CD45, a low SSC signal and expression of CD34. While CD7 is not expressed in normal myeloid precursor cells (*left panel*), CD7 expression can be observed in myelodysplastic myeloid precursor cells (*right panel*)

enumeration of progenitor B cells, it is recommended to report on these cells as a fraction of all CD34-positive progenitor cells. Interestingly, a decreased relative amount of progenitor B cells (<5 % of CD34+ cells) was recently introduced as one of the cardinal parameters in a model to distinguish low-risk MDS from non-clonal cytopenias (Ogata et al. 2009), which has been prospectively validated in a multicenter study within the ELN (Della Porta et al. 2012).

Identification of Immunophenotypic Aberrancies in Myeloid Progenitor Cells

In addition to quantitative aberrancies, dysplastic immature myeloid progenitors in MDS may have an aberrant phenotype that distinguishes them from normal progenitors (van de Loosdrecht et al. 2009). The most widely recognized aberrancies in the immature myeloid compartment in MDS are an abnormal intensity or lack of expression of CD34, CD45, CD13, CD33, CD117, or HLA-DR; abnormal granularity as identified by SSC signal intensity; asynchronous presence of CD11b or CD15; and/or the expression of lineage infidelity markers such as CD5, CD7, CD19, or CD56 (see also Fig. 9.2). Furthermore, a decrease in CD38 expression on CD34-positive myeloid progenitor cells has been reported as a rather typical finding in MDS (Goardon et al. 2009).

9.2.2.2 Definition and Analysis of the Maturing Neutrophil Compartment

The combination of CD45 and SSC is regularly applied to identify maturing neutrophils (CD45intSSCint-bright, Fig. 9.1). In addition, CD33 and CD64 are useful in distinguishing monocytes and hypogranular neutrophils with the former featuring higher expression levels of both markers as compared to the latter.

One of the most frequently reported aberrancies in the maturing neutrophil compartment is an abnormally decreased SSC signal reflecting hypogranularity, a well-known phenomenon in MDS (Fig. 9.3) (Stetler-Stevenson et al. 2001). The SSC of maturing neutrophils is recommended to be expressed as a ratio relative to that obtained for lymphocytes (as an internal reference). Furthermore, dysplastic features in neutrophils include an aberrantly increased or decreased

expression of antigens or an aberrant relationship in the expression of two or more antigens. Most frequently reported are the aberrant relationships between CD13 and CD16 and/or CD13 and CD11b; altered expression of CD45 and CD33; presence of CD34; expression of lineage infidelity markers such as CD2, CD5, CD7, CD19, and CD56; and a decrease in amount of maturing neutrophils as compared to lymphocytes (Fig. 9.4a–d).

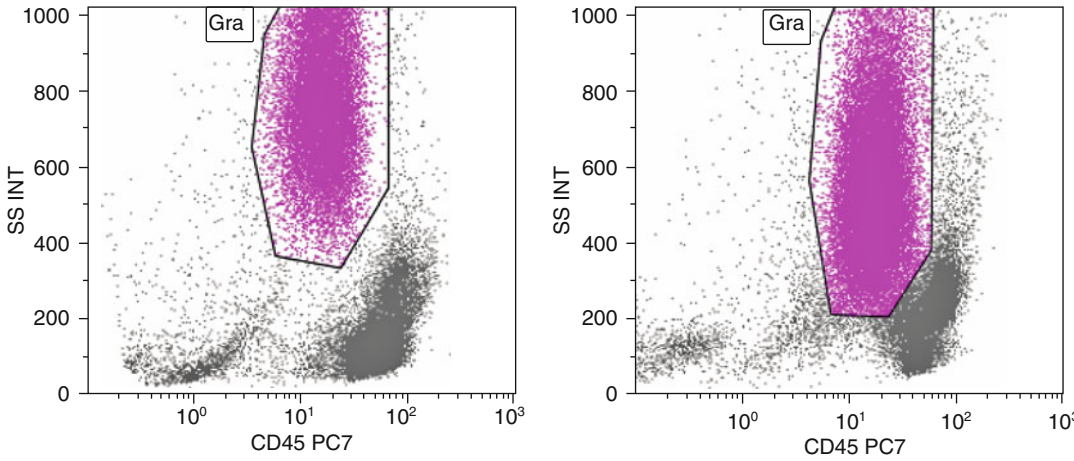

Fig. 9.3 SSC signal in granulocytes (Gra). In MDS (*right panel*), the SSC signal is significantly reduced (median SSC signal, 529.0) as compared to normal bone marrow (median SSC signal, 779.7; *left panel*)

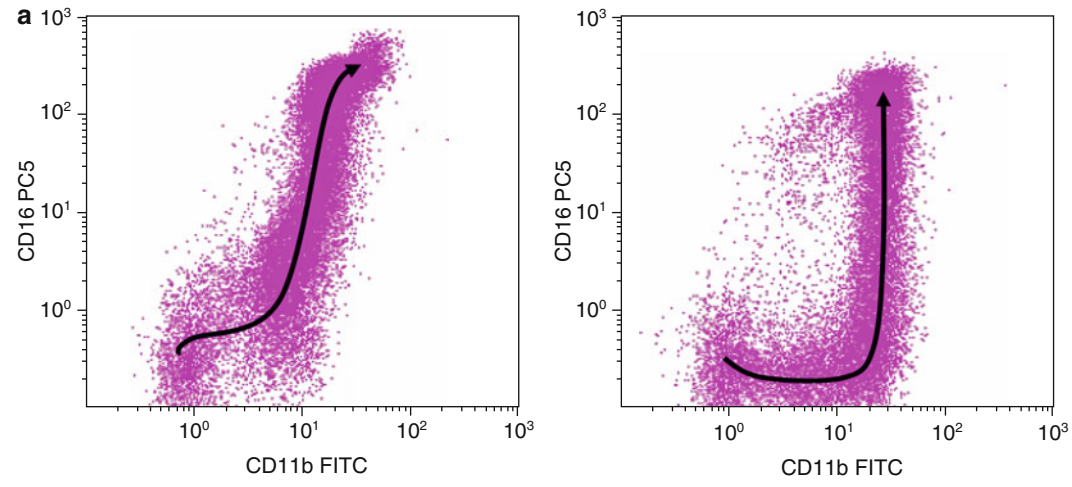

Fig. 9.4 Aberrant antigen expression in granulocytes. Antigen expression in normal bone marrow is shown in the *left panels*, aberrant antigen expression in MDS in the *right panels*, respectively. (**a**) The normal maturation pattern from CD16-CD11b- via CD16-CD11b + to CD16 + CD11b+ (pattern resembling a giraffe) is not followed in MDS: the slight increase in CD16 expression during the first increase in CD11b expression (body of the giraffe) is lacking in MDS; the slight increase in CD11b expression during the steep increase in CD16 expression (neck of the giraffe) is lacking in MDS. (**b**) The normal C-type maturation pattern from CD16-CD13+ via CD16-CD13- via CD16(+)CD13- to CD16+ CD13+ is not followed in MDS. (**c**) In MDS CD56 is aberrantly expressed as compared to no expression in normal bone marrow

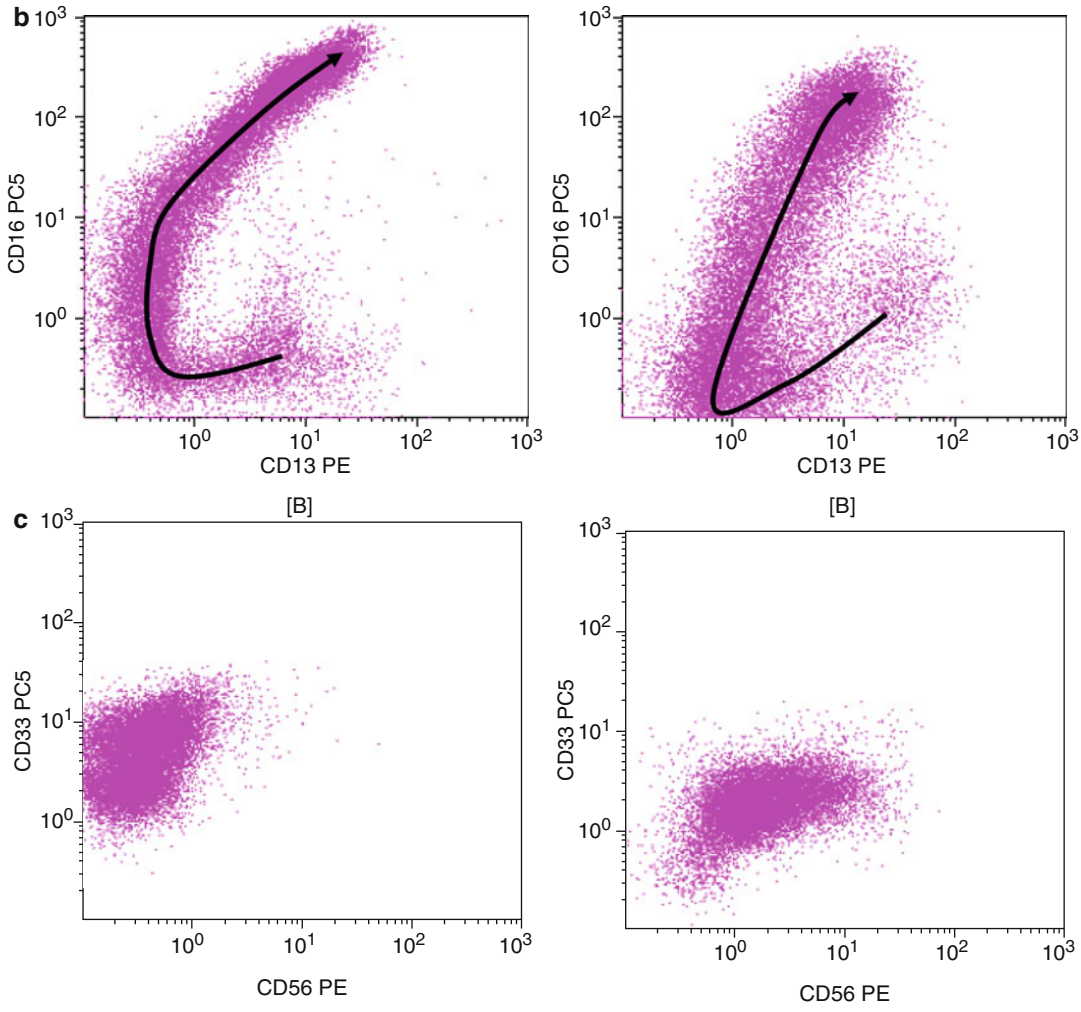

Fig 9.4 (continued)

9.2.2.3 Definition of Monocytes and Aberrancies in the Monocytic Compartment

Morphological assessment of dysmonocytopoiesis in MDS is difficult unless there is marked monocytosis. In contrast, immunophenotyping allows the specific analysis of monocytes as identified in the CD45-SSC plot and useful additional markers (CD14, CD64, CD36, and CD33) for aberrantly expressed antigens.

In MDS the proportion of monocytes may be found abnormally decreased or increased. Furthermore, abnormal CD11b/HLA-DR, CD36/CD14, or CD36/CD33 patterns; abnormal intensity of the more specific markers CD14, CD36, or CD64; and abnormal intensity of the more broadly expressed markers CD13 or CD33 can be found. Other aberrancies are the presence of CD34 or lineage infidelity markers and overexpression of CD56. The aberrant expression of CD56 and CD2 is a characteristic finding particularly in chronic myelomonocytic leukemia (CMML; Fig. 9.5a–d).

9.2.2.4 Definition of and Aberrancies in the Erythroid Compartment

The erythroid population can be defined by its lack of CD45 expression and low FCS and SSC

properties. Based on the knowledge of normal patterns, a decreased reactivity for CD36 and asynchronous expression of CD71 vs. CD235a have been reported in MDS bone marrows (Fig. 9.6). Furthermore, an increased number of erythroid progenitors associated with a larger proportion of immature erythroid cells has been observed. In contrast, a decrease in erythroblasts due to apoptosis can also be observed.

9.3 Clinical Application of Immunophenotyping in MDS

Since MDS is a heterogeneous disease, it is obvious that no single, specific marker can indicate MDS. The presence of multiple aberrancies has been shown to be of higher predictive value for MDS than single aberrancies (Wells et al. 2003; van de Loosdrecht et al. 2008; Kern et al. 2010;

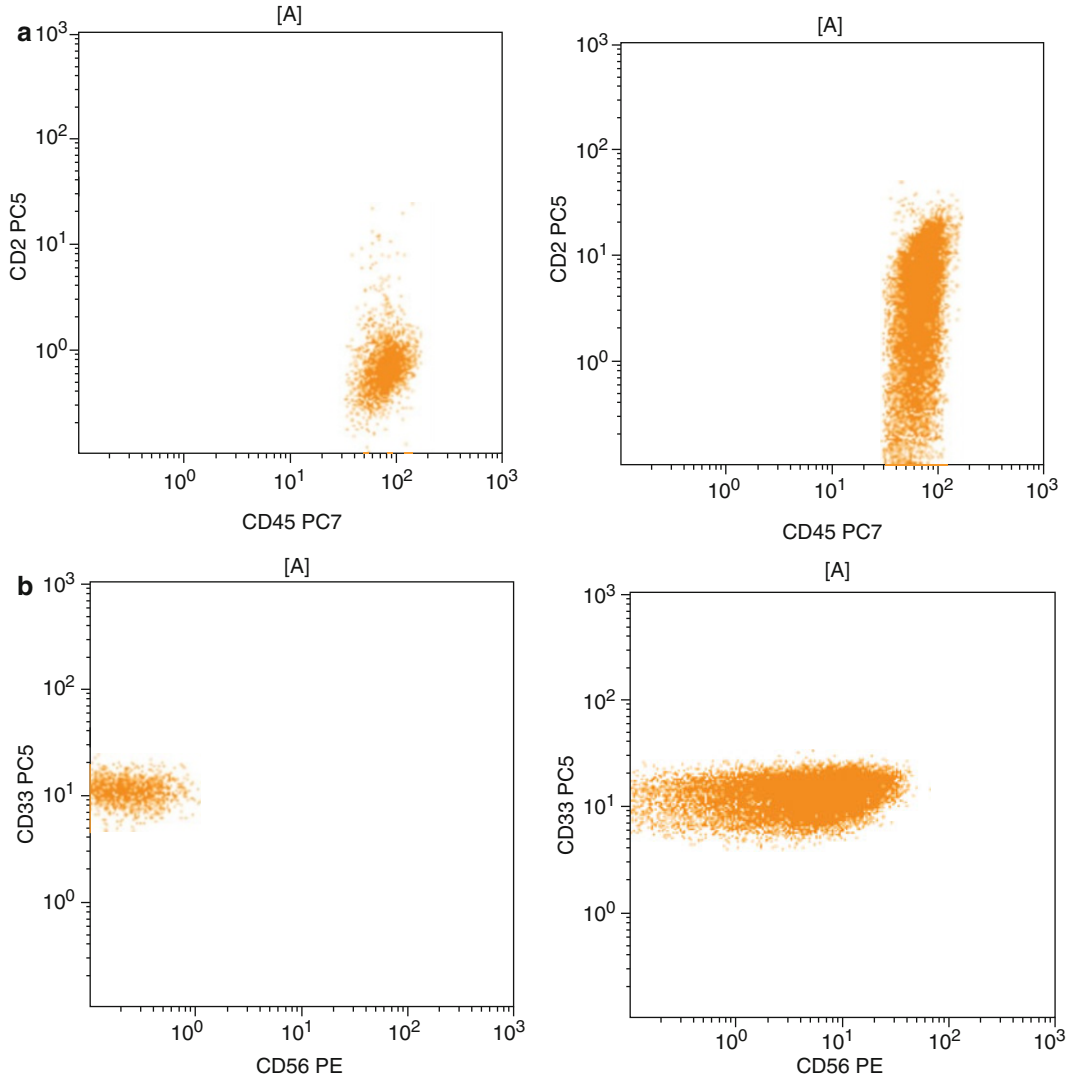

Fig. 9.5 Aberrant antigen expression in monocytes. Antigen expression in normal bone marrow is shown in the *left panels*, aberrant antigen expression in MDS in the *right panels*, respectively. (**a**) Aberrant expression of CD2 in MDS. (**b**) Aberrant expression of CD56 in MDS. (**c**) Decreased expression of CD13 and CD11b in MDS. (**d**) Decreased expression of HLA-DR in MDS

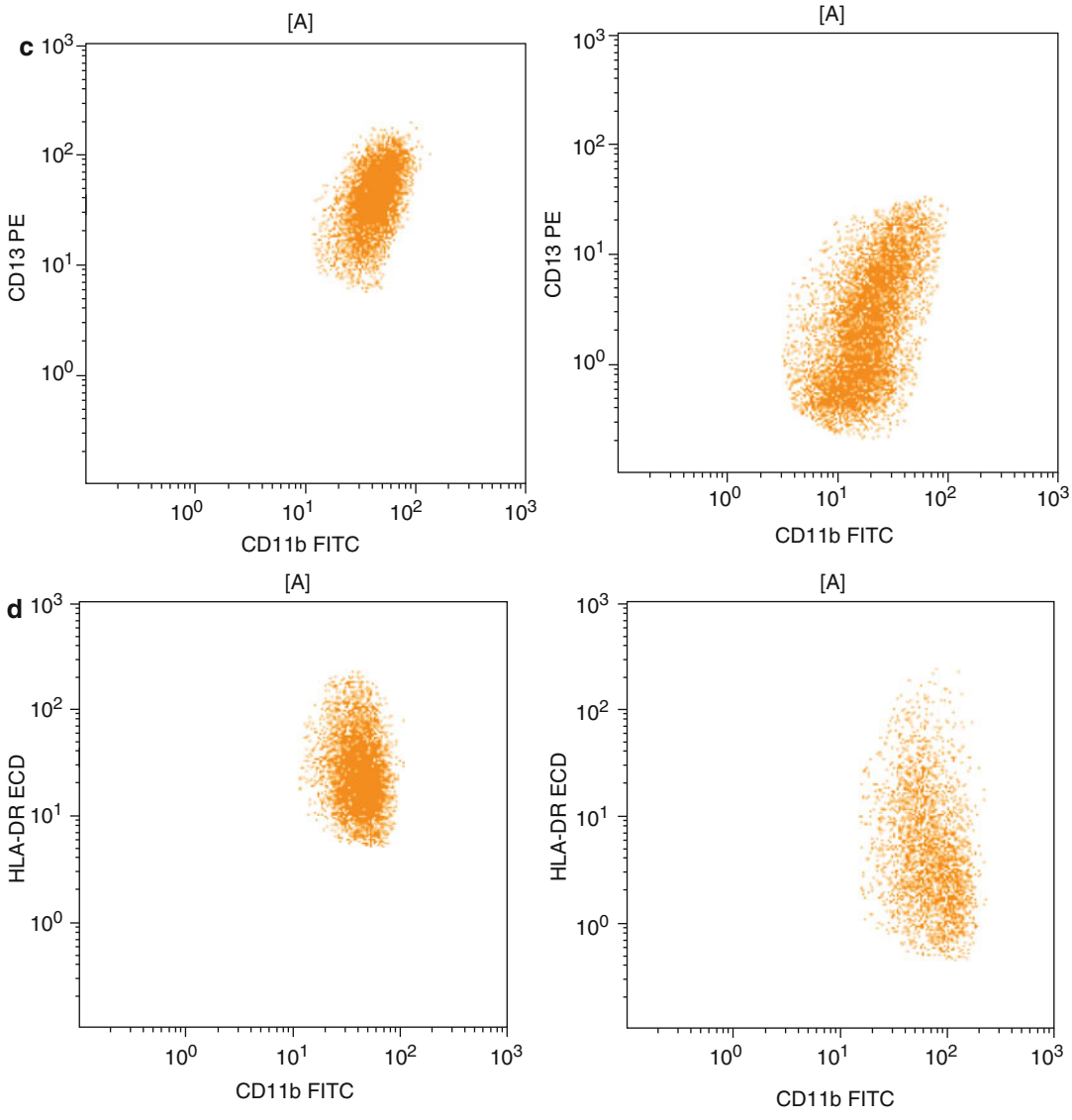

Fig 9.5 (continued)

Scott et al. 2008; Malcovati et al. 2005). Thus, multiparametric assessment of an accumulation of aberrancies might strongly support the diagnosis of MDS by immunophenotyping when applying a panel capable of identifying the most relevant MDS-associated aberrations (Westers et al. 2012) (Table 9.2). While multiple reports are available on the diagnostic use of immunophenotyping in MDS, it is desirable to have validation studies performed supporting this method based on predefined and standardized criteria as defined by the ELN (Westers et al. 2012). As a consequence, immunophenotyping is anticipated to be included in the routine integrated diagnostic work-up of patients with suspected MDS in combination with cytomorphology, cytogenetics, and molecular genetics.

Besides the diagnostic value data generated by immunophenotyping have been shown to correlate with the prognosis of the patients. Thus, a higher number of antigens found aberrantly expressed correlated with a higher risk

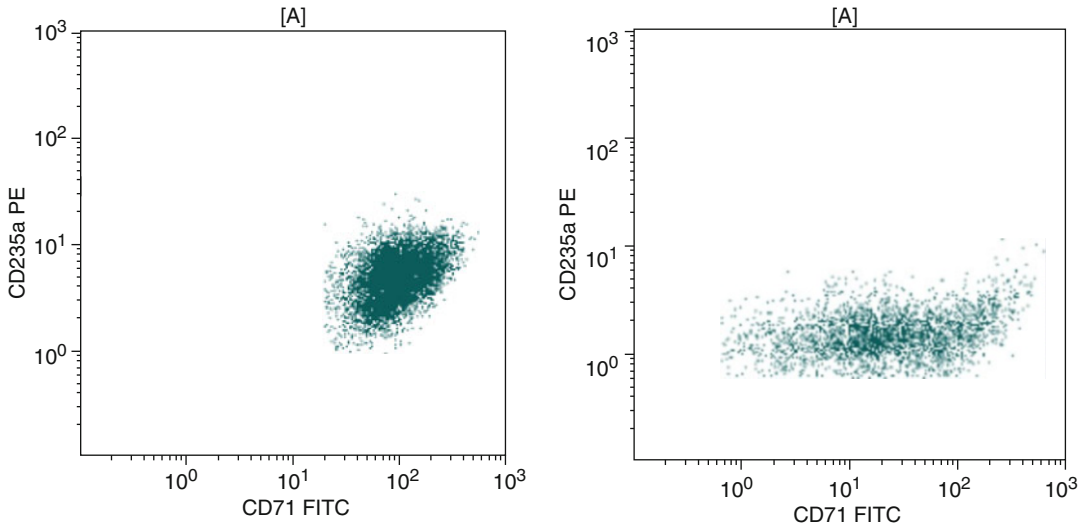

Fig. 9.6 Aberrant antigen expression in the erythroid compartment. While CD71 is homogeneously expressed in normal bone marrow (*left panel*), a weaker expression of CD71 in a portion of erythroid cells is observed in MDS (*right panel*)

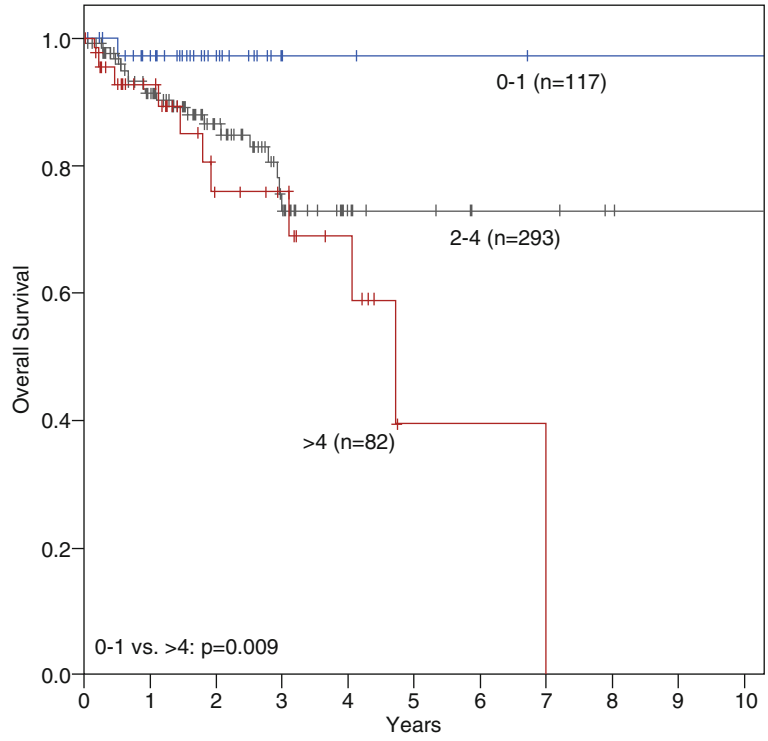

Fig. 9.7 Impact of aberrant antigen expression on overall survival. Patients with MDS proven by cytomorphology are separated according to the numbers of aberrantly expressed antigens in the total of myeloid progenitor cells, granulocytes, monocytes, and erythroid cells. Cases with no or only one aberrantly expressed antigen have a superior survival (97 % at 5 years) as compared to those with two to four aberrantly expressed antigens (survival at 5 years, 73 %) and those with more than four aberrantly expressed antigen (survival at 5 years, 40 %) (Kern et al. 2011)

as indicated by scoring systems like the IPSS as well as with a decreased overall survival (Fig. 9.7) (Wells et al. 2003; van de Loosdrecht et al. 2008; Kern et al. 2010). Furthermore, this impact on the clinical outcome has repeatedly shown to be independent of other prognostic parameters. It is important, therefore, to prove by large prospective studies the prognostic

significance of immunophenotyping findings in order to allow their adequate integration not only in the diagnostic process but also in the prognostication in individual patients and thereby also in treatment stratification. Finally, flow cytometric aberrancies on immature myeloid cells have shown to predict response on standard treatment with erythropoietin in lower-risk patients with MDS (Westers et al. 2010) as well as response on azacitidine in higher-risk patient citation (Alhan et al. 2011).

In conclusion, multiparameter flow cytometry is a promising diagnostic tool in the evaluation of patients with suspected MDS and in anticipated to become an integrated part of the comprehensive diagnostic work-up in combination with cytomorphology, cytogenetics, and molecular genetics. The correlation of flow cytometric findings of myelodysplasia with the prognosis of the patients provides the opportunity to improve current prognostic scoring systems.

References

Alhan C, Westers TM, Cali C, Ossenkoppele GJ, van de Loosdrecht AA (2011) Validation of a flow cytometric scoring system for the prognostication of the myelodysplastic syndromes; a retrospective cohort study. In: The 11th international symposium on myelodysplastic syndromes, Edinburgh, p 250, 18–21 May 2011

Bennett JM, Catovsky D, Daniel MT et al (1982) Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51(2):189–199

Borowitz MJ, Guenther KL, Shults KE, Stelzer GT (1993) Immunophenotyping of acute leukemia by flow cytometric analysis. Use of CD45 and right-angle light scatter to gate on leukemic blasts in three-color analysis. Am J Clin Pathol 100(5):534–540

Brunning RD, Orazi A, Germing U et al (2008) Myelodysplastic syndromes/neoplasms, overview. In: Swerdlow SH, Campo E, Harris NL (eds) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. International Agency for Research on Cancer (IARC), Lyon, pp 88–93

Della Porta MG, Picone C, Pascutto C et al (2012) Multicentre validation of a reproducible flow cytometric score for the diagnosis of low-risk myelodysplastic syndromes: results of a European LeukemiaNET study. Haematologica 97(8):1209–1217

Goardon N, Nikolousis E, Sternberg A et al (2009) Reduced CD38 expression on CD34+ cells as a diagnostic test in myelodysplastic syndromes. Haematologica 94(8):1160–1163

Greenberg P, Cox C, Le Beau MM et al (1997) International Scoring System for evaluating prognosis in myelodysplastic syndromes. Blood 89(6): 2079–2088

Greenberg P, Tuechler H, Schanz J et al (2011) Revised International Prognostic Scoring System (IPSS-R), developed by the International Prognostic Working Group for Prognosis in MDS (IWG-PM). In: The 11th international symposium on myelodysplastic syndromes, Edinburgh, p 274, 18–21 May 2011

Jaffe ES (2001) Pathology and genetics of tumours of haematopoietic and lymphoid tissues, WHO classification of tumours. IARC Press, Lyon

Kern W, Haferlach C, Schnittger S, Haferlach T (2010) Clinical utility of multiparameter flow cytometry in the diagnosis of 1013 patients with suspected myelodysplastic syndrome: correlation to cytomorphology, cytogenetics, and clinical data. Cancer 116(19): 4549–4563

Kern W, Haferlach C, Schnittger S, Haferlach T (2011) Clinical impact of multiparameter flow cytometry in diagnosing myelodysplastic syndromes. In: The 11th international symposium on myelodysplastic syndromes, Edinburgh, p 361, 18–21 May 2011

Malcovati L, Della Porta MG, Lunghi M et al (2005) Flow cytometry evaluation of erythroid and myeloid dysplasia in patients with myelodysplastic syndrome. Leukemia 19(5):776–783

Mufti GJ, Stevens JR, Oscier DG, Hamblin TJ, Machin D (1985) Myelodysplastic syndromes: a scoring system with prognostic significance. Br J Haematol 59(3): 425–433

Ogata K (2011) Flow cytometry will be a routine tool in clinical practice in myelodysplastic syndromes: a real story. Leuk Res 35(7):848–849

Ogata K, Nakamura K, Yokose N et al (2002) Clinical significance of phenotypic features of blasts in patients with myelodysplastic syndrome. Blood 100(12): 3887–3896

Ogata K, la Porta MG, Malcovati L et al (2009) Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study. Haematologica 94(8):1066–1074

Ossenkoppele GJ, van de Loosdrecht AA, Schuurhuis GJ (2011) Review of the relevance of aberrant antigen expression by flow cytometry in myeloid neoplasms. Br J Haematol 153(4):421–436

Scott BL, Wells DA, Loken MR et al (2008) Validation of a flow cytometric scoring system as a prognostic indicator for posttransplantation outcome in patients with myelodysplastic syndrome. Blood 112(7): 2681–2686

Stetler-Stevenson M, Arthur DC, Jabbour N et al (2001) Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. Blood 98(4): 979–987

Swerdlow SH, Campo E, Harris NL et al (2008) WHO classification of tumours of haematopoietic and lymphoid tissues. International Agency for Research on Cancer (IARC), Lyon

van de Loosdrecht AA, Westers TM (2011) Flow cytometry in myelodysplastic syndromes: ready for translation into clinical practice. Leuk Res 35(7): 850–852

van de Loosdrecht AA, Westers TM, Westra AH et al (2008) Identification of distinct prognostic subgroups in low- and intermediate-1-risk myelodysplastic syndromes by flow cytometry. Blood 111(3): 1067–1077

van de Loosdrecht AA, Alhan C, Bene MC et al (2009) Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. Haematologica 94(8): 1124–1134

Wells DA, Benesch M, Loken MR et al (2003) Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. Blood 102(1):394–403

Westers TM, Alhan C, Chamuleau ME et al (2010) Aberrant immunophenotype of blasts in myelodysplastic syndromes is a clinically relevant biomarker in predicting response to growth factor treatment. Blood 115(9):1779–1784

Westers TM, Ireland R, Kern W et al (2012) Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European LeukemiaNet Working Group. Leukemia 26(7):1730–1741

Prognostic Models for Patients with Myelodysplastic Syndromes

10

Guillermo Garcia-Manero

10.1 Introduction

The myelodysplastic syndromes are a very heterogeneous group of myeloid malignancies characterized by bone marrow failure and increased risk of transformation to acute myelogenous leukemia (AML) (Tefferi and Vardiman 2009). This heterogeneity makes the clinical management of patients with MDS very complex particularly when making decisions in terms of when to treat and with what type of therapy. This is particularly important when making decisions such as initial therapy or selecting candidates for stem cell transplantation. Therefore, the need for (1) prognostic models that could aid clinicians in predicting survival without therapy and (2) models to predict outcomes after specific forms of therapy. Since its development, patients with MDS have been evaluated using the IPSS score (Greenberg et al. 1997). This score, in place since 1997, has been fundamental for our understanding and treatment of patients with MDS. Using this model, patients are divided in four subgroups (low, intermediate-1, intermediate-2, and high risk). For practical purposes, patients are divided into lower-risk category (including low and int-1) and higher (including int-2 and high). Based on this concept, and for instance, the NCCN has developed widely accepted treatment guidelines that are considered to represent the standard of care (Greenberg et al. 2011). Despite its importance, the IPSS has several limitations that have prompted the development of new more advanced prognostic models. This has also been promoted by interest in MDS by multiple groups that has resulted in a better understanding of both the clinical characteristics and the molecular bases of MDS. It should be noted that recently the results of a new revised version of the IPSS has been published (IPSS-R) (Greenberg et al. 2012). This, together with the recent description of new multiple molecular lesions in MDS (Bejar et al. 2011a; Shen et al. 2010), results in the fact that this field is in a state of evolution and it is likely that the incorporation of new molecular information is going to have a significant impact in the design of new prognostic models. In this chapter, I provide an update and summary to prognostic models in MDS.

G. Garcia-Manero, MD (✉)
Department of Leukemia,
The University of Texas MD Anderson Cancer Center,
Houston 77025, TX USA

Department of Leukemia, University of Texas MD Anderson Cancer Center,
1515 Holcombe Blvd., 428, Houston, TX 77030-4009, USA
e-mail: ggarciam@mdanderson.org

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_10,

Table 10.1 The IPSS classification for MDS

	Score value				
Prognostic variable	0	0.5	1.0	1.5	2.0
BM blasts (%)	<5	5–10	–	11–20	21–30
Karyotype[a]	Good	Intermediate	Poor		
Cytopenias	0/1	2/3			

[a]Karyotype is classified as follows: good [normal, –Y, del(5q), and del(20q)], poor (complex (≥3 abnormalities) or chromosome 7 anomalies), and intermediate (other abnormalities)

Table 10.2 Age-related survival and AML evolution of MDS patients based on IPSS

	No. of patients	Low	Int-1	Int-2	High
A. Median survival (years)					
Total no. of patients (%)	816	267 (33)	314 (38)	176 (22)	59 (7)
Median (years)		5.7	3.5	1.2	0.4
Age (years)					
≤60	205 (25)	11.8	5.2	1.8	0.3
>60	611	4.8	2.7	1.1	0.5
≤70	445 (54)	9.0	4.4	1.3	0.4
>70	371	3.9	2.4	1.2	0.4
B. 25 % AML evolution (years)					
Total no. of patients (%)	759	235 (31)	295 (39)	171 (22)	58 (8)
Median (years)		9.4	3.3	1.1	0.2
Age (years)					
≤60	187 (25)	>9.4 (NR)	6.9	0.7	0.2
>60	572	9.4	2.7	1.3	0.2
≤70	414 (55)	>9.4 (NR)	5.5	1.0	0.2
>70	345	>5.8 (NR)	2.2	1.4	0.4

Abbreviation: *NR* not reached

10.2 The Standard: The International Prognostic Score System of MDS (IPSS)

For more than one decade, IPSS has been the standard prognostic score utilized by most research centers and clinicians around the world (Greenberg et al. 1997). The importance of this score is derived also from the fact that most of the approved drugs (Kantarjian et al. 2006; Fenaux et al. 2009; List et al. 2006) for patients with MDS have been investigated in the context of the IPSS. Therefore, until we understand the effect of therapy on newer prognostic models, IPSS is still going to remain an important tool for our daily clinical practice. Table 10.1 summarizes the IPSS model and Table 10.2 the impact of age on both the overall survival and transformation to acute myelogenous leukemia (AML) (Greenberg et al. 1997). In summary, IPSS utilizes three characteristics to calculate the outcome: percentage of blasts, cytogenetics, and the number of cytopenias. Details are shown in Table 10.1. Although IPSS is a fundamental tool, it is obvious that this score has several limitations some of which are intuitive and were probably not relevant in 1997 but that are now becoming a major issue regarding our clinical approach to patients with MDS. The first one is that IPSS is a poor model to predict survival of patients with lower-risk disease. A classic example is patients with isolated cytopenia that may not have excess blasts or poor-risk cytogenetics. An example could be a patient with severe thrombocytopenia and no excess blasts or cytogenetic alterations. That patient could have an IPSS of 0 point regardless of whether the

Table 10.3 WPSS classification for MDS

	Score			
Variable	0	1	2	3
WHO category	RA, RARS, 5q–	RCMD, RCMD-RS	RAEB-1	RAEB-2
Karyotype (per IPSS)	Good	Intermediate	Poor	–
Transfusion requirement	No	Regular	–	–

Risk groups are very low (score = 0), low (score = 1), intermediate (score = 2), high (score = 3–4), and very high (score = 5–6)

platelet count requires transfusion replacement or not. It is obvious that the prognosis of these two situations is quite distinct. Indeed, severe thrombocytopenia has been shown to be a predictor of poor survival (Kantarjian et al. 2007). The other significant issue was that the IPSS provided more weight to percentage of blasts relative to cytogenetic alterations. Schanz et al. have clearly shown the fact that specific cytogenetic alterations are associated with distinct prognosis (Schanz et al. 2011). Finally, IPSS was designed to include a very specific group of patients: those with previously untreated disease. It excluded patients with chronic myelomonocytic leukemia (CMML) with elevated white blood cell counts. Finally, IPSS was designed to be used at initial presentation and in principle could not be calculated sequentially. Therefore, IPSS was not designed to assess risk in patients receiving active therapy or in subsequent follow-up evaluations. IPSS was not formally studied in patients with hypoplastic MDS and did not include several other prognostic characteristics that have a significant impact on the natural history of MDS. Therefore, the need for additional models that could account for these limitations.

10.3 The Pavia Model: WPSS

The group at the University of Pavia has been at the forefront in the development of new prognostic models in MDS. The WPSS (Malcovati et al. 2007) was one of the first significant efforts to improve on IPSS. This classification combined IPSS with morphological assessment of the disease MDS using WHO criteria (Vardiman et al. 2009). The proposed model (Table 10.3) was developed using a cohort of 426 patients and was validated in a second cohort of 739 patients. WPSS includes WHO morphologic criteria (Vardiman et al. 2009) together with IPSS-defined cytogenetics (Greenberg et al. 1997) and transfusion dependency. This was defined as red blood cell transfusion in need of at least one transfusion every 8 weeks over a period of 4 months. One potential theoretic limitation of the IPSS was that it was calculated at the time of initial presentation and was never formally tested at sequential later time points. The WPSS was designed as a time-dependent tool that allows calculating prognosis at multiple time points during the natural history of an individual patient. Despite these advantages, WPSS has two major limitations. One is that instead of providing specific hemoglobin thresholds, prognosis was initially based on transfusion dependency, a characteristic that is not always known. This was eventually improved when recently a modified model replaced transfusion needs for specific hemoglobin levels (Malcovati et al. 2011). The second, and major, limitation of WPSS is that it requires WHO morphological assessment of the disease. This is a problem due to the reproducibility of WHO diagnosis in the community (Naqvi et al. 2011a). Although this should not be a problem in most research centers, it is an issue in centers without access to expert hematopathologist. This is important as we have described a significant discrepancy in the diagnosis between centers in the USA (Naqvi et al. 2011a). In summary, WPSS is an excellent model that allows evaluation at multiple time points but is significantly limited by the need of expert morphological assessment of MDS.

10.4 The Global MD Anderson Cancer Center Model

The MDS program at MD Anderson Cancer Center (MDACC) receives close to 300 new patients with MDS annually and has maintained a clinical database that goes back to 1975. This has

Table 10.4 Global MDACC model

Prognostic factor	Coefficient	Points
Performance status		
≥2	0.267	2
Age, years		
60–64	0.179	1
≥65	0.336	2
Platelets, $\times 10^9$/L		
<30	0.418	3
30–49	0.270	2
50–199	0.184	1
Hemoglobin < 12 g/dL	0.274	2
Bone marrow blasts, %		
5–10	0.222	1
11–29	0.260	2
WBC > 20×10^9/L	0.258	2
Karyotype: chromosome 7 abnormality or complex ≥ 3 abnormalities	0.479	3
Prior transfusion, yes	0.107	1

Table 10.5 Estimated overall survival by four levels of prognostic score points

		Survival		
Score	No. of patients (%)	Median (months)	% at 3 years	% at 6 years
0–4	157 (16)	54	63	38
5–6	227 (24)	25	34	13
7–8	233 (24)	14	16	6
≥9	341 (36)	6	4	0.4

allowed investigators at the center to analyze clinical outcomes of patients with MDS in a systematic fashion. Because most patients with MDS referred to MDACC come for a second opinion or have received prior therapy, a population of patients distinct than that of the original IPSS, we developed a new prognostic model using variables not included in IPSS (Kantarjian et al. 2008). The characteristics of this model are shown in Table 10.4 and the relation between survival and different score in Table 10.5. The study included a total population of 1,915 patients and a study group of 958 patients. These patients had been evaluated at MDACC from 1993 to 2005. Patients with CMML or that had received prior therapy were also included in the model. Survival was calculated from the time of referral.

Of particular interest was the observation that the Global MDACC model was able to predict different prognostic subgroups in each IPSS subcategory (Fig. 10.1). This model also allows calculation of survival at different time points during the life of the patient, similar to the time-dependent characteristic of WPSS. The Global MDACC model includes characteristics such as age and performance status that are not included in either WPSS or IPSS. Finally, another major advantage of the model is the fact that it does not require WHO assessment of the disease. The global MDACC model has now been validated by a number of other groups confirming its value outside the context of a major referral center such as MDACC (Komrokji et al. 2012).

10.5 Characteristics Associated with Prognosis Not Included in Most Models

Over the last few years, a number of individual characteristics have been proposed to have prognostic value in MDS. Not all of them have been incorporated in current clinical models but are of interest. Examples include β2-microglobin, ferritin levels, presence of marrow fibrosis, or presence of CD34+ cells. β2-Microglobin is a component of class I MHC molecule. It has been shown to be associated with prognosis in a number of human leukemias. In MDS (Kantarjian et al. 2008) and in CMML (Beran et al. 2007), β2-microglobin has been shown to be associated with worse prognosis. The same was observed in the lower-risk MDACC model discussed below (Garcia-Manero et al. 2008). It has been proposed that patients with evidence of iron accumulation due to red cell transfusions have worse prognosis (Cazzola and Malcovati 2008). At this point, it is not clear whether the impact of this phenomenon is related to end-organ damage or to other pathophysiologic mechanisms. That said, and based on these concepts, a number of groups have evaluated the impact of the number of red cell transfusions or surrogate markers in the prognosis of patients with MDS (Malcovati et al. 2007; Cazzola and Malcovati 2008). Ferritin is

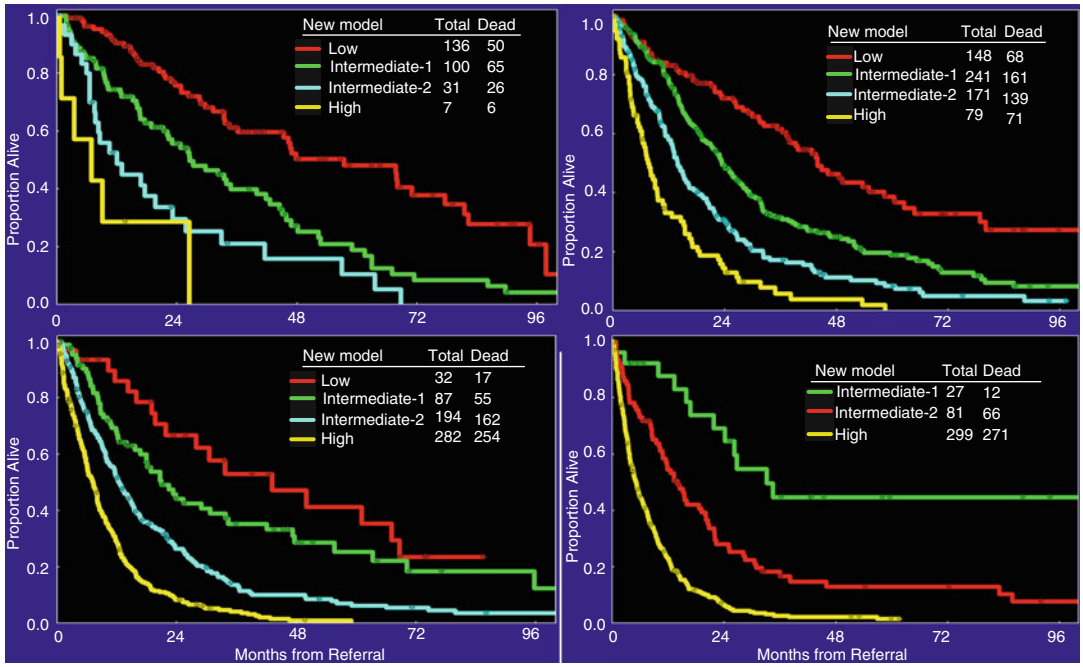

Fig. 10.1 Survival discrepancy between IPSS and the Global MDACC model. In this figure, each quadrant represents a subgroup of risk based on the Global MDACC model. Survival for patients in each quadrant was then calculated using IPSS score. Results show clear heterogeneity for each scenario. The reverse was not observed when the Global model was analyzed in the context of each IPSS category (not shown) (Adapted from Kantarjian et al. (2008))

a surrogate marker of iron stores and several groups have proposed that ferritin levels are also associated with poorer prognosis (Garcia-Manero et al. 2008). For instance, in the lower-risk model, although not incorporated in the final multivariate analysis due to the fact that it was not analyzed in enough patients, it was shown that patients with higher ferritin levels had a worse prognosis (Fig. 10.2). It will be of interest to study the impact of better indicators or iron accumulation, such as hepcidin, in MDS. Finally, the group from Pavia has reported on the potential value of incorporating the analysis of marrow fibrosis or number of CD34+ cells in MDS (Della Porta et al. 2009). In this study, moderate-to-severe bone marrow fibrosis was detected in 17 % of cases and was associated with multilineage dysplasia, higher transfusion needs, and poor-risk cytogenetics. CD34+ cell clusters were detected in 23 % of patients and were associated with excess of blasts and poor-risk cytogenetics. Both had independent negative impact on overall survival

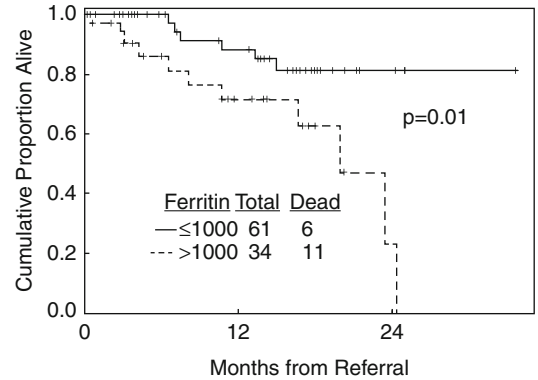

Fig. 10.2 Survival of patients with lower-risk MDS based on ferritin levels (Adapted from Garcia-Manero et al. (2008))

and leukemia-free survival. Incorporation of marrow fibrosis into IPSS resulted in a shift to a one-step more advanced risk group. The analysis of the implications of marrow fibrosis is becoming more complex as it is evident that there is a significant fraction of patients with overlap

myeloproliferative features that have distinct natural histories (Vardiman et al. 2009).

10.6 Incorporating Molecular Information in MDS

A number of groups are performing very significant efforts in the discovery of genetic lesions in MDS. This has been recently reviewed by several groups (Bejar et al. 2011b). At this time, the most comprehensive analysis has been reported by Bejar et al. (2011a) but several groups are performing large-scale whole-genome studies of MDS. The question is whether specific genes or pathways are associated with poorer prognosis independent of other variables. For instance, in the study of Bejar et al. (2011a), an analysis was performed calculating the impact on prognosis of specific genes and their relation to IPSS. The effect of specific mutations on survival is shown in Fig. 10.3. Patients with mutations had worse survival than those without mutations. One important aspect of this study is the confirmation of mutations in p53 as a very poor prognosis identified in 8 % of patients but also the identification of EZH2 as a very poor feature in MDS and specifically also in lower-risk disease (see below). EZH2 is a histone methyltransferase and is a member of the Polycomb-group family (Xu and Li 2012). PcG family members form multimeric protein complexes and are involved in maintaining the transcriptional repressive gene expression state. EZH2 is involved in the addition of three methyl groups to lysine 27 of histone 3. Of importance, EZH2 is located in chromosome 7, a fact that could explain the poor prognosis associated with the deletion of chromosome 7 in MDS. Both activation and inactivation mutations have been described, although in MDS inactivating mutations are more common (Morin et al. 2010).

It is obvious that the next generation of MDS classifications will need to include gene mutational analysis and that we are entering a new phase in the incorporation of molecular data in such models. Already the IPSS-R group (described below) is working on the incorporation of such data.

10.7 Predicting Prognosis of Patients with Lower-Risk MDS

By IPSS, patients with lower-risk MDS are those with low or int-1 disease. Traditionally, a large majority of these patients are observed and most clinical interventions have been targeted towards improving transfusion needs. That said, patients in the lower-risk subgroup constitute a very heterogeneous group including patients with minimal cytopenias and patients heavily transfusion dependent but with low percentage of blasts and normal or intermediate cytogenetic alterations. Based on this, we decided to develop a prognostic model specific for patients with lower-risk MDS (Garcia-Manero et al. 2008). The characteristics of this model are shown in Table 10.6 and the impact on survival on Table 10.7. This model incorporated simple variables such as age, cytogenetics, hemoglobin, platelet count, and percentage of marrow blasts. The model was able to separate patients with low or int-1 disease by IPSS into three distinct survival groups (Fig. 10.4). The lower-risk model allows the calculation of survival based on a score of 0–7 points. As with all models from MDACC (due to the specific type of patients evaluated at the center), it required confirmation by other groups. The most important confirmatory effort has been recently reported indicating that not only the model can be replicated in other patient populations but that patients with poorer prognosis lower-risk disease have distinct gene mutational patterns (Bejar et al. 2012). In this study, it was shown that the frequency of mutational events was lower in patients in the better prognosis subset (Fig. 10.5). Different genes had also distinct prognostic weight (Bejar et al. 2012). For instance, the presence of mutations on EZH2 was associated with very poor prognosis in this subset of patients with lower-risk disease. The results of incorporating molecular data to patients with a priori low-risk disease (based on the percentage of blasts or cytogenetics) have significant importance as it is going to allow the development of early intervention strategies for patients with MDS.

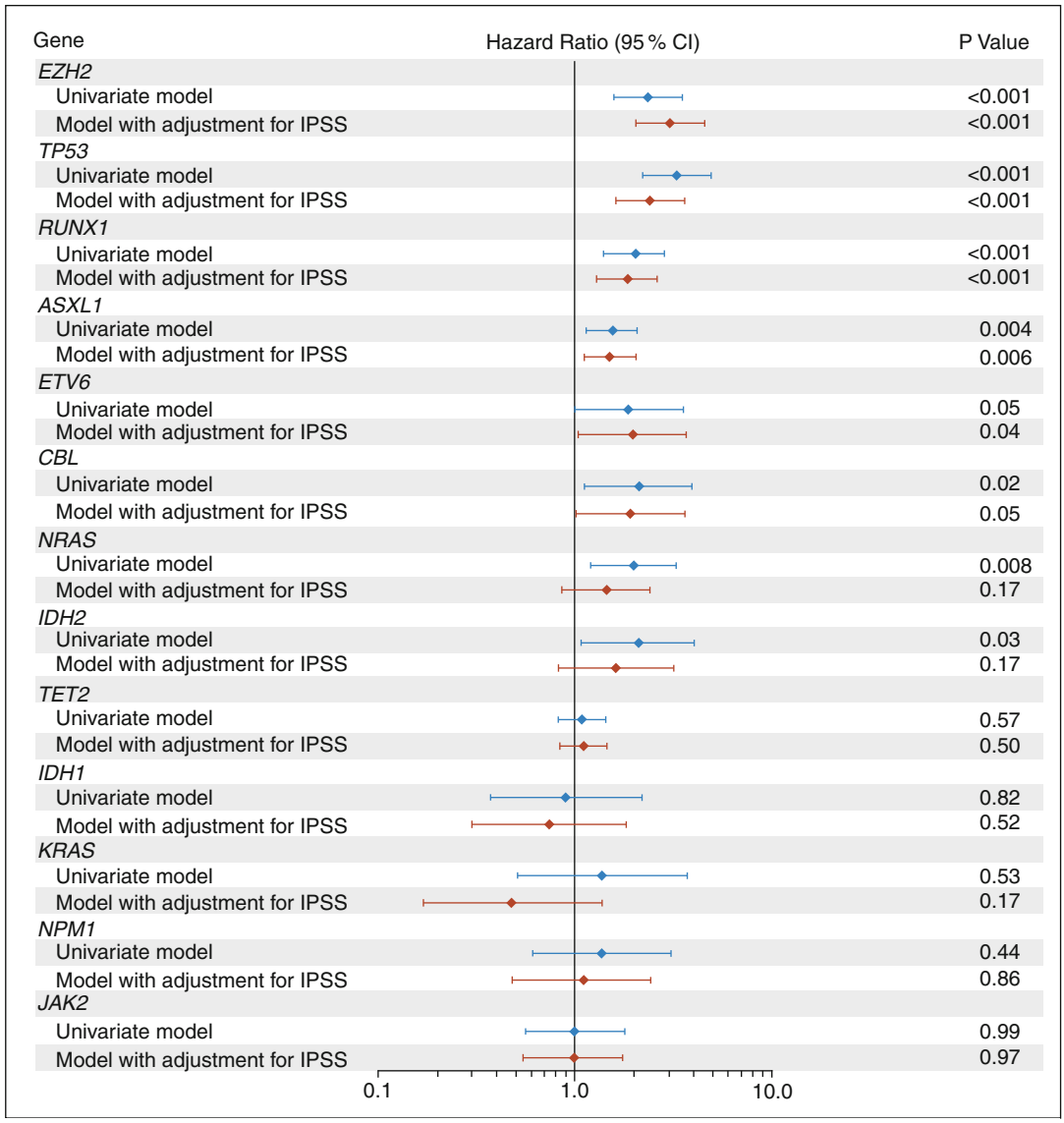

Fig. 10.3 Effect of survival of specific genetic events in patients with MDS (Adapted from Bejar et al. (2011a))

Table 10.6 MDACC MDS lower-risk prognostic model

Characteristics	Points
Unfavorable cytogenetics	1
Age ≥ 60 years	2
Hemoglobin < 10 (g/dL)	1
Platelets	
$< 50 \times 10^9$/L	2
$50–200 \times 10^9$/L	1
Bone marrow blasts ≥ 4 %	1

Table 10.7 Survival per risk score in the lower-risk MDS MDACC model

Score	Median survival	4-year OS (%)
0	NR	78
1	83	82
2	51	51
3	36	40
4	22	27
5	14	9
6	16	7
7	9	N/A

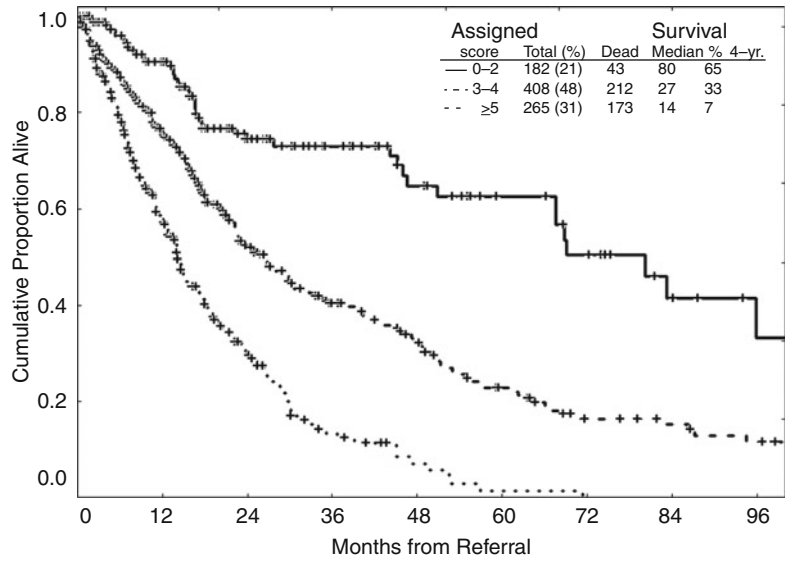

Fig. 10.4 Survival of patients with lower-risk MDS based on the lower-risk MDACC model. Survival is shown for patients with different scores based on Tables 10.6 and 10.7 (Adapted from Garcia-Manero et al. (2008))

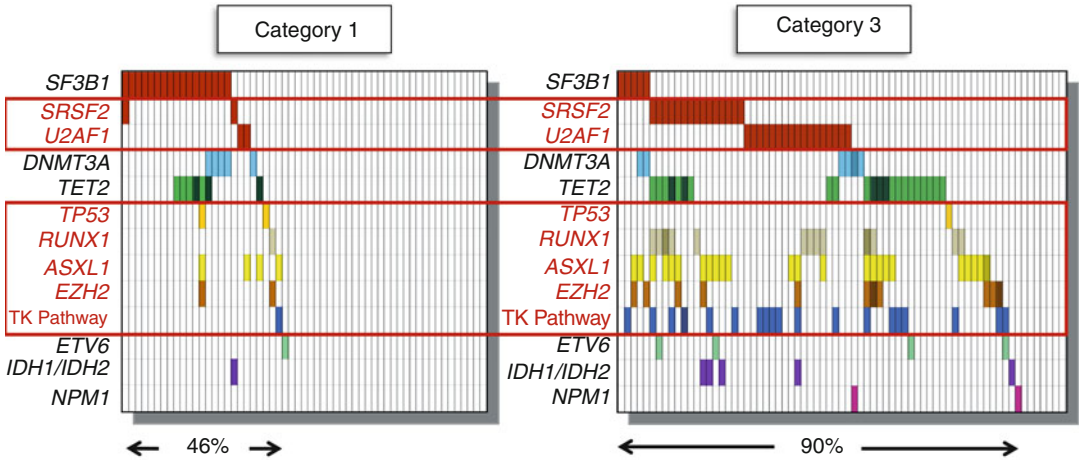

Fig. 10.5 Molecular profile of patients with lower-risk MDS based on lower-risk MDACC model. A retrospective analysis was performed studying the distribution of genetic events in patients with lower-risk MDS. As shown in the figure, patients in the better category subset of the lower-risk MDACC model (category 1) had fewer genetic events than those in category 3. Category 1 – 46 % with mutations, Category 2 – 71 % with mutations, Category 3 – 90 % with mutations (Adapted from Bejar et al. (2012))

10.8 The Impact of Comorbidities

Patients with MDS tend to be older and obviously are at higher risk to have additional concomitant comorbidities. It is known that the presence of other active comorbidities is associated with worse prognosis. It is not clear whether comorbidities have a negative additive effect on survival or whether it has a direct effect on the natural history of the disease. Several groups have performed analysis of the impact of comorbidities in the survival of MDS. A number of studies have addressed this issue using different systems. Wang et al. conducted a population-based study of older persons with MDS that suggested lower survival in those with comorbid ailments, particularly those with congestive heart failure (CHF) and chronic obstructive pulmonary disease (Wang et al. 2009). Moreover, previous studies have shown CHF, pulmonary and liver failure, infections, hemorrhage,

and solid tumors as the main causes of non-leukemic death in MDS (Malcovati et al. 2005; Della Porta and Malcovati 2009; Dayyani et al. 2010). At MDACC, we used the ACE-27 comorbidity score to address this issue (Naqvi et al. 2011b).

The ACE-27 was developed by Piccirillo et al. and was derived from adult cancer patients (Piccirillo et al. 2003). Six hundred patients with MDS were included in this analysis. Approximately half of the patients had a baseline IPSS classification of low or intermediate-1. Baseline ACE-27 comorbidity scores were as follows: none, 137 patients (23 %); mild, 254 (42 %); moderate, 127 (21 %); and severe, 82 (14 %). Approximately 55 % of the patients were diagnosed with disorder of the cardiovascular system, with hypertension being the most common comorbidity (37 %), followed by coronary artery bypass graft (14.3 %). History of prior malignancy was reported in 168 (28 %) patients. Ninety-seven (16 %) patients had diabetes mellitus. Median survival according to the ACE-27 scores was 32 months for no comorbidity, 17 months for mild comorbidity, 15 months for moderate comorbidity, and 9.7 months for severe comorbidity ($p<0.0001$). A prior history of malignancy and cardiovascular disease was associated with the worst survival. Older age, advanced IPSS, and leukemic transformation were associated with greater risk of death, while having undergone stem cell transplantation was associated with longer survival. Comorbidity did not have a significant effect on survival for patients in the low-risk category, whereas patients in the intermediate-1 and intermediate-2 groups ($p=0.001$) and high-risk category ($p=0.04$) had significant worse survival with increasing ACE-27 scores. Comorbidities significantly decreased survival in those younger than 65 years ($p<0.0001$) but had no significant effect in those older than 65. Included in the final model were age, IPSS, and ACE-27 comorbidity scores. The final prognostic model for the overall survival was developed as low (score 0–1), intermediate (score 2–4), and high (score 5–8). Patients in the low-risk category had a median survival of 43.0 months versus 23.0 months and 9.0 months for those in the intermediate-risk and high-risk groups, respectively (Naqvi et al. 2011b).

10.9 A New Cytogenetic Classification of MDS

Metaphase analysis of karyotypic abnormalities continues to be one of the more powerful prognostic tools for patients with human leukemia. The original IPSS score already introduced cytogenetics as one of its prognostic characteristics. In that model, patients were divided in three categories. Schanz et al. have performed a systematic analysis of cytogenetic alterations in MDS (Schanz et al. 2011). First, they demonstrated that prognosis was strongly associated with specific very poor prognostic features, such as deletion of chromosome 7, independent of other characteristics such as blast percent or degree cytopenia (Schanz et al. 2011). This is an important concept: as shown in the lower-risk model described above, there was a subset of patients with very poor prognosis that could be potentially transfusion independent, many of which could have cytogenetic alterations. These authors and in collaboration with the subcommittee on cytogenetics of the IPSS-R reanalyzed the impact of cytogenetics in MDS and developed the new 5-subset cytogenetic classification that forms the bases of the new IPSS-R (Fig. 10.6). This study defined 19 cytogenetic categories. The abnormalities were classified into five prognostic subgroups: very good (median OS, 61 months; hazard ratio [HR], 0.5; $n=81$), good (49 months; HR, 1.0; $n=1{,}809$), intermediate (26 months; HR, 1.6; $n=529$), poor (16 months; HR, 2.6; $n=148$), and very poor (6 months; HR, 4.2; $n=187$). Patients were from an international group of US and European centers. This is a very important effort as it forms the bases of the new IPSS-R (Greenberg et al. 2012) system described below, and it allows a more precise description of cytogenetic alterations in MDS.

10.10 The New Standard: IPSS-R

The development of the new cytogenetic classification described above and the limitations observed after more than two decades of use prompted the development of a new international

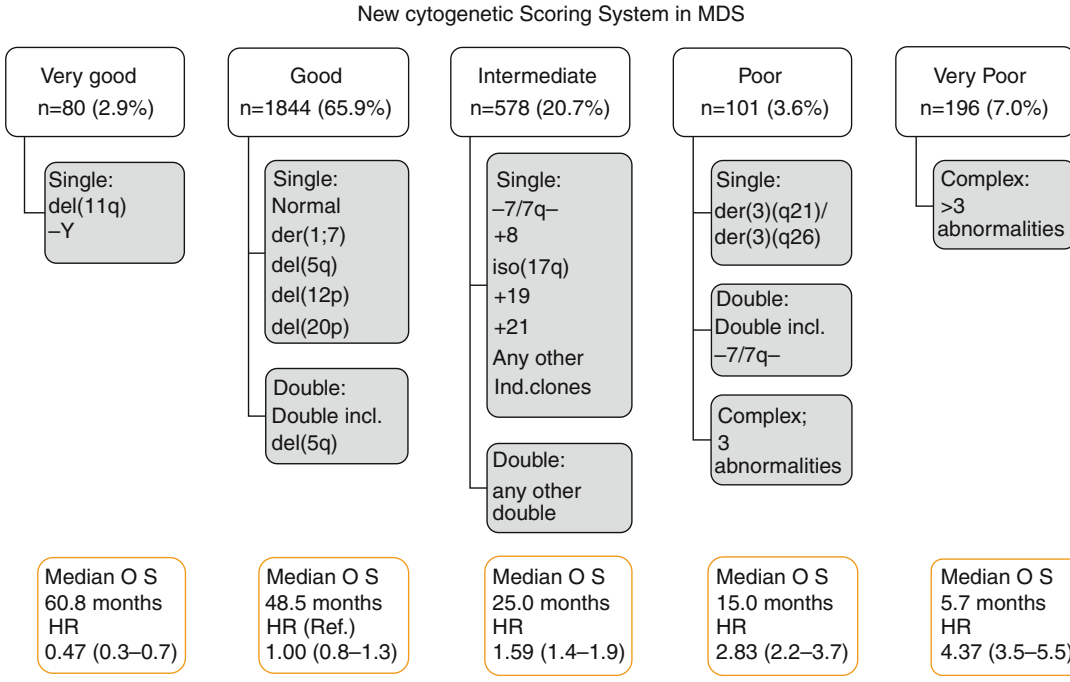

Fig. 10.6 New cytogenetic classification of MDS (Adapted from Greenberg et al. (2012) and Schanz et al. (2011))

Table 10.8 The revised new IPSS score (IPSS-R)

Prognostic variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very good	–	Good	–	Intermediate	Poor	Very poor
BM blast (%)	≤2	–	>2–<5	–	5–10	>10	–
Hemoglobin	≥10	–	8–<10	<8	–	–	–
Platelets	≥100	50–<100	<50	–	–	–	–
ANC	≥0.8	<0.8	–	–	–	–	–

Table 10.9 IPSS-R prognostic risk categories/scores

Risk category	Risk score
Very low	≤1.5
Low	>1.5–3
Intermediate	>3–4.5
High	>4.5–6
Very high	>6

classification for patients with MDS. This effort was led by Peter Greenberg and involved investigators from multiple continents that agreed on sharing data towards the development of a unified classification. The results of this effort were recently published and included data from over 7,000 patients all over the world (Greenberg et al. 2012). The characteristics of this model are shown in Tables 10.8 and 10.9. This model is significantly more complex and predictive than the original IPSS. The major differences include a more complex cytogenetic classification but also different thresholds of blasts percentage and cytopenias. For instance, patients are now divided into those with less than 2 % blasts, 2–5 %, 5–10 %, and more than 10 % (in the initial IPSS the threshold was 10 %). Cytopenias are also more precisely defined. Now a threshold of 50×10^3 k/uL platelets is incorporated; significant neutropenia is computed as an absolute neutrophil count of 800 k/uL, and for hemoglobin, a level of less than 8 g/dL is also incorporated. This translates in five different risk subgroups: very low, low, intermediate, high, and very high. The

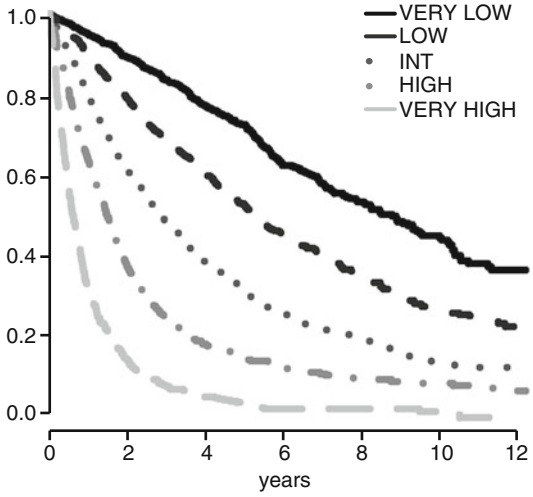

Fig. 10.7 Survival based on IPSS-R prognostic risk-based categories. Survival related to MDS patients' prognostic risk categories (Kaplan-Meier curves, $n=7012$; Dxy 0.43, $p<0.001$). The number of patients in each category and their proportional representation are shown in Table 10.1 (Adapted from Greenberg et al. (2012))

survival of each patient is shown in Fig. 10.7. Two major questions were addressed by the model. The first one was whether the new IPSS-R offered any major advantage compared to IPSS. As shown in Fig. 10.8, IPSS-R was able to further separate subsets of patients in each IPSS subcategory. Finally, there was significant discussion in terms of whether age should be incorporated in the model. At the end, a decision was made not to include age in the model but to offer a nomogram to calculate the impact of age on each subcategory. Until new molecular data is incorporated into these clinical models, the IPSS-R is going to be the standard prognostic model used by most investigators for patients with MDS.

10.11 Specific Subtypes of MDS: Hypoplastic, Overlap Syndromes, and Therapy-Related Disease

There are several subsets of MDS with very specific natural histories. These include patients with hypoplastic MDS, those with features both of MDS and a myeloproliferative disorder (so-called overlap syndromes that include CMML), and those patients treated with prior chemo and/or radiation therapy (therapy-related MDS).

The natural history of patients with hypoplastic MDS is not well understood. Patients with hypoplastic MDS are often difficult to distinguish from those with aplastic anemia, a diagnosis that is commonly excluded based on the presence of clonal abnormal cytogenetic alterations. Hypoplastic MDS is considered when marrow cellularity is less than 30 or 20 %, depending on the age of the patient. There are few retrospective analysis of hypoplastic MDS. Tong et al. performed an analysis of 253 patients with hypocellular MDS (defined as a bone marrow cellularity of less than 20 % regardless of age) (Tong et al. 2012). After analyzing clinical characteristics of this group of patients, the investigators were able to construct a prognostic model. The survival outcomes for patients with each score point are listed in Table 10.9. For clinical practice purposes, patients were divided into three risk groups based on their total risk scores (Table 10.9). Patients with low risk ($n=66$; scores 0–1) had a median survival of 30 months and 2-year/3-year survival of 62 %/44 %. Patients with intermediate risk ($n=44$; score 2) had a median survival of 19.4 months and 2-year/3-year survival of 43 %/20 %. Patients with high-risk disease ($n=59$; scores 3–5) had a median survival of 7.3 months and 2-year/3-year survival of 12 %/6 %.

There are no recent systematic prognostic models for patients with overlap syndrome or those with t-MDS. These models are needed because of the clear distinct natural histories of these patients. For instance, patients with refractory anemia with ring sideroblasts with thrombocytosis appear to have a more benign natural history (Atallah et al. 2008). Patients with CMML are known to have a poorer survival and disease characterized by tissue infiltration, systemic symptoms, and higher frequency of mutations of the Ras family. In 2002, Onida and Beran presented a prognostic model for patients with CMML (Onida et al. 2002). The study group comprised 213 patients. Splenomegaly was observed in 61 patients (29 %). Using different

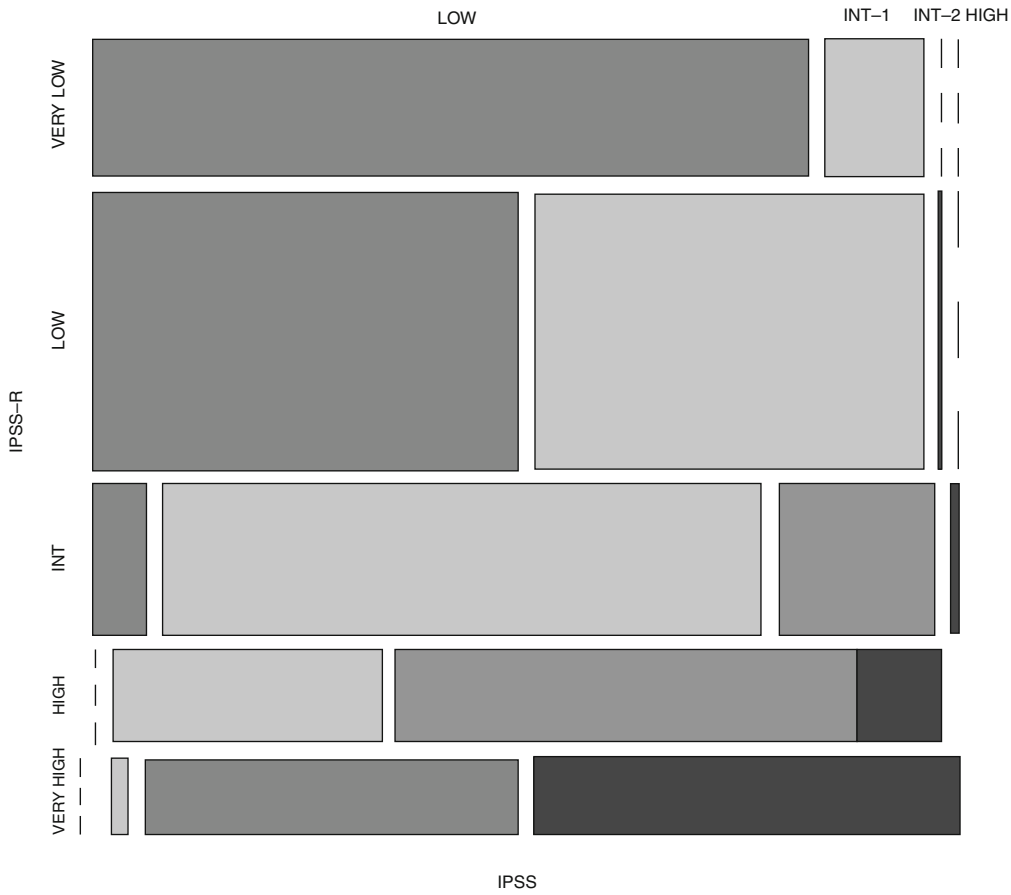

Fig. 10.8 Comparison of IPSS-R and IPSS subgroups within the IWG-PM database patient cohort. Vertical axis represents IPSS-R categories' and horizontal axis, IPSS categories. The proportion of patients in each category is shown in Table 10.9. Kendall $\tau = 0.73$ (Adapted from Greenberg et al. 2012))

methods, the following characteristics were found to be of prognostic significance: hemoglobin less than 12 g/dL, lymphocyte count greater than 2.5×10^9/L, peripheral blood immature monocyte forms 0 %, and bone marrow blasts no more than 10 %. This could then be used to divide the patients into four different categories with distinct survival. It is not clear at this time whether the identification of known genetic events, such as mutations in Ras, adds to this prognostic system.

Finally, patients with secondary or therapy-related disease are known to have poor prognosis due to the very high incidence of cytogenetic alterations as well as their prior history of other malignancies. None of the models discussed above, except for the global MDACC model (Kantarjian et al. 2008), have included therapy-related MDS patients. At MDACC, we evaluated the characteristics of patients with t-MDS. We studied 281 patients with MDS that had received prior chemotherapy and/or radiotherapy for prior malignancy. Multivariate Cox regression analysis identified seven factors that independently predicted short survival in t-MDS: age ≥65 years (HR = 1.63), ECOG performance status 2–4 (HR = 1.86), poor cytogenetics (−7 and/or complex; HR = 2.47), WHO MDS subtype (RARS or RAEB-1/2; HR = 1.92), hemoglobin (<11 g/dL; HR = 2.24), platelets ($<50 \times 10^9$/dL; HR = 2.01), and transfusion dependency (HR = 1.59). These risk factors were used to create a prognostic model that segregated patients into three groups with distinct median overall survival: good (0–2 risk factors;

34 months), intermediate (3–4 risk factors; 12 months), and poor (5–7 risk factors; 5 months) ($p < 0.001$), and 1-year leukemia-free survival (96, 84, and 72 %, respectively, $p = 0.003$). This model also identified distinct survival groups according to t-MDS therapy.

10.12 Models for Response to Therapy, Including Stem Cell Transplant

None of the models that we have discussed above are specifically design to calculate outcomes of patients treated with specific forms of therapy. Over the last few years, we have witnessed a significant improvement in our capacity to treat patients with MDS. A number of clinical characteristics are associated with increased response to lenalidomide therapy for patients with lower-risk MDS (List et al. 2006). These include the presence of an alteration of chromosome 5 in the context of anemia and no significant thrombocytopenia (List et al. 2006). Marrow hypocellularity has been proposed to be a marker of response to ATG-based therapy (Lim et al. 2007). The group at the NIH has developed an algorithm to predict response to immunosuppressive therapy in MDS that includes age, HLA-DR15 positivity, and minimal history of transfusion needs (Saunthararajah et al. 2003). Cytogenetics are known to predict for poor response to standard form of AML-like chemotherapy and more recently stem cell transplantation. That said, we are in need of biomarkers of response to hypomethylating agents. At the present time, no such biomarker exists. Mutations on TET2 have been associated with increased response rates to azacitidine (Itzykson et al. 2010) and miR29b (Blum et al. 2010; Yang et al. 2011) to decitabine. None of these two assays have been validated by other studies. More recently, the French group presented a model of response to azacitidine (Itzykson et al. 2011). Two hundred eighty-two consecutive high or intermediate-2 risk patients with MDS received azacitidine. Diagnosis was RA/RARS/RCMD in 4 %, RAEB-1 in 20 %, RAEB-2 in 54 %, and RAEB-t in 22 %. Cytogenetic risk was good in 31 %, intermediate in 17 %, and poor in 47 %. Patients received azacitidine for a median of 6 cycles. Previous low-dose cytosine arabinoside treatment ($P = 0.009$), bone marrow blasts > 15 % ($P = 0.004$), and abnormal karyotype ($P = 0.03$) independently predicted lower response rates. Complex karyotype predicted shorter responses ($P = 0.0003$). Performance status ≥ 2, intermediate- and poor-risk cytogenetics, presence of circulating blasts, and red blood cell transfusion dependency ≥ 4 units/8 weeks (all $P < 10^{-4}$) independently predicted poorer overall survival. A prognostic score based on those factors discriminated 3 risk groups with median overall survival not reached, 15.0 and 6.1 months, respectively ($P < 10^{-4}$). This prognostic score was validated in an independent set of patients ($P = 0.003$). Achievement of hematological improvement in patients who did not obtain complete or partial remission was associated with improved survival ($P < 10^{-4}$).

Finally, a number of characteristics have been described to predict for outcomes after transplantation. These include a flow cytometry assays and the presence of specific cytogenetic alterations. Deeg et al. studied the impact of the 5-group cytogenetic classification of MDS on outcome after stem cell transplantation. Results were compared with the impact of the original three cytogenetic classification of the IPSS. The effect of monosomal karyotype was also investigated. The study included data on 1,007 patients, median 45 years, transplanted with both related and unrelated donors using diverse conditioning regimens. Both IPSS and 5-group cytogenetic risk classifications were significantly associated with posttransplant relapse and mortality. The 5-group classification discriminated more clearly among the lowest- and highest-risk patients. The presence of a monosomal karyotype also further increased the rates of relapse and mortality, even after considering the IPSS or 5-group classifications. The pathologic disease category correlated with both relapse and mortality. Mortality was also influenced by patient age, donor type, conditioning regimen, platelet count, and etiology of MDS.

Conclusion

A number of prognostic scores are currently available for patients with MDS. Right now the standard should be considered, the IPSS-R. It is likely that these models are going to be rapidly replaced by other systems incorporating molecular information in the near future. We should expect them shortly once molecular analysis becomes standard for patients with MDS. Although these models are powerful tools to predict survival in untreated patients, we are in need of models to predict response to specific forms of therapy or outcome once therapy is started including stem cell transplantation.

References

Atallah E, Nussenzveig R, Yin CC et al (2008) Prognostic interaction between thrombocytosis and JAK2 V617F mutation in the WHO subcategories of myelodysplastic/myeloproliferative disease-unclassifiable and refractory anemia with ringed sideroblasts and marked thrombocytosis. Leukemia 22:1295–1298

Bejar R, Stevenson K, Abdel-Wahab O et al (2011a) Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 364:2496–2506

Bejar R, Levine R, Ebert BL (2011b) Unraveling the molecular pathophysiology of myelodysplastic syndromes. J Clin Oncol 29:504–515

Bejar R, Stevenson KE, Caughey BA et al (2012) Validation of a prognostic model and the impact of mutations in patients with lower-risk myelodysplastic syndromes. J Clin Oncol 30:3376–3382

Beran M, Wen S, Shen Y et al (2007) Prognostic factors and risk assessment in chronic myelomonocytic leukemia: validation study of the M.D. Anderson Prognostic Scoring System. Leuk Lymphoma 48:1150–1160

Blum W, Garzon R, Klisovic RB et al (2010) Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine. Proc Natl Acad Sci USA 107:7473–7478

Cazzola M, Malcovati L (2008) Prognostic relevance of anemia in myelodysplastic syndromes. Am J Hematol 83:761–762

Dayyani F, Conley AP, Strom SS et al (2010) Cause of death in patients with lower-risk myelodysplastic syndrome. Cancer 116:2174–2179

Della Porta MG, Malcovati L (2009) Clinical relevance of extra-hematologic comorbidity in the management of patients with myelodysplastic syndrome. Haematologica 94:602–606

Della Porta MG, Malcovati L, Boveri E et al (2009) Clinical relevance of bone marrow fibrosis and CD34- positive cell clusters in primary myelodysplastic syndromes. J Clin Oncol 27:754–762

Fenaux P, Mufti GJ, Hellstrom-Lindberg E et al (2009) 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 10:223–232

Garcia-Manero G, Shan J, Faderl S et al (2008) A prognostic score for patients with lower risk myelodysplastic syndrome. Leukemia 22:538–543

Greenberg P, Cox C, LeBeau MM et al (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89:2079–2088

Greenberg PL, Attar E, Bennett JM et al (2011) NCCN Clinical Practice Guidelines in Oncology: myelodysplastic syndromes. J Natl Compr Canc Netw 9: 30–56

Greenberg PL, Tuechler H, Schanz J et al (2012) Revised international prognostic scoring system for myelodysplastic syndromes. Blood 120:2454–2465

Itzykson R, Kosmider O, Cluzeau T et al (2010) Presence of TET2 mutation predicts a higher response rate to azacitidine in MDS and AML post MDS. Blood 116(21): Abstract 439

Itzykson R, Thepot S, Quesnel B et al (2011) Prognostic factors for response and overall survival in 282 patients with higher-risk myelodysplastic syndromes treated with azacitidine. Blood 117:403–411

Kantarjian H, Issa JP, Rosenfeld CS et al (2006) Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer 106:1794–1803

Kantarjian H, Giles F, List A et al (2007) The incidence and impact of thrombocytopenia in myelodysplastic syndromes. Cancer 109:1705–1714

Kantarjian H, O'Brien S, Ravandi F et al (2008) Proposal for a new risk model in myelodysplastic syndrome that accounts for events not considered in the original International Prognostic Scoring System. Cancer 113:1351–1361

Komrokji RS, Corrales-Yepez M, Al Ali N et al (2012) Validation of the MD Anderson Prognostic Risk Model for patients with myelodysplastic syndrome. Cancer 118:2659–2664

Lim ZY, Killick S, Germing U et al (2007) Low IPSS score and bone marrow hypocellularity in MDS patients predict hematological responses to antithymocyte globulin. Leukemia 21:1436–1441

List A, Dewald G, Bennett J et al (2006) Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 355:1456–1465

Malcovati L, Porta MG, Pascutto C et al (2005) Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol 23:7594–7603

Malcovati L, Germing U, Kuendgen A et al (2007) Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 25:3503–3510

Malcovati L, Della Porta MG, Strupp C et al (2011) 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 96:1433–1440

Morin RD, Johnson NA, Severson TM et al (2010) Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet 42:181–185

Naqvi K, Jabbour E, Bueso-Ramos C et al (2011a) Implications of discrepancy in morphologic diagnosis of myelodysplastic syndrome between referral and tertiary care centers. Blood 118:4690–4693

Naqvi K, Garcia-Manero G, Sardesai S et al (2011b) Association of comorbidities with overall survival in myelodysplastic syndrome: development of a prognostic model. J Clin Oncol 29(16):2240–2246

Onida F, Kantarjian HM, Smith TL et al (2002) Prognostic factors and scoring systems in chronic myelomonocytic leukemia: a retrospective analysis of 213 patients. Blood 99:840–849

Piccirillo J, Costas I, Claybour P et al (2003) The measurement of comorbidity by cancer registries. J Registry Manage 30:8–14

Sauntharajah Y, Nakamura R, Wesley R et al (2003) A simple method to predict response to immunosuppressive therapy in patients with myelodysplastic syndrome. Blood 102:3025–3027

Schanz J, Steidl C, Fonatsch C et al (2011) Coalesced multicentric analysis of 2,351 patients with myelodysplastic syndromes indicates an underestimation of poor-risk cytogenetics of myelodysplastic syndromes in the International Prognostic Scoring System. J Clin Oncol 29(15):1963–1970

Shen L, Kantarjian H, Guo Y et al (2010) DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes. J Clin Oncol 28:605–613

Tefferi A, Vardiman JW (2009) Myelodysplastic syndromes. N Engl J Med 361:1872–1885

Tong WG, Quintas-Cardama A, Kadia T et al (2012) Predicting survival of patients with hypocellular myelodysplastic syndrome: development of a disease-specific prognostic score system. Cancer 118:4462–4470

Vardiman JW, Thiele J, Arber DA et al (2009) The 2008 revision of the WHO classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 114:937–951

Wang R, Gross CP, Halene S et al (2009) Comorbidities and survival in a large cohort of patients with newly diagnosed myelodysplastic syndromes. Leuk Res 33:1594–1598

Xu F, Li X (2012) The role of histone methyltransferase EZH2 in myelodysplastic syndromes. Expert Rev Hematol 5:177–185

Yang H, Fang Z, Wei Y et al (2011) Levels of miR-29b do not predict for response in patients with acute myelogenous leukemia treated with the combination of 5-azacytidine, valproic acid, and ATRA. Am J Hematol 86:237–238

Part III

Treatment

Management of Low-Risk MDS

11

David T. Bowen

11.1 Introduction

Transformation to acute myeloid leukemia (AML) and overall survival are the endpoints most widely reported for large cohort studies of unselected MDS patients. In the high-risk MDS subtypes (RAEB II, IPSS score INT-2/High), transformation to AML exceeds non-leukemic mortality at all time points from diagnosis (Della Porta and Malcovati 2009). However, the cause of death for those patients who do not transform to AML may be directly related to their MDS, including iatrogenic complications such as iron overload, or alternatively due to comorbid conditions (Della Porta and Malcovati 2009). Thus, the management of low-risk MDS is focussed more on improving quality of life and overall survival and less on an anti-leukemic approach that dominates the management of higher-risk MDS.

11.2 Mechanism of Anemia in MDS

As predicted from the clinical presentation, in vitro studies of clonogenic growth have confirmed erythropoiesis as the hematopoietic lineage expressing the most prominent defect in patients with low-risk MDS (Backx et al. 1993; Sawada et al. 1995). While the anemia of MDS can often be multifactorial, the most prominent pathological processes are ineffective erythropoiesis and hypoproliferative erythropoiesis. Ineffective erythropoiesis is more commonly seen in refractory anemia (RA), refractory anemia with ring sideroblasts (RARS), and refractory anemia with excess blasts (RAEB) (usually <10 % blasts), while patients with >10 % blasts have more hypoproliferative erythropoiesis (Cazzola et al. 1982).

Ineffective erythropoiesis is likely to be affected by both intrinsic and extrinsic factors. Extrinsic factors include erythropoietic suppression by cytokines or suppressor T-cells (Kochenderfer et al. 2002). Gene expression profiling implicates pathways of interferon signaling also (Pellagatti et al. 2006). A previously unrecognized role for the microenvironment may also be relevant for subsets of patients such as del(5q) MDS (SPARC gene) (Pellagatti et al. 2007). Intrinsic abnormalities include haploinsufficiency for the ribosomal protein RPS14 (Ebert et al. 2008) and miR-145 and miR-146a (Starczynowski et al. 2010) in del(5q) MDS or mutation in the splice factor SF3B1 in MDS with ring sideroblasts (Papaemmanuil et al. 2011).

11.2.1 Additional Contributors to Anemia in MDS

Peripheral red cell destruction/loss may also produce anemia in MDS. Red cell loss may result from bleeding associated with thrombocytopenia

D.T. Bowen, MD
Department of Hematology,
Leeds General Infirmary,
Great George St., Leeds LS1 3EX, UK
e-mail: david.bowen@leedsth.nhs.uk

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_11, © Springer-Verlag Berlin Heidelberg 2013

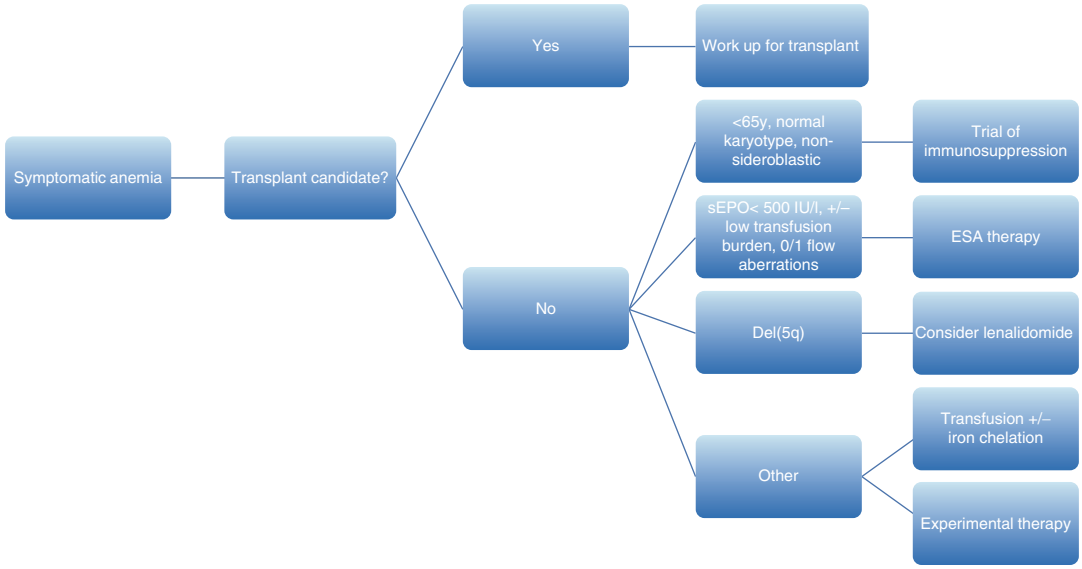

Fig. 11.1 Algorithm for the initial management of patients with low-risk MDS and symptomatic anemia

and/or platelet functional defects. Red cell lifespan is shortened in some MDS patients, and this may be due to hemolysis (often with a positive direct antiglobulin test) (Sokol et al. 1989) or hypersplenism.

11.3 Allogeneic Stem Cell Transplantation: Curative Intent

Although the median age of 74 years precludes consideration of allogeneic stem cell transplantation for the majority of patients with low-risk MDS, there is an increasing recognition that selected younger adult patients should be considered for therapy with curative intent. This is considered in more detail in Chapter 13, but such patients may include:

- Patients <50 years
- Patients <70 years with IPSS Low/INT-1 and a high red cell transfusion burden
- Patients <70 years with IPSS Low/INT-1 and symptomatic thrombocytopenia requiring regular platelet transfusion
- Patients <70 years with IPSS Low/INT-1 with evidence of disease progression (e.g., progressive and symptomatic cytopenias, increasing bone marrow blast count, clonal evolution, or increasing bone marrow fibrosis)

As the molecular landscape of MDS is revealed and the prognostic significance of new molecular abnormalities emerges, transplantation of patients not currently considered for allograft may be explored in clinical trials.

11.4 Management of Anemia

An algorithm for the management of symptomatic anemia is outlined in (Fig. 11.1).

11.4.1 Supportive Care

Although active therapeutic intervention is available for selected patients with MDS, the majority of patients will receive supportive care, either as their only treatment or before and after a therapeutic trial of active intervention. This is a consequence of both a lack of effective and/or durable therapeutic strategies and the relatively high comorbidity incidence (54 %) in this predominantly older patient cohort (Della Porta et al. 2011). The majority of MDS patients are not red cell transfusion dependent at diagnosis, but transfusion dependence is now a well-established poor prognostic factor for overall survival (Malcovati et al. 2007).

Supportive care usually refers to the replacement of deficient blood components (red cells and platelets) in symptomatic patients, treatment

of infection, and iron chelation therapy for transfusional iron overload. These interventions are not intended to alter the natural history of the disease, though there is some evidence that iron chelation therapy may have a disease-modifying effect. High-quality evidence for the effectiveness of supportive care in terms of outcome measures such as quality of life and survival is lacking and has not been systematically studied. It remains paradoxical that supportive care, which is difficult to standardize, is therefore the standard of care against which new therapeutic interventions are compared. Nevertheless, the goal of supportive care is to improve quality of life and, where possible, prolong survival.

11.4.2 Quality of Life at Baseline

Physical quality of life parameters are impaired in MDS patients compared with healthy age-sex-matched subjects. These physical parameters include physical functioning and vitality (Short Form-36 tool) and the physical component of fatigue (EuroQol-5D Visual Analogue Scale instrument). Each of these physical parameters was correlated to hemoglobin concentration in a cohort of regularly transfused MDS patients (Jansen et al. 2003) and at registration in a large low-risk MDS Registry program (EQ-5D only) (Stauder et al. 2010). Mental components of the QOL instruments were not influenced by hemoglobin concentration in either study.

11.4.3 Red Cell Transfusion

Red cell transfusion dependence and also transfusion burden are now established risk factors for poorer outcome compared to non-transfusion-dependent patients (Malcovati et al. 2006; Goldberg et al. 2010). Chronic anemia, though seldom directly life threatening, can nevertheless also produce significant morbidity and is therefore important in relation to quality of life and to comorbid disease. The influence of chronic anemia on cardiac complications in MDS patients remains unknown. Chronic anemia of MDS increases cardiac preload, which leads to left ventricular (LV) hypertrophy and an increased cardiac output (Oliva et al. 2005). In well-chelated patients with β-thalassemia, asymptomatic abnormalities of cardiac function have been documented, including both an increase in left ventricular mass and a reduced contractility (Bosi et al. 2003). LV ejection fraction was reduced in the more iron-loaded patients but was also abnormal in some patients who were well chelated.

Red cell transfusion should be considered in any patient symptomatic of anemia, irrespective of hemoglobin concentration and after exclusion of other causes. Patients with MDS may however compensate well for their anemia, and the hemoglobin trigger for the introduction of red cell transfusion will vary between individuals and also between institutions and indeed countries. The frequency of red cell transfusion is variable, and some evidence suggests that this frequency increases with time (Durairaj et al. 2011), at least in some patients. For patients with short transfusion intervals, bleeding and hemolysis should be considered, but this high transfusion requirement usually simply reflects profound erythroid failure (severe reticulocytopenia) with/without peripheral consumptive processes such as hypersplenism.

Increased amplitude of variation in hemoglobin concentration also correlates with a poorer QOL (Caocci et al. 2007). Maintenance of a consistent hemoglobin concentration >12 g/dl for at least 8 weeks, whether by transfusion or ESA, has also been associated with improved QOL in a further small study (Nilsson-Ehle et al. 2011). In separate cohorts of patients with cancer-related anemia treated with recombinant erythropoietin, a similar improvement in physical QOL parameters has been observed (Fallowfield et al. 2002; Boogaerts et al. 2003). Although most physicians and patients accept the "up-down" cyclical symptoms of a conventional transfusion program, evidence from cancer patients treated with recombinant erythropoietin would indicate the greatest incremental benefit in QOL to occur at a hemoglobin concentration >12 g/dl (Cella 1997; Demetri et al. 1998). Most regularly transfused MDS patients rarely achieve this, even transiently.

The risks associated with red cell transfusion are considerable, and many remain as yet unknown. Some such as transfusion-associated circulatory overload remain unquantified in MDS patient cohorts. Increasing recognition of transfusion-transmitted infection drives the search for

alternative strategies for the management of anemia. Although those transmitted infections with long incubation times appear of little relevance to the majority of MDS patients whose life expectancy is <6 years, there are a group of long-term transfused patients for whom this is a major issue. Red cell alloimmunization is common and increases with increasing numbers of units transfused. Despite this, reactions to red cell transfusion are equally frequent in patients without red cell alloantibodies (Fluit et al. 1990).

11.4.4 Iron Chelation Therapy

There are no convincing data on which to base recommendations for iron chelation therapy in MDS patients. A raised serum ferritin is an established independent risk factor for overall survival in patients with low-risk MDS (Sperr et al. 2010; Malcovati et al. 2005), but as yet there is no evidence that improving iron overload with iron chelation therapy can impact positively on survival. Retrospective, uncontrolled cohort studies suggest a benefit from iron chelation therapy, but these are hampered by methodological limitations (Rose et al. 2010). Anecdotally, all hematologists can recall patients with transfusional iron overload who have died of cardiac failure. For most of these patients, it is difficult to exclude at least a contribution from coexistent cardiac disease or the cardiac effects of chronic anemia (Oliva et al. 2005). For practical purposes, most guidelines have recommended instituting iron chelation therapy for those transfusion-dependent patients with a good prognosis (e.g., 5q− syndrome, WHO RARS), commencing after approximately 25 units transfused (Bowen et al. 2003). Although not licensed for MDS, this author considers that desferrioxamine remains the iron chelator of choice, primarily because of its established safety profile. Desferrioxamine is usually administered subcutaneously by infusion or rarely by twice-daily bolus. The support of an active multidisciplinary team is essential to maximize compliance with this cumbersome and invasive therapy. Desferrioxamine, given as a regular subcutaneous infusion, can reduce serum ferritin and liver iron concentration in MDS patients (Jensen et al. 1995). The addition of vitamin C 100–200 mg daily taken about 1 h prior to desferrioxamine infusion increases the proportional iron excretion but should not be started until approximately 4 weeks after desferrioxamine therapy is initiated. The use of twice-daily subcutaneous bolus injections of desferrioxamine (Franchini et al. 2000) may be considered where infusions are not tolerated, but there is even less information about their potential value in myelodysplasia than for the subcutaneous infusions. Observational studies have also suggested that desferrioxamine therapy may be associated with improved marrow function and reduced transfusion requirements (Haines and Wainscoat 1991; Jensen et al. 1996).

The new oral iron chelator deferasirox is now licensed for the treatment of iron overload in MDS patients. The efficacy and clinical benefit of deferasirox are clearly established for iron-overloaded patients with β-thalassemia major (Cappellini et al. 2011). Large phase 2 studies in patients with low-risk MDS have confirmed a reduction in serum ferritin and in labile plasma iron (Gattermann et al. 2010), and a large phase 3 study is ongoing to assess clinical benefit. Safety and tolerability of long-term administration is also to be established.

The oral iron chelator deferiprone is occasionally used but is not licensed for use in MDS and is contraindicated in neutropenic patients due to the rare side effect of agranulocytosis. Large studies demonstrating the efficacy and safety of deferiprone in MDS patients are lacking.

The main challenges are to clearly link transfusional iron overload in MDS to life-threatening complications, such as cardiac failure, and to develop reliable and reproducible methods for monitoring iron status in relevant end organs, such as the liver and heart (Pennell et al. 2011). If this can be achieved, then if iron chelation therapy improves clinical outcome in MDS, the mechanism may also be elucidated.

11.4.5 Hematopoietic Growth Factors

11.4.5.1 Introduction

Although red cell transfusion remains the most appropriate intervention for most patients, an improved understanding of the optimal use of interventional agents, coupled with the availability of new drugs, will lead to an increasing proportion

of patients treated actively with the intention of eliminating transfusion need. The most widely used pharmacological stimulants of erythropoiesis in anemic MDS patients are the recombinant erythropoietins (alpha and beta) and the longer-acting derivative erythropoietin compounds (e.g., darbepoetin alfa), collectively now referred to as erythropoiesis-stimulating agents (ESA).

The aim of interventional therapy for anemia is to improve quality of life, benchmarked either against the baseline untreated state or against best supportive care in the form of red cell transfusions. These interventional therapies have the potential to produce *sustained* increases in hemoglobin concentration and to thus avoid the up-and-down lifestyle accepted by so many regularly transfused patients and their physicians who set relatively low thresholds for transfusion trigger. A small study suggests that quality of life can be improved if a high hemoglobin concentration (>12 g/dl) is maintained by either red cell transfusion or ESA (Nilsson-Ehle et al. 2011).

An International Working Group has defined clinically relevant criteria for erythroid response evaluation in MDS clinical trials (Cheson et al. 2006). Erythroid response mandates a baseline hemoglobin concentration <9 g/dl for red cell transfusion-dependent patients (<11 g/dl for non-transfusion dependent) and a reduction in red cell transfusion by at least 4 units in an 8-week period compared with the 8 weeks prior to initiating therapy.

11.4.5.2 Erythropoiesis-Stimulating Agents (ESA)

The therapeutic efficacy of the erythropoiesis-stimulating agents including recombinant erythropoietin (EPO), alone or combined with granulocyte colony-stimulating factor (G-CSF), in the treatment of anemia is now well established for selected patients with MDS. Despite the vast literature and multitude of clinical studies, the precise role of hematopoietic growth factors in the management of MDS patients remains to be clearly defined. Definitive clinical trials, powered to demonstrate improved quality of life and improved survival, are still required but are now ongoing. Cohort studies have clearly demonstrated responses to ESA, and two small randomized studies have indicated superior erythroid response rates compared with either best supportive care plus placebo (vs. EPO alone) (Italian Cooperative Study Group 1998) or best supportive care alone (vs. EPO + G-CSF) (Casadevall et al. 2004a). In a recent cross-sectional survey of the management of MDS from 74 French centers, 40 % of low-risk patients had received an ESA within the preceding 6 months (Kelaidi et al. 2010).

ESA therapy is generally well tolerated with the most common side effects of flu-like symptoms and occasional splenic pain and enlargement. Thrombocytopenia can be accentuated in nonresponders to EPO, but rarely with adverse clinical consequences (Gabrilove et al. 2008).

However, several key questions remain (partially) unanswered:

Which Patients Will Respond?

Early indications of response predictors to ESA therapy as a single agent were identified in a meta-analysis covering trials of EPO for MDS patients up to 1994 and including 205 patients from 17 trials (Hellstrom-Lindberg et al. 1998). The overall response rate was 16 %, using 100 % reduction of transfusion need as a minimal response criterion. Factors predictive of response were non-RARS FAB subtype, pretreatment serum erythropoietin (sEPO) concentration of less than 200 u/l, and absent need for red cell transfusion. Earlier studies suggested that patients with sideroblastic erythropoiesis (WHO subtypes RARS and RCMD-RS) responded less well to ESA therapy alone, but more recent large studies indicate comparable responses across all low-risk WHO subgroups (Park et al. 2008) with no apparent difference between patients with predominant erythroid dysplasia compared with those with multilineage dysplasia. The largest cohort study of ESA-treated MDS patients has identified the following independent predictors of response in multivariate analysis: <10 % bone marrow blasts, IPSS category Low/INT-1, red cell transfusion independence, sEPO <200 IU/l, and shorter time from diagnosis (Park et al. 2008). A large phase 2 study also indicates that response rates may be higher in red cell transfusion-independent patients (Gabrilove et al. 2008).

The synergistic therapeutic effect of G-CSF added to EPO has been clearly demonstrated (Hellstrom-Lindberg et al. 1998; Remacha et al. 1999; Negrin et al. 1996). This effect was most pronounced in patients with RARS, who have

shown the best response rate to the combination (~50 %). The combination therapy was well tolerated. Using pretreatment erythropoietin as a ternary variable (<100, 100–500, or >500 u/l) and RBC transfusion requirement (<2 or ≥2 units per month) as a binary variable, a predictive model for response was developed from the data of 98 patients treated in two multicenter studies. Three groups were identified with predicted response rates of 74, 23, and 7 % (Hellstrom-Lindberg et al. 1997a). The model remained predictive of response in a prospective validation of the model, although response rates were predictably lower at 61 and 14 % in the "good" and "intermediate" predictive groups, respectively (Hellstrom-Lindberg et al. 2003). It is important to emphasize that this predictive model was derived for patients treated with a therapeutic trial of 12-week duration only.

Despite the availability of a validated predictive model for response prediction, 39 % patients with a high predictive score still fail to respond. Improved predictors of response or earlier indicators of therapeutic response are clearly required. A small study suggests that reticulocyte response at 1 week post-initial ESA dose strongly predicts response (Bowen et al. 2006). Preliminary data indicate that absent/minimal aberrant myeloid antigen expression by flow cytometry is a strong predictor of response to ESA in low-risk MDS (Westers et al. 2010). This is now under study in a prospective randomized trial.

When Should Therapy Be Initiated?

A shorter interval from diagnosis to ESA initiation (<6 months vs. > 6 months) is associated both with an improved response rate to ESA and also with a longer duration of response (Park et al. 2010). In addition there is some evidence that earlier ESA therapy is associated with delayed onset of transfusion dependence and median interval from diagnosis to transfusion dependency of 80 months versus 35 months, respectively, in patients with onset of ESA <6 months and >6 months from diagnosis (Park et al. 2010). These data are perhaps supported by the high response rate to ESA in non-transfused or minimally transfused patients (Gabrilove et al. 2008).

Optimal Dosing Schedule?

The majority of published studies have used dosing schedules for erythropoietin alpha/beta of approximately 50,000–70,000 units/week in 3–5 divided doses for a minimum of 6 weeks. Small studies have indicated equivalent efficacy of once-weekly dosing of EPO alpha/beta for MDS patients, usually at a total dose of approximately 40,000 U/week (Garypidou et al. 2003), but larger studies are required to confirm this. Darbepoetin alfa doses are typically equivalent to 150 mcg/week, usually initiating at 300 mcg every 2 weeks or 450 mcg every 3 weeks and reducing the dose and/or increasing the interval to the optimal schedule for maintenance of response. Given the potentially higher or at least faster response rate of RARS patients to combination therapy, it is reasonable to initiate treatment with ESA plus G-CSF for this group. G-CSF should be added at a dose to normalize (and at least double) the neutrophil count if it is less than 1.5×10^9/l or double the neutrophil count if it is more than 1.5×10^9/l. As for all other patients on ESA treatment, functional iron deficiency has to be considered in nonresponders, though this has not been systematically studied as a cause of ESA treatment failure in MDS.

How Prolonged a Therapeutic Trial?

Two studies with less stringent response criteria than the Scandinavian trials have demonstrated an increased response rate with prolonged growth factor treatment. Responses to therapy with EPO + G-CSF increased from 61 % at 12 weeks to 80 % at 36 weeks (Mantovani et al. 2000), while responses to EPO therapy alone increased from 18 % at 12 weeks to 45 % at 26 weeks in another cohort (Terpos et al. 2002). Both studies indicate that RARS (FAB classification) patients benefit most from these prolonged therapeutic trials, but the quality of response is not described. The extensive French experience calls these observations into question (Park et al. 2008).

For practical purposes a therapeutic trial of ESA alone for 12 weeks in red cell transfusion-dependent patients and 8 weeks in non-transfusion-dependent patients is recommended. Nonresponse to ESA alone should invoke addition of G-CSF in

patients with RARS for a further 12-week trial of therapy. A smaller proportion of patient with non-RARS low-risk MDS will respond to the addition of G-CSF.

How Durable Is Erythroid Response to Growth Factors?

The response criteria used for different studies have varied in their stringency, and those using the most stringent criteria (complete response = achievement of Hb >11.5 g/dl and transfusion independence, partial response => 2 g/dl increment in Hb concentration and independence from transfusion) (Hellstrom-Lindberg et al. 2003; Casadevall et al. 2004b) will be most likely to identify durable responses.

Quality of response determines durability with median response duration for complete responders (achievement of Hb >11.5 g/dl and transfusion independence) of 29 months versus 5.5 months for partial responders (>2 g/dl increment in Hb concentration and independence from transfusion) (Hellstrom-Lindberg et al. 2003). Prolonged responses are reported with similar durability in some cohorts (Mantovani et al. 2000; Hast et al. 2001), but not others (Casadevall et al. 2004a).

Is There a Quality of Life Improvement?

No randomized studies have been conducted with sufficient statistical power to demonstrate differences in quality of life (QOL). Small cohort studies have identified an increase in global QOL (EORTC QLQ-C30 instrument) in responding patients (Nilsson-Ehle et al. 2011; Hellstrom-Lindberg et al. 2003), while a small randomized study failed to show any difference in QOL (Functional Assessment of Cancer Therapy-Anemia tool) between cohorts treated with ESA + G-CSF versus best supportive care (Casadevall et al. 2004a).

Can ESA Therapy Impact on Overall Survival?

Two comparative cohort studies suggest an improved overall survival in patients treated with ESA with no difference in transformation to acute myeloid leukemia (Park et al. 2008; Jadersten et al. 2008). In the French study 5-year OS was 64 % for ESA-treated patients versus 39 % for historical controls, but OS benefit was restricted to ESA responders (Park et al. 2008). In the Nordic study the hazard ratio for overall survival for ESA-treated patients compared with untreated matched controls was 0.66, with the survival benefit most evident in patients with lower transfusion burden (<2 units/month) (Jadersten et al. 2008). These studies both compared a historical cohort of patients treated with ESA with a matched historical cohort treated with supportive care only. Such studies are subject to methodological limitations, and given the widespread use of ESA as the accepted standard of care in suitable low-risk MDS patients, there will never be a prospective randomized controlled trial to definitively confirm or refute this survival advantage. Given the controversy surrounding the possible decrement in overall survival for ESA-treated patients with solid tumors (Rizzo et al. 2010), it is reassuring to know that this seems highly unlikely in MDS and indeed the converse appears more likely.

Safety Monitoring

The risk of thrombosis with ESA therapy in MDS appears relatively low, 2 % in a recent study (Gabrilove et al. 2008). Nevertheless, it seems prudent to interrupt therapy if the hemoglobin concentration or hematocrit rises rapidly or if hemoglobin concentration exceeds 12 g/dl (Rizzo et al. 2010).

Mechanism of Action?

A response to ESA + G-CSF therapy is morphologically associated with a reduction of bone marrow apoptosis, reduced (but more effective) bone marrow erythropoiesis, and, in RARS, a reduced number of ring sideroblasts (Hellstrom-Lindberg et al. 1997b). In vitro, EPO + G-CSF reduces mitochondria-mediated proapoptotic pathway activation in erythroid culture systems from both RARS and RA patients (Tehranchi et al. 2003). A preferential stimulation of non-clonal hematopoietic cells in patients responding to ESA alone has also recently been reported (Fig. 11.2a, b) (Rigolin et al. 2002). Apparently off-target immunological mechanisms may also be relevant (Lifshitz et al. 2010).

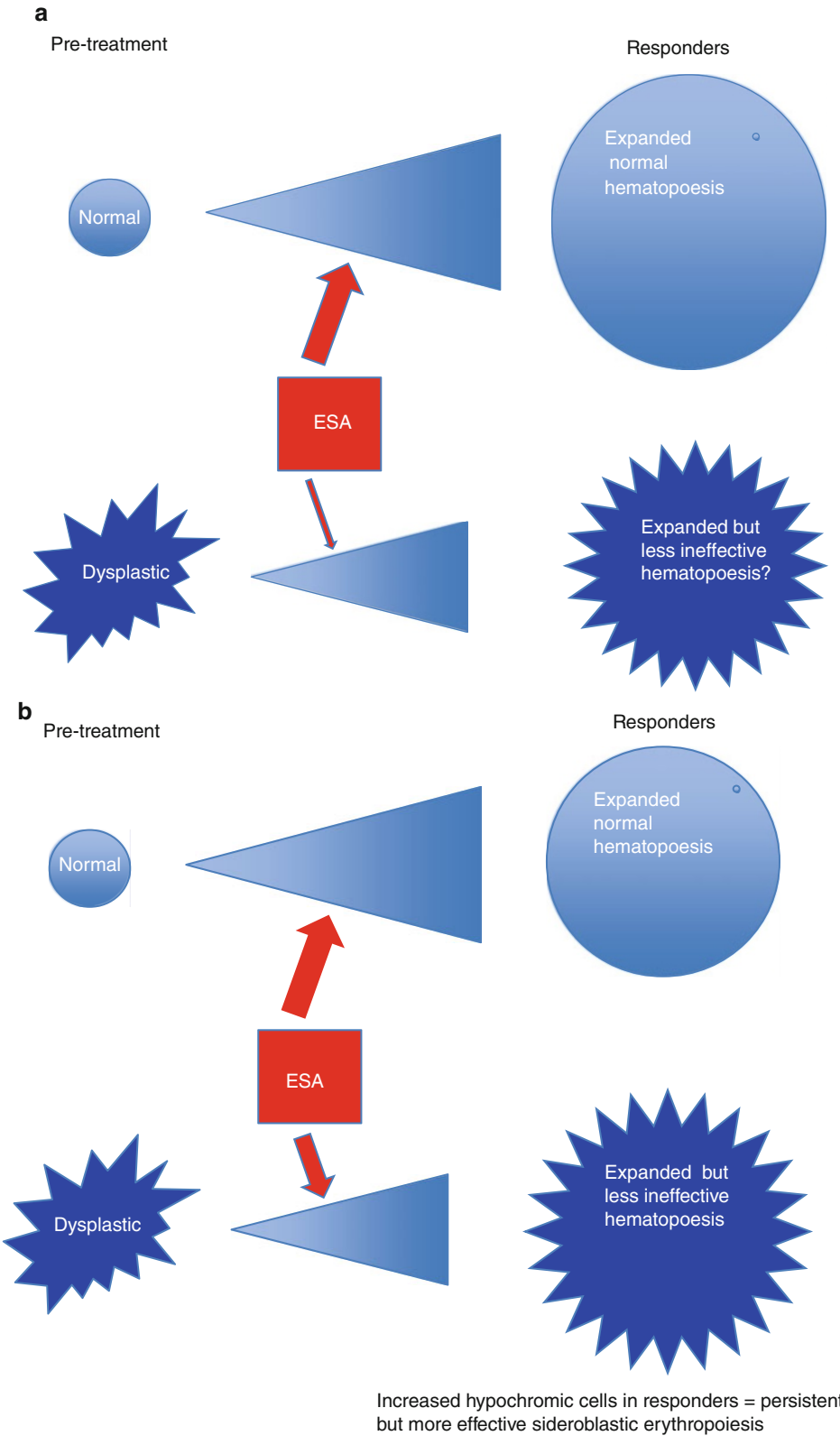

Fig. 11.2 Simplified mechanisms of action of selected agents in low-risk MDS. (**a**) ESA for non-sideroblastic low-risk MDS. (**b**) ESA for sideroblastic low-risk MDS. (**c**) Immunosuppressive therapy. (**d**) Lenalidomide

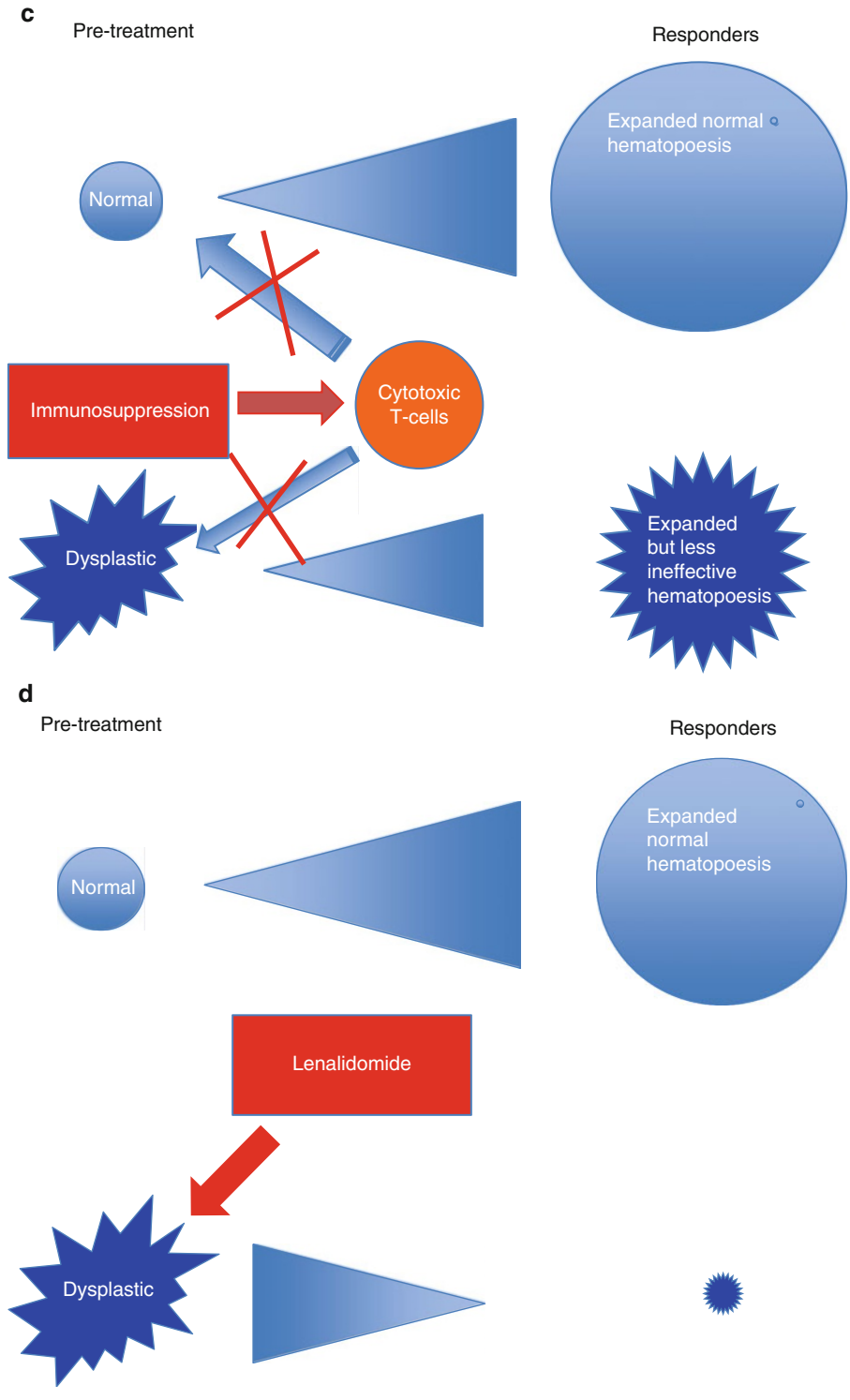

Fig. 11.2 (contitnued)

11.4.6 Erythroid Response to Combination Therapy with Erythropoiesis-Stimulating Agents

Several smaller cohort studies have indicated that response rates to the combination of erythropoietin + granulocyte-macrophage colony-stimulating factor (GM-CSF) are comparable to those with erythropoietin + G-CSF (Stasi et al. 1999). However, the only randomized study of GM-CSF + placebo versus GM-CSF + erythropoietin showed low response rates (<10 % in each arm) and little difference between both arms (Thompson et al. 2000). GM-CSF has more side effects than G-CSF, and there is little evidence to recommend GM-CSF therapy in combination with erythropoietin.

No combination studies of ESA plus other cytokines or differentiating agents have demonstrated evidence of potential superiority to ESA therapy alone. ESA plus ATRA may induce responses in patients previously failing ESA or with other factors predictive of nonresponse to frontline ESA (high transfusion burden and/or serum EPO) (Itzykson et al. 2009).

11.4.7 Immunosuppressive Therapy

Several biological observations indicate a component of immunological dysregulation in at least some patients with low-risk MDS. These include a greater than expected incidence of autoimmune abnormalities (clinical and serological) (Hamblin 1996), augmented cytotoxic T-cell activity (Kochenderfer et al. 2002), and dysregulation of regulatory T-cells (Kordasti et al. 2007). Finally there is an overlap between low-risk MDS and bone marrow failure syndromes such as aplastic anemia and paroxysmal nocturnal hemoglobinuria. These provide the rationale for immunosuppressive therapeutics, which are now an established and effective treatment in a small subgroup of patients:

11.4.7.1 Antilymphocyte/Antithymocyte Globulin (ALG/ATG)

The response rate to ATG in unselected low-risk MDS patients is approximately 30 % (Molldrem et al. 2002; Lim et al. 2007), recognizing that all patient series reported thus far have a considerably lower median age than for population-based MDS patient series. ATG is associated with a considerably higher morbidity/mortality in older patients with aplastic anemia (Tichelli et al. 1999), and its use in MDS should be restricted to relatively younger patients (typically < 65 years). In contrast to aplastic anemia, modeling data suggest a lower response rate to ATG with increasing age in MDS patients (Sloand et al. 2008), although patient numbers are small. Table 11.1 indicates optimal patient selection criteria to maximize response rate. The initial assumption that this therapy would be effective only in patients with hypocellular bone marrow is incorrect, and responses in patients with normocellular and even hypercellular marrows have been reported. ATG must be administered as an inpatient in units experienced with such therapy. The preferred source of ATG is horse derived which now has more limited availability in Europe. In aplastic anemia there is emerging evidence for a lower efficacy and higher morbidity/mortality in patients treated with rabbit ATG compared with horse ATG (Scheinberg et al. 2011). The evidence for this differential efficacy and toxicity in MDS is lacking, and indeed one small MDS study showed no significant difference between the two (Aivado et al. 2002). Biologically, response is associated with a derepression of oligoclonal T-cells on hematopoietic production with restoration of polyclonal T-cells in responders (Fig. 11.2c) (Kochenderfer et al. 2002). The mechanism of action of ATG is however multifactorial and may include direct hematopoietic stimulation. A significant advantage of ATG therapy is that it is a "one-hit" option. Ciclosporin should be introduced following ATG (Sloand et al. 2008) and continued for 6 months, but following withdrawal of ciclosporin, the median duration of response is 2 years. Most responders have a trilineage response, although red cell transfusion independence is usually the most clinically significant. Retrospective cohort comparator population analysis suggests a survival advantage for responders to ATG (Sloand et al. 2008), but such analyses have many statistical

Table 11.1 Response predictors for interventional therapy in low-risk MDS (IPSS Low/INT-1) identified as independent variables in multivariate analyses

	ESA ± G-CSF	ATG	Lenalidomide
sEPO	<500 IU/l[a]	No	No
Transfusion dependence	≤2 units per 4 weeks (Hellstrom-Lindberg et al. 1997a) No transfusions (Park et al. 2008)	Shorter duration (Sauntharajah et al. 2003)	N/A[b]
Time from diagnosis	<6 months (Park et al. 2010)	No[c]	>2 years from diagnosis
Flow cytometry	Number of myeloid aberrancies (Westers et al. 2010)	No	No
Bone marrow karyotype	No	Normal	Del(5q) as sole abnormality or with additional abnormality
WHO classification	No	No	MDS with del(5q)
Platelet count	No	Lower count (Molldrem et al. 2002)	Platelets > 150×10^9/l
Age	No	≤60 years (Sloand et al. 2008) No (Lim et al. 2007)	No
HLA subtype	No	DR15 yes (Sloand et al. 2008) DR15 no (Lim et al. 2007)	No

[a]<100 IU/l predicts for highest response rate
[b]No trials have recruited transfusion-independent patients
[c]Time from diagnosis not significant but duration of red cell transfusion dependent inversely correlated with response

flaws. Finally whether ATG therapy impacts on subsequent outcome following allogeneic stem cell transplantation remains unknown. This is an important question as the increasing recognition of the prognostic significance of transfusion dependence creates a dilemma for the management of such relatively young low-risk MDS patients (e.g., <65 years, transfusion dependent with a normal karyotype).

11.4.7.2 Ciclosporin

Ciclosporin monotherapy may have a niche role for older patients with associated evidence of autoimmune phenomena or hypocellular bone marrow. Response is lower than for ATG, and earlier studies indicating a high response rate (Okamoto et al. 2000) have not been easily reproduced.

11.4.7.3 CAMPATH

Following the efficacy of anti-CD52 monoclonal antibody therapy in aplastic anemia, early indications are for high efficacy in low-risk MDS (Sloand et al. 2010).

11.4.8 Management of Del(5q) MDS: A Special Case

The classical MDS patient with del(5q) is an older female with a macrocytic anemia and thrombocytosis. Erythroid hypoplasia is common (46 % patients) (Giagounidis et al. 2004). Biological data implicate haploinsufficiency of RPS14 (perhaps via p53 activation) in the erythroid defect (Ebert et al. 2008), haploinsufficiency of miR-145 and miR-146a (Starczynowski et al. 2010) in the thrombocytosis, and haploinsufficiency of SPARC in the clonal dominance (Pellagatti et al. 2007). Del(5q) as an isolated abnormality is associated with a good prognosis with an overall survival of approximately 12 years (Giagounidis et al. 2004).

Prior to the mid-2000s, the management of the anemia associated with del(5q) was similar to other low-risk MDS. However, it had long been recognized that this subtype of MDS had a higher response rate to the (rarely used) low-dose cytarabine than others (Juneja et al. 1994). The Groupe Francophone des Myélodysplasies

(GFM) retrospectively reviewed the response rates for del(5q) MDS treated with ESA or thalidomide. Response rates to ESA were lower (39 %) than for non-del(5q) MDS (52 %) and of shorter duration (13 vs. 27 months) but were comparable for thalidomide therapy, although again of relatively short duration (9 months).

A serendipitous observation in an early-phase clinical trial of lenalidomide for low-risk MDS has changed the management of anemia in del(5q) low-risk MDS. In this phase 2 study, 83 % patients with del(5q) MDS had an erythroid response to lenalidomide (List et al. 2005). A subsequent expanded phase 2 study showed a 67 % rate of red cell transfusion independence, with 38/62 patients having a complete cytogenetic response (List et al. 2006). A recently completed phase 3 trial has confirmed the superior erythroid response rate of lenalidomide compared with placebo and that 10 mg daily (compared with 5 mg daily) for 21 of 28 days is the optimal dose (Fenaux et al. 2011). Medium-/long-term safety and overall survival advantage have yet to be confirmed.

11.4.9 Other Therapies

11.4.9.1 Immunomodulatory Therapy for Non-del(5q) MDS

Initial studies with thalidomide demonstrated clear biological activity in low-risk MDS, but tolerance is poor in this older patient population (Raza et al. 2001). Lenalidomide monotherapy for low-risk MDS patients lacking the del(5q) karyotypic abnormality has significant activity and is currently in phase 3 study. In a large phase 2 trial of transfusion-dependent patients, 26 % achieved transfusion independence with a median duration of response of 10 months and with limited toxicity (predominantly manageable myelosuppression) (Raza et al. 2008). Factors associated with an increased likelihood of achievement of transfusion independence included lower transfusion burden, shorter duration of MDS, and higher baseline platelet count (Table 11.1).

11.4.9.2 Hypomethylating Agents

The US Federal Drugs Administration has licensed two hypomethylating agents, azacitidine and decitabine, for the treatment of patients with low-risk MDS, although neither is licensed for this indication in Europe. Subcutaneous azacitidine has clear activity in low-risk MDS, producing transfusion independence in a similar proportion of patients as in high-risk MDS in one large US-based randomized trial (Silverman et al. 2002). Recent retrospective studies have confirmed these early data (Musto et al. 2010). Oral azacitidine is promising in low-risk MDS with response rates in early-phase study that are at least comparable to the subcutaneous formulation and acceptable tolerability (Garcia-Manero et al. 2011).

11.4.9.3 Sodium Valproate

The oral histone deacetylase inhibitors sodium valproate and sodium phenylbutyrate have some clinical activity in low-risk MDS. Responses are short lived and toxicity is significant, principally fatigue (Kuendgen et al. 2004). More selective HDACIs with more potent HDAC inhibition are now in clinical trial and perhaps promise more than the first-generation inhibitors. In vitro studies indicate at least additive effects when combined with hypomethylating agents, and combination clinical trials are ongoing.

11.4.10 New Agents

Several novel agents are in early-phase clinical trial including neddylation inhibitors (MLN4924) for lenalidomide failure in del(5q) MDS, interleukin-6 inhibitors (siltuximab) for ESA failure, GST-P1-1 inhibitor (TLK199), p38 MAP kinase inhibitors (SCIO-469), and indoleamine 2,3-dioxygenase inhibitors to inhibit myeloid-derived suppressor cells. As yet, efficacy remains unclear.

11.5 Supportive Management of Thrombocytopenia and Neutropenia

11.5.1 Thrombocytopenia and Bleeding

Bleeding is usually coincident with severe thrombocytopenia due to bone marrow failure, but may

rarely also be a manifestation of MDS-associated platelet functional defects. In a large single institutional study, thrombocytopenia was more common in high-risk MDS patients and, in all MDS patients, was considered directly causative of death (hemorrhage) in 10 % of patients (Kantarjian et al. 2007). Prophylactic platelet transfusion is not indicated in asymptomatic thrombocytopenic patients and should be reserved to cover symptomatic bleeding or planned interventional procedures such as surgery or dental extraction. The efficacy of danazol in transiently improving platelet counts in MDS patients is controversial (Chan et al. 2002; Chabannon et al. 1994). Recent optimism that single-agent thrombomimetics used successfully in immune-mediated thrombocytopenia would safely increase platelet counts in patients with MDS has now been dampened by safety concerns. Romiplostim demonstrated impressive short-term efficacy in the dose-finding study, but transient blast proliferation was noted at higher doses (Kantarjian et al. 2010). The follow-on phase 3 study was stopped prematurely following concerns that transient blast proliferations were relatively frequent and the rate of AML transformation was greater in the romiplostim arm compared to placebo. Efficacy was clear, as assessed by platelet counts, reduced platelet transfusions, and a reduction in thrombocytopenic (bleeding) events.

11.5.2 Neutropenia and Infection

Neutropenia is common in MDS patients, and infection is one of the major causes of death (Table 11.1). Most MDS patients treated with granulocyte colony-stimulating factor (G-CSF) increase their blood neutrophil counts, indicating reasonable marrow reserve. No randomized studies have yet demonstrated a clear benefit for routine use of G-CSF, although in one cohort study, patients with neutrophil counts maintained at $>1.5 \times 10^9$/l had fewer infections than those with lower values (Negrin et al. 1990). Prophylactic G-CSF therapy may have some role in patients with severe chronic neutropenia and recurrent infections. There is no evidence for prophylactic antibiotic therapy in this context.

References

Aivado M, Rong A, Stadler M, Germing U, Giagounidis A, Strupp C et al (2002) Favourable response to antithymocyte or antilymphocyte globulin in low-risk myelodysplastic syndrome patients with a 'non-clonal' pattern of X-chromosome inactivation in bone marrow cells. Eur J Haematol 68(4): 210–216

Backx B, Broeders L, Touw I, Lowenberg B (1993) Blast colony-forming cells in myelodysplastic syndrome: decreased potential to generate erythroid precursors. Leukemia 7(1):75–79

Boogaerts M, Coiffier B, Kainz C (2003) Impact of epoetin beta on quality of life in patients with malignant disease. Br J Cancer 88(7):988–995

Bosi G, Crepaz R, Gamberini MR, Fortini M, Scarcia S, Bonsante E et al (2003) Left ventricular remodelling, and systolic and diastolic function in young adults with beta thalassaemia major: a Doppler echocardiographic assessment and correlation with haematological data. Heart 89(7):762–766

Bowen D, Culligan D, Jowitt S, Kelsey S, Mufti G, Oscier D et al (2003) Guidelines for the diagnosis and therapy of adult myelodysplastic syndromes. Br J Haematol 120(2):187–200

Bowen D, Hyslop A, Keenan N, Groves M, Culligan D, Johnson P et al (2006) Predicting erythroid response to recombinant erythropoietin plus granulocyte colony-stimulating factor therapy following a single subcutaneous bolus in patients with myelodysplasia. Haematologica 91(5):709–710

Caocci G, Baccoli R, Ledda A, Littera R, La Nasa G (2007) A mathematical model for the evaluation of amplitude of hemoglobin fluctuations in elderly anemic patients affected by myelodysplastic syndromes: correlation with quality of life and fatigue. Leuk Res 31(2):249–252

Cappellini MD, Bejaoui M, Agaoglu L, Canatan D, Capra M, Cohen A et al (2011) Iron chelation with deferasirox in adult and pediatric patients with thalassemia major: efficacy and safety during 5 years' follow-up. Blood 118(4):884–893

Casadevall N, Durieux P, Dubois S, Hemery F, Lepage E, Quarre MC et al (2004) Health, economic, and quality-of-life effects of erythropoietin and granulocyte colony-stimulating factor for the treatment of myelodysplastic syndromes: a randomized, controlled trial. Blood 104(2):321–327

Casadevall N, Durieux P, Dubois S, Hemery F, Lepage E, Quarre MC et al (2004b) Effects of erythropoietin (rHuEpo) plus granulocyte colony stimulating factor (rHuG-CSF) for the treatment of myelodysplastic syndromes (MDS) on anemia, costs and quality of life: a randomized controlled trial. Blood 104:321–327

Cazzola M, Barosi G, Berzuini C, Dacco M, Orlandi E, Stefanelli M et al (1982) Quantitative evaluation of erythropoietic activity in dysmyelopoietic syndromes. Br J Haematol 50(1):55–62

Cella D (1997) The Functional Assessment of Cancer Therapy-Anemia (FACT-An) Scale: a new tool for the

assessment of outcomes in cancer anemia and fatigue. Semin Hematol 34(3 Suppl 2):13–19
Chabannon C, Molina L, Pegourie-Bandelier B, Bost M, Leger J, Hollard D (1994) A review of 76 patients with myelodysplastic syndromes treated with danazol. Cancer 73:3073–3080
Chan G, DiVenuti G, Miller K (2002) Danazol for the treatment of thrombocytopenia in patients with myelodysplastic syndrome. Am J Hematol 71(3):166–171
Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD et al (2006) Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 108(2):419–425
Della Porta MG, Malcovati L (2009) Clinical relevance of extra-hematologic comorbidity in the management of patients with myelodysplastic syndrome. Haematologica 94(5):602–606 [Comment Editorial]
Della Porta MG, Malcovati L, Strupp C, Ambaglio I, Kuendgen A, Zipperer E et al (2011) Risk stratification based on both disease status and extra-hematologic comorbidities in patients with myelodysplastic syndrome. Haematologica 96(3):441–449 [Research Support, Non-U.S. Gov't]
Demetri GD, Kris M, Wade J, Degos L, Cella D (1998) Quality-of-life benefit in chemotherapy patients treated with epoetin alfa is independent of disease response or tumor type: results from a prospective community oncology study. J Clin Oncol 16:3412–3425
Durairaj S, Chew S, Hyslop A, Keenan N, Groves MJ, Tauro S (2011) Predicted costs of iron-chelators in myelodysplastic syndromes: a 10-year analysis based on actual prevalence and red cell transfusion rates. Am J Hematol 86(5):406–410
Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N et al (2008) Identification of RPS14 as a 5q− syndrome gene by RNA interference screen. Nature 451(7176):335–339 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]
Fallowfield L, Gagnon D, Zagari M, Cella D, Bresnahan B, Littlewood TJ et al (2002) Multivariate regression analyses of data from a randomised, double-blind, placebo-controlled study confirm quality of life benefit of epoetin alfa in patients receiving non-platinum chemotherapy. Br J Cancer 87(12):1341–1353
Fenaux P, Giagounidis A, Selleslag D, Beyne-Rauzy O, Mufti G, Mittelman M et al (2011) A randomized phase 3 study of lenalidomide versus placebo in RBC transfusion-dependent patients with Low-/Intermediate-1-risk myelodysplastic syndromes with del5q. Blood 118(14):3765–3776
Fluit CR, Kunst VA, Drenthe-Schonk AM (1990) Incidence of red cell antibodies after multiple blood transfusion. Transfusion 30(6):532–535
Franchini M, Gandini G, de Gironcoli M, Vassanelli A, Borgna-Pignatti C, Aprili G (2000) Safety and efficacy of subcutaneous bolus injection of deferoxamine in adult patients with iron overload. Blood 95(9): 2776–2779
Gabrilove J, Paquette R, Lyons RM, Mushtaq C, Sekeres MA, Tomita D et al (2008) Phase 2, single-arm trial to evaluate the effectiveness of darbepoetin alfa for correcting anaemia in patients with myelodysplastic syndromes. Br J Haematol 142(3):379–393 [Clinical Trial, Phase II Multicenter Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]
Garcia-Manero G, Gore SD, Cogle C, Ward R, Shi T, Macbeth KJ et al (2011) Phase I study of oral azacitidine in myelodysplastic syndromes, chronic myelomonocytic leukemia, and acute myeloid leukemia. J Clin Oncol 29(18):2521–2527 [Clinical Trial, Phase I Research Support, Non-U.S. Gov't]
Garypidou V, Verrou E, Vakalopoulou S, Perifanis V, Tziomalos K, Venizelos I (2003) Efficacy of a single, weekly dose of recombinant erythropoietin in myelodysplastic syndromes. Br J Haematol 123(5):958
Gattermann N, Finelli C, Porta MD, Fenaux P, Ganser A, Guerci-Bresler A et al (2010) Deferasirox in iron-overloaded patients with transfusion-dependent myelodysplastic syndromes: results from the large 1-year EPIC study. Leuk Res 34(9):1143–1150 [Clinical Trial Multicenter Study Research Support, Non-U.S. Gov't]
Giagounidis AA, Germing U, Haase S, Hildebrandt B, Schlegelberger B, Schoch C et al (2004) Clinical, morphological, cytogenetic, and prognostic features of patients with myelodysplastic syndromes and del(5q) including band q31. Leukemia 18(1):113–119
Goldberg SL, Chen E, Corral M, Guo A, Mody-Patel N, Pecora AL et al (2010) Incidence and clinical complications of myelodysplastic syndromes among United States Medicare beneficiaries. J Clin Oncol 28(17): 2847–2852 [Review]
Haines ME, Wainscoat JS (1991) Relapsing sideroblastic anaemia. Br J Haematol 78(2):285–286
Hamblin TJ (1996) Immunological abnormalities in myelodysplastic syndromes. Semin Hematol 33(2): 150–162
Hast R, Wallvik J, Folin A, Bernell P, Stenke L (2001) Long-term follow-up of 18 patients with myelodysplastic syndromes responding to recombinant erythropoietin treatment. Leuk Res 25(1):13–18
Hellstrom-Lindberg E, Negrin R, Stein R, Krantz S, Lindberg G, Vardiman J et al (1997a) Erythroid response to treatment with G-CSF plus erythropoietin for the anaemia of patients with myelodysplastic syndromes: proposal for a predictive model. Br J Haematol 99(2):344–351 [Clinical Trial Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't]
Hellstrom-Lindberg E, Kanter-Lewensohn L, Ost A (1997b) Morphological changes and apoptosis in bone marrow from patients with myelodysplastic syndromes treated with granulocyte-CSF and erythropoietin. Leuk Res 21(5):415–425 [Clinical Trial Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't]

Hellstrom-Lindberg E, Ahlgren T, Beguin Y, Carlsson M, Carneskog J, Dahl IM et al (1998) Treatment of anemia in myelodysplastic syndromes with granulocyte colony-stimulating factor plus erythropoietin: results from a randomized phase II study and long-term follow-up of 71 patients. Blood 92(1):68–75

Hellstrom-Lindberg E, Gulbrandsen N, Lindberg G, Ahlgren T, Dahl IM, Dybedal I et al (2003) 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 120(6):1037–1046

Italian Cooperative Study Group (1998) A randomized double-blind placebo-controlled study with subcutaneous recombinant human erythropoietin in patients with low-risk myelodysplastic syndromes. Italian Cooperative Study Group for rHuEpo in Myelodysplastic Syndromes. Br J Haematol 103(4):1070–1074

Itzykson R, Ayari S, Vassilief D, Berger E, Slama B, Vey N et al (2009) Is there a role for all-trans retinoic acid in combination with recombinant erythropoietin in myelodysplastic syndromes? A report on 59 cases. Leukemia 23(4):673–678 [Clinical Trial Multicenter Study]

Jadersten M, Malcovati L, Dybedal I, Della Porta MG, Invernizzi R, Montgomery SM et al (2008) Erythropoietin and granulocyte-colony stimulating factor treatment associated with improved survival in myelodysplastic syndrome. J Clin Oncol 26(21):3607–3613 [Research Support, Non-U.S. Gov't]

Jansen AJ, Essink-Bot ML, Beckers EA, Hop WC, Schipperus MR, van Rhenen DJ (2003) Quality of life measurement in patients with transfusion-dependent myelodysplastic syndromes. Br J Haematol 121(2):270–274

Jensen PD, Jensen FT, Christensen T, Ellegaard J (1995) Evaluation of transfusional iron overload before and during iron chelation by magnetic resonance imaging of the liver and determination of serum ferritin in adult non-thalassaemic patients. Br J Haematol 89(4):880–889

Jensen PD, Heickendorff L, Pedersen B, Bendix-Hansen K, Jensen FT, Christensen T et al (1996) The effect of iron chelation on haemopoiesis in MDS patients with transfusional iron overload. Br J Haematol 94(2):288–299

Juneja HS, Jodhani M, Gardner FH, Trevarthen D, Schottstedt M (1994) Low-dose ARA-C consistently induces hematologic responses in the clinical 5q− syndrome. Am J Hematol 46:338–342

Kantarjian H, Giles F, List A, Lyons R, Sekeres MA, Pierce S et al (2007) The incidence and impact of thrombocytopenia in myelodysplastic syndromes. Cancer 109(9):1705–1714 [Research Support, Non-U.S. Gov't Review]

Kantarjian H, Fenaux P, Sekeres MA, Becker PS, Boruchov A, Bowen D et al (2010) Safety and efficacy of romiplostim in patients with lower-risk myelodysplastic syndrome and thrombocytopenia. J Clin Oncol 28(3):437–444 [Clinical Trial, Phase I Clinical Trial, Phase II Multicenter Study Research Support, Non-U.S. Gov't]

Kelaidi C, Stamatoullas A, Beyne-Rauzy O, Raffoux E, Quesnel B, Guerci A et al (2010) Daily practice management of myelodysplastic syndromes in France: data from 907 patients in a one-week cross-sectional study by the Groupe Francophone des Myélodysplasies. Haematologica 95(6):892–899, Epub 2009 Dec 16

Kochenderfer JN, Kobayashi S, Wieder ED, Su C, Molldrem JJ (2002) Loss of T-lymphocyte clonal dominance in patients with myelodysplastic syndrome responsive to immunosuppression. Blood 100(10):3639–3645

Kordasti SY, Ingram W, Hayden J, Darling D, Barber L, Afzali B et al (2007) CD4+CD25high Foxp3+ regulatory T-cells in Myelodysplastic Syndrome (MDS). Blood 110(3):847–850

Kuendgen A, Strupp C, Aivado M, Bernhardt A, Hildebrandt B, Haas R et al (2004) Treatment of myelodysplastic syndromes with valproic acid alone or in combination with all-trans retinoic acid. Blood 104(5):1266–1269, Epub 2004 May 20

Lifshitz L, Tabak G, Gassmann M, Mittelman M, Neumann D (2010) Macrophages as novel target cells for erythropoietin. Haematologica 95(11):1823–1831 [Research Support, Non-U.S. Gov't]

Lim ZY, Killick S, Germing U, Cavenagh J, Culligan D, Bacigalupo A et al (2007) Low IPSS score and bone marrow hypocellularity in MDS patients predict hematological responses to antithymocyte globulin. Leukemia 21(7):1436–1441, Epub 2007 May 17

List A, Kurtin S, Roe DJ, Buresh A, Mahadevan D, Fuchs D et al (2005) Efficacy of lenalidomide in myelodysplastic syndromes. N Engl J Med 352(6):549–557

List A, Dewald G, Bennett J, Giagounidis A, Raza A, Feldman E et al (2006) Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 355(14):1456–1465

Malcovati L, Porta MGD, Pascutto C, Invernizzi R, Boni M, Travaglino E et al (2005) Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol 23(30):7594–7603

Malcovati L, Della Porta MG, Cazzola M (2006) Predicting survival and leukemic evolution in patients with myelodysplastic syndrome. Haematologica 91(12):1588–1590

Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R et al (2007) Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 25(23):3503–3510 [Multicenter Study Research Support, Non-U.S. Gov't]

Mantovani L, Lentini G, Hentschel B, Wickramanayake PD, Loeffler M, Diehl V et al (2000) Treatment of anaemia in myelodysplastic syndromes with prolonged administration of recombinant human granulocyte

colony-stimulating factor and erythropoietin. Br J Haematol 109(2):367–375

Molldrem JJ, Leifer E, Bahceci E, Saunthararajah Y, Rivera M, Dunbar C et al (2002) Antithymocyte globulin for treatment of the bone marrow failure associated with myelodysplastic syndromes. Ann Intern Med 137(3):156–163

Musto P, Maurillo L, Spagnoli A, Gozzini A, Rivellini F, Lunghi M et al (2010) Azacitidine for the treatment of lower risk myelodysplastic syndromes: a retrospective study of 74 patients enrolled in an Italian named patient program. Cancer 116(6):1485–1494 [Evaluation Studies Research Support, Non-U.S. Gov't]

Negrin RS, Haeuber DH, Nagler A, Kobayashi Y, Sklar J, Donlon T et al (1990) Maintenance treatment of patients with myelodysplastic syndromes using recombinant human granulocyte colony-stimulating factor. Blood 76(1):36–43

Negrin RS, Stein R, Doherty K, Cornwell J, Vardiman J, Krantz S et al (1996) Maintenance treatment of the anemia of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor and erythropoietin: evidence for in vivo synergy. Blood 87(10):4076–4081

Nilsson-Ehle H, Birgegård G, Samuelsson J, Antunovic P, Astermark J, Garelius H et al (2011) Quality of life, physical function and MRI T2* in elderly low-risk MDS patients treated to a haemoglobin level of ≥120 g/L with darbepoetin alfa ± filgrastim or erythrocyte transfusions. Eur J Haematol 87(3):244–252

Okamoto T, Okada M, Yamada S, Takatsuka H, Wada H, Tamura A et al (2000) Good response to cyclosporine therapy in patients with myelodysplastic syndromes having the HLA-DRB1*1501 allele. Leukemia 14(2):344–346

Oliva EN, Dimitrov BD, Benedetto F, D'Angelo A, Nobile F (2005) Hemoglobin level threshold for cardiac remodeling and quality of life in myelodysplastic syndrome. Leuk Res 29(10):1217–1219, Epub 2005 Apr 7

Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D et al (2011) Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med 365(15):1384–1395

Park S, Grabar S, Kelaidi C, Beyne-Rauzy O, Picard F, Bardet V et al (2008) Predictive factors of response and survival in myelodysplastic syndrome treated with erythropoietin and G-CSF: the GFM experience. Blood 111(2):574–582 [Clinical Trial Comparative Study Multicenter Study]

Park S, Kelaidi C, Sapena R, Vassilieff D, Beyne-Rauzy O, Coiteux V et al (2010) Early introduction of ESA in low risk MDS patients may delay the need for RBC transfusion: A retrospective analysis on 112 patients. Leuk Res 34(11):1430–1436

Pellagatti A, Cazzola M, Giagounidis AA, Malcovati L, Porta MG, Killick S et al (2006) Gene expression profiles of CD34+ cells in myelodysplastic syndromes: involvement of interferon-stimulated genes and correlation to FAB subtype and karyotype. Blood 108(1):337–345 [Comparative Study Research Support, Non-U.S. Gov't]

Pellagatti A, Jadersten M, Forsblom AM, Cattan H, Christensson B, Emanuelsson EK et al (2007) Lenalidomide inhibits the malignant clone and upregulates the SPARC gene mapping to the commonly deleted region in 5q− syndrome patients. Proc Natl Acad Sci USA 104(27):11406–11411 [Research Support, Non-U.S. Gov't]

Pennell DJ, Porter JB, Cappellini MD, Chan LL, El-Beshlawy A, Aydinok Y et al (2011) Continued improvement in myocardial T2* over two years of deferasirox therapy in beta-thalassemia major patients with cardiac iron overload. Haematologica 96(1):48–54 [Multicenter Study Research Support, Non-U.S. Gov't]

Raza A, Meyer P, Dutt D, Zorat F, Lisak L, Nascimben F et al (2001) Thalidomide produces transfusion independence in long-standing refractory anemias of patients with myelodysplastic syndromes. Blood 98(4):958–965

Raza A, Reeves JA, Feldman EJ, Dewald GW, Bennett JM, Deeg HJ et al (2008) Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and intermediate-1 risk myelodysplastic syndromes with karyotypes other than deletion 5q. Blood 111(1):86–93

Remacha AF, Arrizabalaga B, Villegas A, Manteiga R, Calvo T, Julia A et al (1999) Erythropoietin plus granulocyte colony-stimulating factor in the treatment of myelodysplastic syndromes. Identification of a subgroup of responders. The Spanish Erythropathology Group. Haematologica 84(12):1058–1064

Rigolin GM, Porta MD, Bigoni R, Cavazzini F, Ciccone M, Bardi A et al (2002) rHuEpo administration in patients with low-risk myelodysplastic syndromes: evaluation of erythroid precursors' response by fluorescence in situ hybridization on May-Grunwald-Giemsa-stained bone marrow samples. Br J Haematol 119(3):652–659

Rizzo JD, Brouwers M, Hurley P, Seidenfeld J, Arcasoy MO, Spivak JL et al (2010) American Society of Clinical Oncology/American Society of Hematology clinical practice guideline update on the use of epoetin and darbepoetin in adult patients with cancer. J Clin Oncol 28(33):4996–5010 [Practice Guideline Review]

Rose C, Brechignac S, Vassilief D, Pascal L, Stamatoullas A, Guerci A et al (2010) 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 34(7):864–870

Saunthararajah Y, Nakamura R, Wesley R, Wang QJ, Barrett AJ (2003) A simple method to predict response to immunosuppressive therapy in patients with myelodysplastic syndrome. Blood 102(8):3025–3027

Sawada K, Sato N, Notoya A, Tarumi T, Hirayama S, Takano H et al (1995) Proliferation and differentiation

of myelodysplastic cd34(+) cells - phenotypic subpopulations of marrow cd34(+) cells. Blood 85: 194–202

Scheinberg P, Nunez O, Weinstein B, Biancotto A, Wu CO, Young NS (2011) Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. N Engl J Med 365(5):430–438 [Comparative Study Randomized Controlled Trial Research Support, N.I.H., Intramural]

Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R et al (2002) Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol 20(10): 2429–2440

Sloand EM, Wu CO, Greenberg P, Young N, Barrett J (2008) Factors affecting response and survival in patients with myelodysplasia treated with immunosuppressive therapy. J Clin Oncol 26(15):2505–2511 [Comparative Study]

Sloand EM, Olnes MJ, Shenoy A, Weinstein B, Boss C, Loeliger K et al (2010) Alemtuzumab treatment of intermediate-1 myelodysplasia patients is associated with sustained improvement in blood counts and cytogenetic remissions. J Clin Oncol 28(35):5166–5173 [Clinical Trial, Phase I Clinical Trial, Phase II Research Support, N.I.H., Intramural]

Sokol RJ, Hewitt S, Booker DJ (1989) Erythrocyte autoantibodies, autoimmune haemolysis, and myelodysplastic syndromes. J Clin Pathol 42(10): 1088–1091

Sperr WR, Wimazal F, Kundi M, Baumgartner C, Nosslinger T, Makrai A et al (2010) Comorbidity as prognostic variable in MDS: comparative evaluation of the HCT-CI and CCI in a core dataset of 419 patients of the Austrian MDS Study Group. Ann Oncol 21(1):114–119 [Multicenter Study Research Support, Non-U.S. Gov't]

Starczynowski DT, Kuchenbauer F, Argiropoulos B, Sung S, Morin R, Muranyi A et al (2010) Identification of miR-145 and miR-146a as mediators of the 5q− syndrome phenotype. Nat Med 16(1):49–58 [Research Support, Non-U.S. Gov't]

Stasi R, Pagano A, Terzoli E, Amadori S (1999) Recombinant human granulocyte-macrophage colony-stimulating factor plus erythropoietin for the treatment of cytopenias in patients with myelodysplastic syndromes. Br J Haematol 105(1):141–148

Stauder R, Smith A, Witte T, Droste J, Fenaux P, Symeonidis A et al (2010) Health-related quality of life in newly diagnosed low risk and intermediate-1 risk MDS: report on the first 683 patients from the European LeukemiaNet Registry. ASH Annu Meet Abstr 116(21):3999

Tehranchi R, Fadeel B, Forsblom AM, Christensson B, Samuelsson J, Zhivotovsky B et al (2003) Granulocyte colony-stimulating factor inhibits spontaneous cytochrome c release and mitochondria-dependent apoptosis of myelodysplastic syndrome hematopoietic progenitors. Blood 101(3):1080–1086

Terpos E, Mougiou A, Kouraklis A, Chatzivassili A, Michalis E, Giannakoulas N et al (2002) Prolonged administration of erythropoietin increases erythroid response rate in myelodysplastic syndromes: a phase II trial in 281 patients. Br J Haematol 118(1): 174–180

Thompson JA, Gilliland DG, Prchal JT, Bennett JM, Larholt K, Nelson RA et al (2000) Effect of recombinant human erythropoietin combined with granulocyte/macrophage colony-stimulating factor in the treatment of patients with myelodysplastic syndrome. GM/EPO MDS Study Group. Blood 95(4): 1175–1179

Tichelli A, Socie G, Henry-Amar M, Marsh J, Passweg J, Schrezenmeier H et al (1999) Effectiveness of immunosuppressive therapy in older patients with aplastic anemia. European Group for Blood and Marrow Transplantation Severe Aplastic Anaemia Working Party. Ann Intern Med 130(3):193–201

Westers TM, Alhan C, Chamuleau ME, van der Vorst MJ, Eeltink C, Ossenkoppele GJ et al (2010) Aberrant immunophenotype of blasts in myelodysplastic syndromes is a clinically relevant biomarker in predicting response to growth factor treatment. Blood 115(9):1779–1784 [Research Support, Non-U.S. Gov't]

Management of High-Risk Myelodysplastic Syndrome

12

Amer M. Zeidan and Steven D. Gore

12.1 Overview of Management of High-Risk Myelodysplastic Syndrome

Myelodysplastic syndromes (MDS) comprise a group of heterogeneous clonal hematopoietic stem cell disorders characterized by dysplastic changes and ineffective hematopoiesis leading to peripheral cytopenias in one or more blood cell lines combined with a variable risk of leukemic progression. For prognostic and therapeutic purposes, MDS has been generally divided into two major risk categories, low- and high-risk groups, according to the International Prognostic Scoring System (IPSS) depending on the percentage of blasts in the bone marrow (BM), the karyotype, and the number of cell lines affected by cytopenias (Greenberg et al. 1997). High-risk myelodysplastic syndrome (HR-MDS) is usually defined by the IPSS risk categories of intermediate-2 (INT-2) and high (Greenberg et al. 1997), by the French-American-British (FAB) categories of refractory anemia with excess blasts (RAEB) and refractory anemia with excess blasts in transformation (RAEB-T) (Bennett et al. 1982), or by the World Health Organization (WHO) classification categories of refractory cytopenia with multilineage dysplasia (RCMD) and refractory anemia with excess blasts 1 and 2 (RAEB-1 and RAEB-2) (Malcovati et al. 2005, 2007). The FAB category of RAEB-T (with BM blasts of 20–30 %) was reclassified in the new WHO classification into AML with myelodysplastic features or trilineage dysplasia, but many studies of HR-MDS, especially older studies of that predated the WHO classification, have included patients with RAEB-T. The prognosis of patients with HR-MDS is generally poor with high risk of progression to acute myeloid leukemia (AML) and limited survival in absence of active therapy (Greenberg et al. 1997; Germing et al. 2000).

The goal of therapy for HR-MDS is to alter the natural history of the disease and prolong survival. The use of traditional chemotherapeutic agents for HR-MDS has been largely disappointing. Aside from enrollment into clinical trials and supportive care, the 3 standard therapeutic options for HR-MDS include allogeneic hematopoietic stem cell transplantation (alloHSCT), intensive AML-type chemotherapy, and DNA methyltransferase inhibitor (DNMTi) therapy. Although alloHSCT is the only known curative therapy for HR-MDS, only a minority of patients are considered eligible for the procedure given the high transplant-related

A.M. Zeidan (✉)
Department of Oncology,
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University,
1650 Orleans St., CRBI-186,
Baltimore, MD 21231, USA
e-mail: azeidan1@jhmi.edu

S.D. Gore, MD
Department of Pediatrics,
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins,
Division of Hematologic Malignancies,
Johns Hopkins School of Medicine,
1650 Orleans St., CRBI-288,
Baltimore, MD 21231, USA
e-mail: gorest@jhmi.edu

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_12,

mortality (TRM) and morbidity and the fact that most patients with HR-MDS are elderly and/or medically infirm. Despite recent advances in the field of alloHSCT, such as improvements in supportive care, introduction of reduced-intensity conditioning (RIC) and nonmyeloablative regimens, and increased alternative donor options that have expanded the applicability of alloHSCT to patients with HR-MDS, the majority of patients still do not proceed to alloHSCT. In addition, the use of alloHSCT for HR-MDS therapy is associated with limited efficacy, and many such transplanted patients suffer from high rates of disease relapse and/or significant transplant-related morbidity and mortality. Until the recent approval of DNMTi therapy, treatment for patients with HR-MDS who were ineligible for HSCT was largely supportive, including transfusions, hematopoietic growth factors, and antibiotics as needed. In this chapter, we will discuss the current therapeutic options for HR-MDS with a focus on DNMTi therapy and provide an overview of the main investigational agents that are in advanced stages of evaluation in HR-MDS. The role of alloHSCT in the management of HR-MDS and the management of low-risk MDS (LR-MDS) including supportive care, growth factor, and iron chelation use is discussed elsewhere in this book.

12.2 DNA Methyltransferase Inhibitor (DNMTi) Therapy in HR-MDS

12.2.1 Mechanisms of Action of DNMTi

To date, two DNMTi have been approved by the Food and Drug Administration (FDA) for the management of HR-MDS: 5-azacytidine (AZA, Vidaza®, Celgene) and decitabine (DAC, Dacogen®, Eisai). The two drugs are chemically closely related, naturally occurring analogs of the pyrimidine nucleoside cytidine. The mechanism of action of AZA and DAC is not fully understood; at high concentrations, they act as classic cytotoxic chemotherapeutic agents, especially active against myeloid tumors, while at lower doses, they reverse cytosine methylation, potentially causing reactivation of previously silenced genes (Griffiths and Gore 2008a, b). DNMTi therapy with AZA has also been shown to cause immunomodulation by affecting different subtypes of T cells, including the regulatory T cells, and by causing re-expression of tumor-associated antigens on leukemic cells (Sanchez-Abarca et al. 2010; Goodyear et al. 2010). The relative contribution of these mechanisms to the observed clinical benefit observed in HR-MDS is currently unknown (Steensma and Stone 2010).

Both AZA and DAC are potent inhibitors of DNA methyltransferases (DNMTs), a group of enzymes responsible for cytosine methylation and maintenance of such methylation in cellular progeny. Methylation of cytosine in CpG-rich islands within the promoter regions of certain genes, including tumor suppressor genes, leads to their transcriptional silencing (Griffiths and Gore 2008a, b). Aberrant DNA methylation has been shown to confer an adverse prognosis in patients with MDS independent of age, sex, and IPSS group (Shen et al. 2010). As MDS progresses, the malignant clone acquires an increasing number of methylated tumor suppressor genes, which may result in progressive downregulation of expression of tumor suppressor genes and possibly resistance to classic cytotoxic chemotherapy (Ye et al. 2009). The clinical activity of DNMTi suggests that reversal of DNA methylation may potentially restore aberrant cellular processes resulting in dysregulated cell growth, differentiation, and death (Griffiths and Gore 2008b; Boultwood and Wainscoat 2001; Kuendgen and Lubbert 2008). Despite this attractive biological hypothesis, several studies have failed to successfully correlate reversal of promoter methylation and clinical outcomes (Fandy et al. 2009; Garcia-Manero 2011).

12.2.2 Randomized Trials of DNMTi Therapy in HR-MDS

12.2.2.1 Randomized Trials of AZA in HR-MDS

AZA has been shown in randomized phase 3 trials to prolong overall survival (OS) and reduce the risk of leukemic progression in patients with HR-MDS (Silverman et al. 2002; Kantarjian et al. 2006; Fenaux et al. 2009). In the Cancer and

Leukemia Group B (CALGB) 9221 study, Silverman et al. (2002) randomized 191 patients with MDS to receive AZA at 75 mg/m^2 subcutaneously (SQ) daily for 7 days in 28-day cycles or best supportive care (BSC). The median age of patients was 68 years, and crossover from BSC arm to the AZA arm was allowed for disease progression. The overall response rate (ORR) was 60 % in the AZA arm compared to 5 % in the BSC group ($P<0.001$). In the AZA arm, 7 % achieved complete response (CR), 16 % partial response (PR), and 37 % hematological improvement (HI), compared to 5 % HI and no CR or PR in the BSC arm. Median time to leukemic progression or death was 21 months for the AZA arm compared to 13 months for BSC ($P=0.007$). Progression to AML occurred as the first event in 15 % of patients in the AZA arm compared to 38 % in the BSC arm ($P=0.001$). There was no statistically significant OS difference noted in the trial. Nonetheless, a landmark analysis that accounted for the early crossover to the AZA arm showed median survival of an additional 18 months for the AZA arm compared to 11 months for BSC ($P=0.03$).

The landmark study AZA001 confirmed the role of AZA as an important therapeutic intervention for patients with HR-MDS (Fenaux et al. 2009). In this trial, HR-MDS patients were randomly assigned in a 1:1 fashion to receive AZA at a similar schedule to CALGB 9221 or conventional care (BSC, low-dose cytarabine, or AML-type induction chemotherapy as selected by investigators before randomization). Median age of patients was 69 years. Patients were stratified by the FAB and IPSS classifications, and the results were analyzed by intention-to- treat methodology. A total of 358 patients were randomized (179 to AZA arm and 179 to conventional care regimens). After a median follow-up of 21.1 months, the median OS was 24.5 months for the AZA group versus 15 months for the conventional care group (hazard ratio (HR), 0.58; 95 % confidence interval (95%CI), 0.43–0.77; stratified log rank $P=0.0001$). At 2 years, using Kaplan-Meier estimates, 50.8 % (95%CI 42.1–58.8) of patients in the AZA arm were alive compared with 26.2 % (95%CI 18.7–34.3) in the conventional care arm ($P<0.0001$). The survival advantage with AZA held up irrespective of age or cytogenetics. Other benefits seen in the AZA-treated group included improvements in transfusion requirements and infection rates and a significant delay in leukemic transformation (Fenaux et al. 2009).

While the AZA001 was not designed to assess survival differences between the AZA arm and the individual conventional care regimens, a post hoc analysis was performed to compare patients who received AZA to those who were preselected by their physicians to receive low-dose cytarabine (Fenaux et al. 2010b). Similar to the overall study results, the patients who received AZA had twice the 2-year survival of the low-dose cytarabine-treated patients. At 2 years of follow-up, 54 and 27 % of patients who received AZA and low-dose cytarabine, respectively, were still alive. Compared to low-dose cytarabine, AZA especially prolonged OS in patients with poor cytogenetic risk, presence of chromosome 7 deletions, and patients with excess blasts. AZA-treated patients had significantly more and longer hematological responses and increased transfusion independence. In addition, AZA-treated patients had fewer grade 3–4 cytopenias and shorter hospitalizations. Another post hoc analysis from the study showed that clinical benefits extended to the AZA-treated older patients with RAEB-T (patients with 20–30 % BM blasts, now considered to have AML with myelodysplastic features under current WHO classification) (Fenaux et al. 2010c). At a median follow-up of 20.1 months, median OS for those AZA-treated patients was 24.5 months compared with 16.0 months for similar patients in the conventional care arm (HR, 0.47; 95%CI, 0.28–0.79; $P=0.005$), and 2-year OS rates were 50 and 16 %, respectively ($P=0.001$). The 2-year OS rates were also higher for AZA versus conventional care in patients considered unfit for intensive chemotherapy ($P=0.0003$). AZA was associated with fewer total days in hospital ($P<0.0001$) compared to conventional care for this group as well.

12.2.2.2 Randomized Trials of DAC in HR-MDS

In contrast to AZA, two large randomized studies failed to show an OS benefit with DAC in HR-MDS. Kantarjian et al. (2006) randomized 170 patients with MDS to receive either DAC (15 mg/m^2 given intravenously (IV) over 3 h every

8 h for 3 days for a total dose of 135 mg/m^2 per course and repeated every 6 weeks) or BSC. The patients who received DAC achieved a 17 % ORR (including 9 % CR), compared with 0 % for the BSC group ($P<0.001$). An additional 13 % of patients treated with DAC achieved HI. Responses had a median duration of 10.3 months and were associated with transfusion independence. Time to AML progression or death was not significantly different between the two groups (DAC vs. supportive care 12.1 vs. 7.8 months, respectively; $P=0.16$). In unplanned post hoc analyses, the trend was statistically significant for patients with INT-2 and high IPSS risk categories, treatment-naïve patients, and patients with de novo MDS. In another trial, Lubbert et al. (2011) randomized 233 patients with HR-MDS who were 60 years or older and considered ineligible for intensive chemotherapy to receive a similar regimen of DAC or BSC. The median age of the patients was 70 years, and 53 % of patients had poor-risk cytogenetics. There was no statistically significant prolongation in OS with DAC compared to BSC (median OS, 10.1 vs. 8.5 months, respectively; HR, 0.88; 95%CI, 0.66–1.17; $P=0.38$). The progression-free survival (PFS) was significantly prolonged with DAC compared to BSC (median PFS, 6.6 vs. 3.0 months, respectively; HR, 0.68; 95%CI, 0.52–0.88; $P=0.004$), and AML progression was significantly reduced at 1 year (from 33 % with BSC to 22 % with DAC, $P=0.036$). Short MDS duration was found to be an independent adverse prognosticator, and more patients in the DAC arm achieved CR (13 % vs. 0 %), PR (6 % vs. 0 %), and HI (15 % vs. 2 %) compared to the BSC arm.

12.2.2.3 Summary of Results of Randomized Trials of DNMTi Therapy in HR-MDS

In summary, both AZA and DAC were associated with improved patient outcomes in HR-MDS, including higher hematologic response rates, increased transfusion independence, decreased risk of leukemic progression, and improvements in patient-reported quality-of-life measures, but only AZA has been shown to date to prolong OS in a randomized fashion (Kantarjian et al. 2006; Fenaux et al. 2009; Silverman et al. 2002; Lubbert et al. 2011). No published study to date has compared AZA directly to DAC in HR-MDS. Some of the discrepancy between the OS results of AZA and DAC could partly be the result of lower median number of cycles of DAC administered and higher prevalence of patients with poor-risk karyotypes in the EORTC randomized DAC trial compared to the AZA001 trial (Fenaux et al. 2009; Lubbert et al. 2011; Itzykson and Fenaux 2012). At the present time, although AZA and DAC are felt to be mechanistically similar, AZA is considered the preferred agent for HR-MDS given that randomized data to date demonstrated survival advantage only with AZA and not with DAC (Greenberg et al. 2011).

12.2.3 Duration of DNMTi Therapy in HR-MDS

In contrast to conventional intensive chemotherapy kinetics, a long time lag exists between the start of DNMTi therapy and the first observation of a clinical response (Gore 2011; Silverman et al. 2011). In a combined analysis of 3 CALGB trials, the median number of cycles to first hematologic response was 3, with 90 % of responses seen by cycle 6 (Silverman et al. 2006). A secondary analysis of the AZA001 study showed that the median number of AZA cycles was 9, while the AZA-responding patients received a median of 14 treatment cycles (range, 2–30) (Silverman et al. 2011). Although the median time to first response was two cycles (range, 1–16), 91 % of first responses occurred by six cycles. In addition, continued AZA beyond first response improved response category in 48 % of patients, and the best response was achieved by 92 % of responders by 12 cycles. Median time from first response to best response was 3.5 cycles (95%CI, 3.0–6.0) in 30 patients who ultimately achieved a CR and 3.0 cycles (95%CI, 1.0–3.0) in 21 patients who achieved a PR. The authors concluded that continued AZA therapy may enhance clinical benefit in patients with HR-MDS (Silverman et al. 2011).

Clinical observations indicate that the survival benefit associated with AZA therapy appears to

extend beyond patients who achieve CR and PR to those who achieve HI as well. Comparison between AZA-treated patients who achieved HI in the AZA001 versus those who received conventional care and achieved HI as well showed an improved survival for the AZA-treated group (Gore et al. 2010; Itzykson et al. 2011b). Whether patients whose best response is stable disease at 6 months of treatment or greater have improved survival is unclear (Gore 2011; Greenberg et al. 2011; Garcia-Manero and Fenaux 2011). When AZA-treated patients whose best response was stable disease were compared to patients in the conventional care arm who had a similar best response, no survival difference could be detected. When interpreting these results, it should be taken into consideration that AZA-treated patients whose best response was stable disease had higher prevalence of poor-risk cytogenetics and severe thrombocytopenia compared to patients in the conventional care arm who also achieved stable disease as best response (Gore 2011; Gore et al. 2010). Similar treatment principles may also apply to DAC therapy (Garcia-Manero and Fenaux 2011).

Therefore, therapy with AZA or DAC for HR-MDS is recommended to continue for at least four to six cycles before failure of therapy is considered if there is no evidence of CR, PR, or HI, unless frank progression to AML or excessive toxicity occurs (Steensma and Stone 2010; Greenberg et al. 2011). There is no data to support stopping AZA therapy in responding patients before progression of their disease (Itzykson and Fenaux 2012). Therapy can be extended as much as possible if CR, PR, or HI has been achieved, probably until disease progression (Steensma and Stone 2010; Garcia-Manero and Fenaux 2011), while continuation beyond six cycles in case of stable disease still warrants further investigation.

12.2.4 Schedules and Routes of Administration of DNMTi Therapy

The standard of care FDA-approved schedule of AZA (75 mg/m^2/day for 7 days every 28 days) poses logistic problems as it requires weekend administration, but it is the only schedule that has been shown to date to result in a survival advantage in HR-MDS. In an effort to develop a regimen that is easier to administer, a multicenter, community-based study was initiated to evaluate 3 alternative AZA dosing schedules without weekend dosing (Lyons et al. 2009). One hundred fifty-one patients with MDS were randomly assigned to one of three regimens every 4 weeks for six cycles: AZA 5-2-2 (75 mg/m^2/day SQ for 5 days, followed by 2 days no treatment, then 75 mg/m^2/day for 2 more days, $n=50$), AZA 5-2-5 (50 mg/m^2/day SQ for 5 days, followed by 2 days no treatment, then 50 mg/m^2/day for 5 more days, $n=51$), or AZA 5 (75 mg/m^2/day SQ for 5 days, $n=50$). The majority of the randomized patients (66 %) had LR-MDS by FAB criteria. HI was achieved by 44, 45, and 56 %, and transfusion independence was achieved in 50, 55, and 64 % of AZA 5-2-2, AZA 5-2-5, and AZA 5 arms, respectively. Grade 3 or 4 toxicity was experienced in 84, 77, and 58 % in the AZA 5-2-2, AZA 5-2-5, and AZA 5. The authors concluded that all three alternative dosing regimens produced HI, transfusion independence, and safety profiles similar to the currently FDA-approved AZA regimen (Lyons et al. 2009). However, patients with baseline thrombocytopenia were more likely to achieve transfusion independence if treated with the 5-2-5 schedule. The effects on BM responses, survival, and progression to AML were not studied. These regimens have not been directly compared with the FDA-approved AZA regimen and need further testing in patients with HR-MDS. The 5-2-5 schedule was adopted after phase I study that selected 50 mg/m^2/day for 10 days as a clinically tolerable schedule particularly effective in reversing promoter methylation (Gore et al. 2006). It has been suggested that prolonged therapy with low doses of DNMTi may cause less inhibition of the cell cycle and therefore increase incorporation of the nucleoside into the DNA, with subsequent increased cell replication necessary for effective methylation reversal and potentially enhancing the activity of DNMTi therapy (Gore 2011).

The pharmacokinetics of IV AZA is almost identical to those of SQ AZA, and despite limited

published response data with IV AZA, it is reasonable to administer IV AZA for patients who suffer significant injection site reactions with SQ AZA or who are cachectic with limited SQ tissue reserve (Steensma and Stone 2010; Fenaux et al. 2010a). A recently reported phase 1 study evaluated the use of an oral formulation of AZA in patients with MDS, CMML, or AML (Garcia-Manero et al. 2011). Patients received 1 cycle of SQ AZA (75 mg/m^2) on the first 7 days of cycle 1, followed by oral AZA daily (120–600 mg) on the first 7 days of each additional 28-day cycle. A total of 41 patients received SQ and oral AZA (MDS, $n=29$; CMML, $n=4$; AML, $n=8$). Dose-limiting toxicity (grade 3/4 diarrhea) occurred at the 600-mg dose, and the maximally tolerated dose (MTD) was 480 mg. Most common grade 3/4 adverse events were diarrhea, nausea, vomiting, febrile neutropenia, and fatigue. AZA exposure increased with escalating oral doses, and the mean relative oral bioavailability ranged from 6.3 to 20 %. At the MTD, oral bioavailability was approximately 13 % that of parenteral AZA. Nonetheless, methylation reversal was similar to that seen with SQ AZA, with maximum effect at day 15 of each cycle, although fewer loci were significantly demethylated. Hematologic responses occurred in patients with MDS and CMML with an ORR of 35 % in previously treated patients and 73 % in previously untreated patients. This oral preparation is being studied in daily schedules for 14 or 21 days, further evaluating the concept of prolonged administration of lower doses of DNMTi (Gore 2011).

The two previously mentioned randomized trials of DAC used a 3-day infusional regimen that requires hospitalization. Researchers from MD Anderson reported a randomized phase 2 trial in which adults with advanced MDS or CMML were randomly assigned to 1 of 3 DAC schedules: (1) 20 mg/m^2 IV daily for 5 days, (2) 20 mg/m^2 SQ daily for 5 days, and (3) 10 mg/m^2 IV daily for 10 days (Kantarjian et al. 2007). A total of 95 patients were treated (77 MDS and 18 CMML). The overall CR rate was 34 %, with a total of 73 % achieved objective responses. The 5-day IV regimen was considered the optimal as the CR rate in this arm was 39 %, compared with 21 % in the 5-day SQ arm and 24 % in the 10-day IV arm ($P<0.05$). The 5-day IV arm was also superior at inducing hypomethylation at day 5 and at activating P15 expression at days 12 or 28 after therapy. The safety of this 5-day IV regimen was confirmed in another phase 2 study, but it showed significantly lower response rates than the MD Anderson study, with a CR rate of 17 % (Steensma et al. 2009). To date, no survival studies have performed using this 5-day outpatient regimen of DAC in HR-MDS. Similar to AZA, SQ administration of DAC has been reported with other hematological disorders, and an oral formulation of DAC has been developed, but neither has been systematically tested in MDS to date (Saunthararajah et al. 2003; Lavelle et al. 2007).

To date, no dose or time threshold for DNMTi therapy in HR-MDS has been established, so the importance of following a strict dosing schedule is not clear at this point (Steensma and Stone 2010). Since the 7-day schedule of 75 mg/m^2 daily was the only schedule associated with survival advantage in randomized trials, many experts suggest it should be the recommended schedule, especially for HR-MDS. Nonetheless, the manufacturers recommend dose delays and/or reductions for the commonly observed therapy-related cytopenias based on expert opinion, but many other experts do not agree with such delays or reductions for the mere development of severe cytopenias in the absence of life-threatening toxicities (Fenaux et al. 2010a; Steensma and Stone 2010). Many questions remain to be answered about the optimal dosing, scheduling, and management of treatment-associated cytopenias with DNMTi therapy.

12.2.5 Response Prediction for DNMTi Therapy in HR-MDS

Although DNMTi therapy leads results in clinically meaningful hematologic responses in 40–60 % (Silverman et al. 2002; Kantarjian et al. 2006; Fenaux et al. 2009; Lubbert et al. 2011), predicting the individual patients who would respond a priori has proved a challenging task (Moon et al. 2010). The ability to predict who is

likely to respond to DNMTi therapy is important especially due to the long time lag and the need to administer four to six cycles before failure of therapy is declared. To date, no reliable biomarkers have been identified that predict response to DNMTi therapy in HR-MDS. Multiple biomarkers have been suggested to predict response to DNMTi in individual studies, but none have been validated on a wider scale or gained wide acceptance yet. Although the main mechanism of action of DNMTi is believed to be induction of hypomethylation of promoters of genes, there has been no clear association between clinical responses and reversal of methylation at the cellular level (Fandy et al. 2009; Garcia-Manero 2011). The ten-eleven-translocation 2 (TET2) mutations have been reported to be possible genetic predictors of response to AZA in HR-MDS and AML with low blast counts, independent of the karyotype (Itzykson et al. 2011a). Blum et al. showed that higher levels of miR-29b were associated with clinical response to a 10-day schedule of DAC in a poor-risk cohort of older AML patients, but these findings need validation in HR-MDS (Blum et al. 2010). A recent 10-gene methylation signature has been reported to predict outcomes in MDS to DAC therapy independent of the IPSS score, but it needs further validation and technical standardization to be useful clinically (Shen et al. 2010).

The prognostic and predictive factors associated with the probability of response and OS in patients with HR-MDS who receive AZA have not been clearly defined. Recently, a French prognostic scoring system to predict response to AZA in HR-MDS patients has been developed from a cohort of patients who received AZA on compassionate-use program and was subsequently validated in a cohort of patients in the AZA001 study and in another cohort of patients from a single institution in Italy (Itzykson et al. 2011b; Breccia et al. 2012). The French group performed an analysis of 282 patients with HR-MDS who received AZA in a compassionate, patient-named program for a median of 6-cycle evaluated prognostic factors of response and survival (Itzykson et al. 2011b). In this analysis, previous low-dose cytarabine, BM blasts > 15 %, and abnormal karyotype independently predicted lower response rates, while complex karyotype predicted shorter responses. Performance status (PS) ≥2, intermediate- and poor-risk cytogenetics, presence of circulating blasts, and transfusion dependency ≥4 units/8 weeks independently predicted worse OS, and the authors developed a prognostic score based on these four factors that separated the patients into three risk categories with median OS of not reached, 15.0 and 6.1 months, respectively. This prognostic score was subsequently validated in an independent set of patients receiving AZA in the AZA001 trial. This analysis also verified the observation that achievement of HI in patients who did not obtain CR or PR was also associated with improved OS (Itzykson et al. 2011b).

This French prognostic score was subsequently validated externally in an independent cohort of 60 patients with HR-MDS who were treated with AZA in a single institution in Italy (Breccia et al. 2012). Using this scoring system, 12, 38, and 10 patients were identified as having low, intermediate, and high risk, respectively. A statistically significant difference in median OS was found in these three subgroups of patients with an OS of 21 months for low-risk, 15 months for intermediate-risk, and 11 months for high-risk patients ($P = 0.001$). In addition, this score was identified in patients who achieved CR with AZA after four cycles. CR was achieved in 6/12 low-risk patients (50 %), in 9/38 intermediate-risk patients (23.6 %), and in none of the high-risk patients ($P = 0.0001$). The median OS was 23 months for patients who reached a CR and 11 months for patients who did not achieve a response. Lastly, the French score identified patients at risk of progression to AML. Leukemic transformation developed during AZA therapy in 2/12 low-risk patients (16 %), in 9/38 intermediate-risk patients (23.6 %), and in 4/10 high-risk patients (40 %) ($P = 0.003$).

12.2.6 Adverse Events of DNMTi Therapy in HR-MDS

The main side effects of DNMTi therapy are hematologic in nature in the form of development of new or worsening cytopenias. Grade 3 or 4

neutropenia and thrombocytopenia are seen in the vast majority of AZA- and DAC-treated patients (Kantarjian et al. 2006, 2007; Fenaux et al. 2009). The hematologic adverse events are most frequently seen during the first few cycles of therapy with AZA and generally decreased during subsequent cycles and were usually managed with dosing delays (23–29 %) (Santini et al. 2010). Although the manufacturer recommends dose delay and/or reduction for persistent and significant neutropenia or thrombocytopenia, some researchers recommend staying on schedule and keeping the same dose regardless of cytopenias for at least the first few cycles, except in the presence of life-threatening complications such as major infections (Steensma and Stone 2010; Fenaux et al. 2010a). If it is decided to modify the AZA regimen due to severe toxicity, some investigators recommend increasing the interval between cycles rather than decreasing daily doses (Itzykson and Fenaux 2012; Santini et al. 2010). In one view, since cytopenias could be reflective of response to therapy at the level of malignant clones, dose delays or reductions are probably not warranted and could result in emergence of resistant clones (Steensma and Stone 2010; Fenaux et al. 2010a). On the other hand, opting for dose reductions or delays for therapy-associated cytopenias can be supported by the lack of evidence for a threshold dose or time interval for DNMTi therapy given that gene expression modulation can occur at very low doses of DNMTi (Garcia-Manero et al. 2011; Saunthararajah et al. 2003). Although some providers use prophylactic antibiotics and growth factors to manage the cytopenias that commonly emerge during DNMTi therapy, there are no established evidence-based guidelines for the use of prophylactic antibiotics or antifungals or for the use of granulocyte colony-stimulating factor (G-CSF) in this setting (Fenaux et al. 2010a; Lee et al. 2011).

Aside from cytopenias and related complications such as infections, DNMTi is generally well tolerated. The most common nonhematologic side effects of DNMTi therapy include injection site local reactions, fatigue, and gastrointestinal adverse events such as nausea, vomiting, and diarrhea, but they are typically not severe (Steensma and Stone 2010). Symptomatic management for gastrointestinal side effects is usually sufficient, while the drugs can be administered IV instead of SQ if the injection site local reactions cannot be managed with supportive measures (Fenaux et al. 2010a). Most adverse events noted with AZA therapy in the AZA001 and CALGB 9221 were transient and resolved during ongoing therapy (>83 %) (Santini et al. 2010).

12.2.7 Other Uses of DNMTi Therapy in HR-MDS

12.2.7.1 DNMTi as Maintenance Therapy After Intensive Chemotherapy Induction for HR-MDS

Gene promoter methylation status was shown to have a significant effect on the outcome of intensive chemotherapy in HR-MDS and AML arising following MDS (Grovdal et al. 2007). To assess the therapeutic implications of this observation, a phase 2 study was conducted to assess the feasibility and efficacy of maintenance AZA for older patients with HR-MDS, CMML, and MDS-AML syndromes in CR after induction intensive chemotherapy (Grovdal et al. 2010). From the 60 patients enrolled, 24 (40 %) achieved CR after induction chemotherapy, and 23 started maintenance treatment with AZA on a modified 5/28 schedule until relapse. The median CR duration was 13.5 months, >24 months in 17 % of the patients, and 18–30.5 months in the four patients with trisomy 8. The CR duration was not associated with CDKN2B methylation status or karyotype. The median OS was 20 months. Hypermethylation of CDH1 was significantly associated with low CR rate, early relapse, and short OS ($P = 0.003$). AZA therapy, at a dose of 60 mg/m^2, was well tolerated. Grade 3–4 thrombocytopenia and neutropenia occurred after 9.5 and 30 % of the cycles, respectively, but hemoglobin levels increased during treatment. The authors concluded that AZA therapy was safe and feasible and may be of benefit in a subset of patients, but it is not clear that the outcome of AZA-treated patients in this study was superior to historical experience.

12.2.7.2 DNMTi Therapy in Transplant Setting in HR-MDS

As started earlier, alloHSCT is the only known curative therapy for HR-MDS to date, but only a minority of patients with HR-MDS are transplanted, largely due to the older age, limited donor availability, and higher prevalence of comorbidities in this patient population (Lim et al. 2010). The issue of timing of alloHSCT in eligible patients with HR-MDS is discussed in detail elsewhere in this book. A commonly referenced Markov decision analysis from the International Bone Marrow Transplantation Registry concluded that earlier alloHSCT in HR-MDS is associated with longer life expectancy (Cutler et al. 2004). Nonetheless, this analysis was conducted before the widespread use of DNMTi and was not validated in a prospective fashion. In the setting of alloHSCT, DNMTi therapy has been used to induce a hematologic response prior to proceeding to the transplant, to maintain response after alloHSCT, and as salvage therapy for relapse after alloHSCT. In addition, DNMTi therapy has been evaluated as part of the conditioning regimen of alloHSCT in some studies of HR-MDS.

Should DNMTi Therapy Be Used as a Bridge Prior to AlloHSCT?

Several studies evaluated this question, but no randomized studies were performed to date. Kim et al. analyzed 19 patients who received DNMTi therapy followed by alloHSCT (Kim et al. 2011). Twelve patients were classified as HR-MDS according to the WHO classification at the time of DNMTi therapy. AZA was administered to 10 patients, while DAC was given to the other 9. After DNMTi therapy, 2 patients achieved CR, 6 had marrow CR, 3 had HI, and 6 achieved SD as best response. DNMTi therapy did not change the WHO classification in 15 patients (79 %), whereas 1 patient (5 %) improved and 3 (16 %) progressed to AML. Most patients (95 %) received a nonmyeloablative conditioning regimen based on fludarabine, busulfan, and anti-thymocyte globulin, followed by PB-mobilized stem cells. Neutrophil and platelet engraftments were achieved in 95 and 79 % of patients, respectively. The incidences of acute and chronic GVHD were 42 and 26 %, respectively. The 2-year OS rate was 68 %, and those patients who achieved CR or marrow CR with DNMTi therapy tended to fare better than those who did not. The authors concluded that DNMTi followed by alloHSCT was a feasible and effective treatment strategy for patients with HR-MDS. Similar conclusions of feasibility of DNMTi therapy for HR-MDS prior to alloHSCT were reached in another retrospective analysis (Cogle et al. 2010).

Field et al. (2010) retrospectively evaluated 54 consecutive patients with MDS or CMML who received alloHSCT from HLA-compatible donors who were analyzed according to pre-transplant AZA exposure. Thirty patients received a median of 4 (1–7) cycles of AZA, while the other 24 patients did not receive AZA prior to the transplant. The 1-year OS, relapse-free survival, and cumulative incidence of relapse rates were 47, 41, and 20 % for the AZA-treated patients compared to 60, 51, and 32 %, respectively, for the patients who did not receive AZA prior to alloHSCT. There was no difference in GVHD occurrence between the two groups. These findings suggested similar outcomes in both groups with a trend toward decreased early relapse in patients who received AZA (Field et al. 2010). In addition to its use prior to alloHSCT, DNMTi therapy has been incorporated as part of the conditioning regimens, and this subject is discussed in detail elsewhere in this book.

Is DNMTi Maintenance Therapy After AlloHSCT Beneficial?

In an effort to reduce relapse rates after alloHSCT for AML and HR-MDS, a study looked at using low-dose AZA as maintenance therapy after alloHSCT for high-risk patients (de Lima et al. 2010). The study enrolled 45 such patients, of whom 67 % were not in remission. The median age was 60 years. Escalating doses of AZA were used in four different schedules, each with 5 days of drug and 25 days of rest. Cycle 1 started on day +40. Reversible thrombocytopenia was the DLT. No dose significantly affected DNA global methylation. The optimal schedule was determined to be 32 mg/m^2 given for four cycles. The 1-year event-free survival (EFS) and OS rates were 58

and 77 %, respectively, therefore providing basis for more studies to estimate long-term responses. Factors associated with improved OS and EFS included greater number of cycles of AZA administered, although the dose of AZA received was not associated with survival. AZA administration was not associated with increased incidence of GVHD or other toxicities (de Lima et al. 2010).

Can DNMTi Therapy Be Used for Salvage After Relapse Following AlloHSCT for HR-MDS?

Relapse following alloHSCT for HR-MDS is associated with very poor outcomes. Bolanos-Meade et al. on behalf of the Hopkins group reported outcomes of 10 patients with myeloid malignancies that received AZA after a failed alloHSCT (Bolanos-Meade et al. 2011). Of the 10 patients, 6 achieved CR, 1 had stable disease, and 3 progressed after a median of 6 cycles of AZA. Only 1 patient has died (of disease progression), and no flares of GVHD were observed with administration of AZA. At the last follow-up, the median OS for the group was about 14 months. Of the 6 patients who achieved CR, 4 also achieved restoration of complete donor chimerism, and 5 of the 6 remained alive and disease-free at a median follow-up of 624 days. Three of these patients received donor lymphocyte infusions (DLI) following AZA. Two German groups also reported encouraging results in a 22- and 26-patient series in which AZA was used for relapse after alloHSCT for AML, CMML, and MDS (Czibere et al. 2010; Lubbert et al. 2010). It can be concluded from these reports that a non-intensive outpatient regimen of AZA followed by DLI is feasible with an acceptable acute GVHD rate and is associated with meaningful clinical responses, including continuous complete donor chimerism, even in some patients with poor-risk cytogenetics (Lubbert et al. 2010). A recent early-phase study reported encouraging results using preemptive AZA therapy for molecular relapse after alloHSCT for MDS or AML where AZA use was associated with an acceptable safety profile and prevented or delayed hematologic relapse in some patients (Platzbecker et al. 2012).

12.2.8 Failure of DNMTi Therapy in HR-MDS

While AZA and, to a lesser extent, DAC have become widely accepted first-line therapies for HR-MDS, the current outcomes using conventional dosing regimens of these agents leave room for significant improvement (Gore 2011). The currently FDA-approved regimens of HMAs in HD-MDS patients as a single agent result in ORR in the range of 40–60 %; the CR rates are much lower (in the range of 10–20 %), with a limited median duration of the achieved CR in the range of 10–14 months (Silverman et al. 2002; Kantarjian et al. 2006; Fenaux et al. 2009; Lubbert et al. 2011). In addition, without alloHSCT, cure is not possible, and almost all patients will progress and/or succumb to the complications of the disease. The outcomes of patients with HR-MDS whose disease fails to respond to AZA or DAC therapy or lose their initial responses are very dismal. It has been suggested that the typical failures to DNMTi therapy can be divided generally into approximately three-thirds: one-third progress to AML, another third develop to progressive disease with worsening cytopenias, and the last third include patients who refuse further therapy or die from complications (Kadia et al. 2011). A retrospective analysis showed that prior therapy with DNMTi was an independent negative predictive factor for response and OS after induction intensive chemotherapy in patients with secondary AML arising from prior MDS (Bello et al. 2011). The median OS for patients with HR-MDS after primary or secondary failure of AZA or DAC therapy were 5.6 and 4.3 months, respectively, with an estimated 1-year survival probability of 28 % in both groups and 21- to 24-month survival probability of 15 % in both groups (Prebet et al. 2011; Jabbour et al. 2010).

It has been suggested that those patients who develop resistance to one DNMTi are usually resistant to the other DNMTi and to cytarabine-based chemotherapy, although a small percentage of patients may respond to switching from AZA to DAC (Garcia-Manero 2011; Kadia et al. 2011). In a small prospective study, 14 patients with MDS who received AZA but had primary

or secondary failure or intolerance to the drug received DAC (Borthakur et al. 2008). The ORR was 28 % (3 patients had CR, and 1 had HI). Of the responders, one had stopped prior AZA due to disease progression, 2 for no response, and 1 for severe skin toxicity. DAC was well tolerated with minimal side effects, and global methylation studies from patient samples showed decrease of methylation after treatment with DAC. However, these results should be taken with caution given that several of these patients were intolerant rather than resistant to AZA.

The mechanisms of resistance to DNMTi therapy are poorly understood, and theories have ranged from variable pharmacokinetics of the approved drug doses between individual patients, to changes in proteins involved in transport, phosphorylation, and catabolism of AZA and DAC, to alternations of the target enzyme DNMT (Kadia et al. 2011). In vitro studies in malignant cell lines suggested preexisting and DAC-induced genetic instability may lead to mutations in key proteins involved in drug metabolism or transport, resulting in insufficient incorporation into DNA and therefore conferring resistance to these clones, which subsequently expand under selection pressure (Qin et al. 2009). Obviously, more work is needed to study the exact mechanisms of resistance of DNMTi in HR-MDS, and combination therapies can be rationally designed or chosen to prevent or overcome such resistance. Until the mechanisms of resistance to DNMTi therapy are better deciphered, the choice of subsequent therapy will continue to be largely empirical, and enrollment in clinical trials should be strongly considered for such patients.

It follows that there is a clear need for effective and novel therapeutic options for this patient population with HR-MDS who are ineligible or have relapsed after alloHSCT and those who have failed or relapsed after the use of DNMTi therapy. Examples of the agents that have been or currently are undergoing evaluations for this indication in clinical studies include the novel oral deoxycytidine nucleoside sapacitabine (Kantarjian et al. 2010), the new agent ON 01910. Na (Seetharam et al. 2012; Reddy et al. 2011), oral and IV formulations of clofarabine (Faderl et al. 2012, 2010), arsenic trioxide (Schiller et al. 2006; Vey et al. 2006), different farnesyltransferase inhibitors (Fenaux et al. 2007; Itzykson and Fenaux 2009; Jabbour et al. 2011), lenalidomide (Ades et al. 2009), various histone deacetylase inhibitors (Griffiths and Gore 2008b), the oral novel drug glutathione S-transferase P1-1 inhibitor ezatiostat (TLK199) (Raza et al. 2009), and the humanized anti-CD52 monoclonal antibody alemtuzumab (Sloand et al. 2010). In addition, different combinations between these agents and with DNMTi have been also evaluated in clinical trials. We will discuss these agents further later in this chapter, except for ezatiostat and alemtuzumab who have been studied mainly for MDS patients with lower IPSS risk categories.

12.3 Intensive AML-Type Chemotherapy for HR-MDS

The use of cytarabine-based intensive chemotherapy regimens in HR-MDS similar to those used for AML therapy has generally yielded disappointing results with lower rates of CR (41–58 %), higher failure rates, shorter CR durations (mean duration of 10–12 months), and more prolonged aplasia and higher induction mortality rates (16–21 %) compared to their use in de novo AML (Wattel et al. 1997; Beran et al. 2001; Garcia-Manero 2011). The use of intensive chemotherapy in HR-MDS has been limited by the older age and higher prevalence of comorbidities in this patient population. A retrospective study of patients with HR-MDS who received five different regimens of intensive chemotherapy found that achievement of CR was significantly associated with karyotype, PS, treatment in the laminar airflow room, duration of antecedent hematologic disorder, and age, but not with the FAB, IPSS risk categories, or with the particular regimen (Beran et al. 2001). A multivariate of time to death identified cytogenetic status (deletions of chromosome 5 and/or 7), increasing age, and PS > 2 as significant independent unfavorable prognostic factors. The authors concluded that innovative post-remission therapeutic options are needed because improvement in outcome is not likely to

come from intensified therapy (Beran et al. 2001). Although other studies noted higher CR rates with intensive chemotherapy in HR-MDS and AML (70 %), the response duration was still short, especially in patients with poor-risk karyotype (Knipp et al. 2007).

To date, intensive AML-type chemotherapy has not been compared directly with DNMTi therapy in a randomized fashion. The small number of patients who received intensive chemotherapy in AZA001 trial prevented a meaningful comparison with the AZA-treated group. Given that unfavorable karyotype (complex or chromosome 7 deletions) in HR-MDS patients tends to be associated with lower CR rates and short response duration with intensive chemotherapy (Knipp et al. 2007) and given the therapeutic advantage of AZA and DAC in patients with HR-MDS and chromosome 7 deletions (Fenaux et al. 2009; Blum et al. 2010), it seems that such patients, especially older ones, may benefit more from AZA therapy. In general, intensive chemotherapy in HR-MDS tends to be reserved for young, fit patients, especially those with normal or favorable cytogenetics, especially for debulking in those patients with HR-MDS with excess BM blasts (>10 %) who are planned to proceed to alloHSCT (Garcia-Manero and Fenaux 2011). Whether debulking intensive chemotherapy should be administered to patients with HR-MDS prior to alloHSCT, especially for those without excess BM blasts, is not clear at this point.

12.4 Other Single-Agent Therapies in HR-MDS

12.4.1 Histone Deacetylase Inhibitor (HDACi) Therapy in HR-MDS

HDACi modulate gene expression, induce apoptosis and cell-cycle arrest, and appear to also cause differentiation in neoplastic cells (Griffiths and Gore 2008b). The John Hopkins group reported clinical activity for the HDACi phenylbutyrate in MDS and AML, mainly in the form of HI, but further development of this agent was limited by the need for prolonged infusion and central nervous system toxicity (Gore et al. 2002). While these agents do not exhibit prominent activity in HR-MDS as monotherapy, several of these agents, such as panobinostat, vorinostat, and entinostat, have been studied in combination regimens with DNMTi (see below for details). As a class, these agents cause predominately gastrointestinal and constitutional side effects.

12.4.2 Lenalidomide in HR-MDS

Low-dose lenalidomide has been shown in the MDS 003 trial to reduce the need for transfusions and lead to transfusion independence in 76 and 67 %, respectively, of 148 patients with transfusion-dependent LR-MDS and chromosome 5q31 deletion regardless of the karyotype complexity, with a median duration of longer than 104 weeks (List et al. 2006). In addition, 73 % of the evaluable patients had partial or complete cytogenetic responses consistent with selective cytotoxicity of the 5q deletion clone. Only 8 patients (5 %) in this trial had HR-MDS by IPSS criteria, and 3 of them achieved transfusion independence. This trial led to the approval of lenalidomide by the FDA for use in LR-MDS with 5q deletion and transfusion-dependent anemia. The randomized placebo-controlled MDS 004 trial confirmed the clinical benefits in transfusion-dependent patients with LR-MDS and 5q deletion, although the rates of erythroid and cytogenetic responses were somewhat lower than the MDS 003 trial, and the trial failed to show a survival benefit (Fenaux et al. 2011). In contrast, only 26 % of patients with LR-MDS who lacked the 5q deletion achieved transfusion independence in the MDS 002 trial, and the median duration of response was lower at 41 weeks (Raza et al. 2008). In addition, the occurrence of lenalidomide-induced thrombocytopenia and neutropenia was associated with subsequent achievement of transfusion independence in patients with LR-MDS with 5q deletion but not in those without it (Sekeres et al. 2008). These findings were suggestive of a direct cytotoxic effect of lenalidomide specific to the 5q deletion clone. The exact mechanism of action of lenalidomide in this setting is still a subject of debate. A plethora of pleiotropic mechanism may play a role, including stimulation of erythropoiesis,

immunomodulation, anti-inflammatory effects, BM microenvironment changes, and angiogenesis inhibition (Heise et al. 2010). New evidence has emerged supporting a direct suppression of the 5q deletion clone by inhibition of haplodeficient cell-cycle regulatory and ribosomal proteins coded within the commonly deleted region in chromosome 5q (Oliva et al. 2010).

To examine whether a similar therapeutic effect of lenalidomide extends to patients with HR-MDS and 5q deletion, Ades et al. (2009) enrolled 47 such patients in a phase 2 trial that used a 10-mg daily dose of lenalidomide. The deletion 5q was isolated, with 1 additional and >1 additional abnormality in 19, 23, and 58 % patients, respectively. In contrast to the MDS 003 trial, hematologic responses were seen only in 27 % of patients and in 41 % if analysis was restricted to WHO-defined MDS patients (excluding RAEB-T). The responses included 7 CR (4 of whom had complete and 3 had partial cytogenetic response), 2 marrow CR, and 4 HI. Twelve patients achieved transfusion independence for a median duration of 6.5 months compared to 26 months in the MDS003 trial. Median CR duration was 11.5 months. Six of 9 (67 %) patients with isolated 5q deletion achieved CR, versus 1 of 11 and none of 27 patients with 1 or >1 additional abnormality, respectively ($P < 0.001$). Notably, 35 % of patients with initial platelets counts >100,000/μL obtained CR, while none of the 27 patients with platelet counts <100,000/μL achieved CR ($P = 0.001$). In contrast to the MDS 003 trial, the authors could not find a relation between lenalidomide-induced cytopenias and subsequent response, but this finding could have been confounded by the high rates of baseline cytopenias. The authors concluded that their findings suggest a direct cytotoxicity of lenalidomide to the dysplastic clone in patients with isolated 5q deletion and excess blasts and that the drug may play a therapeutic rule in this setting.

It has been postulated that using higher doses of lenalidomide may lead to better responses in patients HR-MDS with excess blasts and 5q deletion. A recent phase 2 study suggested tolerability and activity of high-dose lenalidomide (50 mg daily) in previously untreated older AML patients with 5q deletion who declined standard chemotherapy (Sekeres et al. 2011b). In this study, patients were treated with lenalidomide 50 mg daily for 28 days as induction therapy and 10 mg daily for 21 days of a 28-day cycle as maintenance until disease progression or unacceptable toxicity. The median age of 37 evaluable patients was 74 years, and 19 patients (51 %) had prior MDS. Of 30 patients (81 %) for whom central confirmation of pretreatment cytogenetic studies was obtained, 6 had isolated 5q deletion, 1 had 5q and +8 deletions, and 23 had complex cytogenetics. The other 7 patients had 5q deletion identified locally. Fourteen patients (38 %) completed induction therapy, 7 patients died during induction therapy, 8 had disease progression, 7 had nonfatal adverse events, and 1 entered hospice. Eight patients started maintenance therapy. Five patients (14 %) achieved PR or CR, 2 with isolated 5q deletion, and 3 with complex cytogenetics, and median relapse-free survival was 5 months, but the median OS for the entire cohort was 2 months for the entire population. The authors concluded that lenalidomide as a single agent has modest activity in older AML patients with 5q deletion. Another phase 2 study showed that high-dose lenalidomide (50 mg) was relatively well tolerated and exhibited clinical activity as initial therapy for older AML patients, especially those with lower WBC count and lower PB and BM blast percentages (Fehniger et al. 2011). Both of these studies suggested that further evaluation of high-dose lenalidomide in HR-MDS with excess blasts is warranted, and there are ongoing studies evaluating this question.

12.4.3 Clofarabine in HR-MDS

Clofarabine (CLO) is a second-generation nucleoside analog that exerts an antileukemic cytotoxic effect by inhibiting DNA synthesis and repair and by disrupting mitochondrial membrane with subsequent release of pro-apoptotic proteins (Ghanem et al. 2010). A single arm, phase 2 study evaluated the feasibility and efficacy of orally administered CLO in 32 patients with HR-MDS (Faderl et al. 2010). Median age of patients was 70 years. Three doses of CLO were evaluated, 20, 30, or 40 mg/m^2 daily for 5 days, and the

courses were repeated every 4–8 weeks. The ORR was 43 %, with 25 % CR, 9 % HI, and 9 % had "clinical benefit." In the 20 patients who had failed prior DNMTi therapy, responses included CR in 10 %, HI in 10 %, and "clinical benefit" in 10 %. No patients died within 6 weeks of induction, but 4 patients developed renal failure in the context of aplasia-associated infections. The most common side effects were gastrointestinal and hepatic, but prolonged myelosuppression (>42 days) was rare. The toxicity profile was better with lower doses of CLO, while response rates did not differ significantly. These results indicate that oral CLO in HR-MDS is well tolerated with meaningful clinical activity, but the optimal dosing and scheduling and target patient population will need further studies (Faderl et al. 2010).

A recently published trial evaluated the IV formulation of CLO in HR-MDS (Faderl et al. 2012). A total of 58 patients with HR-MDS were randomized adaptively to receive two doses of IV CLO: 15 or 30 mg/m^2 administered daily for 5 days. The median age of the group was 68 years, and 60 % of the patients had received prior DNMTi therapy. The ORR was 36 % (including 26 % CR). At the 15 mg/m^2 dose, the ORR was 41 % versus 29 % for the 30 mg/m^2 dose. Responses were lower in patients who failed DNMTi therapy (ORR, 17 %; CR rate, 14 %). The median survival was 7.4 months for all patients (13.4 months for responders vs. 21.7 months for those CR). The 8-week mortality rate was 19 %. More severe hepatic and renal adverse effects were noted at the 30 mg/m^2 dose, and myelosuppression and infections were frequent in both groups. These results suggest that the 15 mg/m^2 dose has comparable clinical activity but seems less toxic than the higher dose and can be evaluated further, possibly in alternative schedules (Faderl et al. 2012).

12.4.4 ON 01910.Na in HR-MDS

ON 01910.Na. is a multi-tyrosine-kinase inhibitor that exerts anticancer activity by multiple mechanisms including alterations in the PI3K-Akt-mTOR pathway (Reddy et al. 2011). In a phase 1/2 study, 13 patients with transfusion-dependent HR-MDS that experienced failure of response to DNMTi therapy received ON 01910. Na. In this study, responses included 4 patients with marrow CR among 8 with stable disease. These responses occurred in all morphologic, prognostic, and cytogenetic risk subgroups. The drug was well tolerated. These results suggest meaningful clinical activity and drug tolerance for ON 01910.Na in patients with HR-MDS who failed DNMTi therapy. ON 01910.Na is currently in phase 3 trials for HR-MDS associated with aberrant expression of cyclin D proteins who failed DNMTi therapy (Reddy et al. 2011).

12.4.5 Sapacitabine in HR-MDS

Sapacitabine is an oral deoxycytidine nucleoside analog that is chemically close to cytarabine but with a different mechanism of action. Sapacitabine contains a cyano group within its ring that results in rearrangement of the nucleotide while it is incorporated in the DNA, creating single-strand DNA breaks (Kadia et al. 2011). A dose-escalation phase 1 study with a classical 3+3 design enrolled 47 patients with refractory-relapsed AML and MDS (Kantarjian et al. 2010). Sapacitabine was given orally twice daily for 7 days every 3–4 weeks or twice daily for 3 days for 2 weeks every 3–4 weeks+3 design. The ORR was 28 % (9 % CR, 4 % CRp, CRi 15 %). The drug was generally well tolerated with a 4 % estimated 4-week mortality. The DLT was gastrointestinal. Ongoing phase 2 and 3 studies are evaluating the optimal dose schedules of 325 mg twice daily for 7 days and 425 mg twice daily for 3 days on days 1–3 and days 8–10.

12.4.6 Farnesyltransferase Inhibitor Therapy in HR-MDS

Farnesylation is a posttranslational modification required for many proteins to anchor to cell membranes, and one of these proteins is the oncoprotein Ras which is mutated in 10–40 % of

MDS (Itzykson and Fenaux 2009). A multicenter phase 2 trial evaluated the farnesyltransferase inhibitor tipifarnib in 82 patients with HR-MDS (Fenaux et al. 2007). Tipifarnib was given orally at 300 mg twice daily for the first 21 days of each 28-day cycle. The ORR was 32 % (15 % CR, 17 % HI), and 45 % of patients had stable disease. For the complete responders, the median response duration was 11.5 months, and 7 were still alive at time of analysis (all > 3 years). The median OS was 11.7 months (95%CI, 9.4–15.0). The most common side effects were neutropenia and thrombocytopenia, while severe nonhematologic adverse events were rarely reported. It was concluded that tipifarnib resulted in durable responses and acceptable toxicity in patients with HR-MDS. Similarly, encouraging findings were demonstrated in another early-phase study in HR-MDS and CMML that used lonafarnib, another oral farnesyltransferase inhibitor (Feldman et al. 2008). Tipifarnib was also studied in an early-phase trial in combination with cytarabine-based intensive chemotherapy for AML and HR-MDS with encouraging results (Jabbour et al. 2011).

12.4.7 Arsenic Trioxide in HR-MDS

Two phase 2 studies reported clinical activity for arsenic trioxide monotherapy in patients with HR-MDS (Schiller et al. 2006; Vey et al. 2006). In the first study (Schiller et al. 2006), patients received arsenic trioxide (0.25 mg/kg/day) on 5 consecutive days per week for 2 weeks, followed by rest for 2 weeks (= 1 cycle). The study enrolled both LR-MDS and HR-MDS patients. Among patients who completed ≥2 cycles ($n = 51$), the HI rates were 9 % in HR-MDS, and 1 patient (3 %) with HR-MDS achieved a CR. Two patients died during the study due to treatment-related toxicities. In the second study, 115 patients with MDS (including 64 patients with HR-MDS) received a loading dose of 0.3 mg/kg/day of arsenic trioxide for 5 days followed by a maintenance dose of 0.25 mg/kg twice weekly for 15 weeks (Schiller et al. 2006; Vey et al. 2006). The ORR in the study was 19 %, including 1 CR and 1 PR in the HR-MDS cohort. The hematologic response rate was 17 % for patients with HR-MDS. In both studies, responses were seen in all 3 hematologic lineages, and therapy was well tolerated. These results indicate a modest activity of arsenic trioxide, mainly in the form of HI, in HR-MDS, with a manageable toxicity profile.

12.5 Combination Therapies in HR-MDS

Given the limited responses seen with DNMTi therapy in HR-MDS, there has been a growing interest in combining AZA or DAC with other drugs with the goal of increasing response rate, duration, depth, and, ultimately, survival.

12.5.1 DNMTi and HDACi Combination in HR-MDS

Studies showed that densely methylated DNA associates with transcriptionally repressive chromatin characterized by the presence of underacetylated histones in a dynamic fashion (Cameron et al. 1999). Findings from epigenetic studies demonstrated that in vitro re-expression by reversing promoter methylation of silenced genes was achieved optimally by applying a DNMTi and sequentially following it by an HDACi (Cameron et al. 1999). Therefore, HDACi therapy was a logical choice for a combination strategy with DNMTi therapy in HR-MDS (Gore et al. 2006). Synergistic antileukemic effect was demonstrated in vitro by combining the HDACi valproic acid with AZA or DAC (Yang et al. 2005). The safety and clinical activity of valproic acid combinations with AZA and DAC and the combination of the HDACi sodium phenylbutyrate with AZA were confirmed in early-phase clinical studies (Gore et al. 2006; Garcia-Manero et al. 2006; Soriano et al. 2007). Valproic acid requires millimolar concentrations, which can be only transiently achieved in vivo, to demonstrate HDAC inhibitory activity (Gore 2011). Based on the encouraging results of early-phase trials, several randomized

trials are examining this combination (Garcia-Manero and Fenaux 2011). Several trials are evaluating combinations of DNMTi with other HDACi such as entinostat, vorinostat, panobinostat, and belinostat. Preliminary results of some of these trials reported in abstract forms in which valproic acid or entinostat was added to AZA or DAC have not shown increased response rates, but preliminary results from an AZA combination with vorinostat seem encouraging (Itzykson and Fenaux 2012). So far, early epigenetic changes and DNA damage have not been shown to predict clinical responses with these combinations (Fandy et al. 2009).

12.5.2 DNMTi and Lenalidomide Combination in HR-MDS

Due to their activity in HR-MDS that is mediated by different mechanisms of action targeting both the BM microenvironment and epigenetic changes of the dysplastic clone, the combination of lenalidomide and DNMTi for HR-MDS is an appealing one (Garcia-Manero and Fenaux 2011). A multicenter single arm phase 1 study was conducted to assess the feasibility of this combination in patients with HR-MDS (Sekeres et al. 2010). Patients were enrolled on a traditional "3 + 3" schema, with cycles lasting 28 days, and patients received a maximum of 7 cycles. The median age of 18 enrolled patients was 68 years, and follow-up was for 7 months (range, 1–26 months). No DLT occurred, and an MTD was not reached. Grade 3–4 nonhematologic toxicities included febrile neutropenia ($n = 5$), cardiac ($n = 2$), and CNS hemorrhage ($n = 2$). The ORR was 67 % (44 % CR, 17 % HI, and 6 % marrow CR). Patients achieving CR were more likely to have normal cytogenetics and lower methylation levels. The authors concluded that this combination in HR-MDS is well tolerated with encouraging clinical activity. Further studies are evaluating the go-forward regimen from this study (AZA 75 mg/m^2/day on days 1–5 and lenalidomide 10 mg on days 1–21) (Sekeres et al. 2010; Sekeres et al. 2011a).

12.5.3 DNMTi and Intensive Chemotherapy Combination in HR-MDS

Relapsed and refractory AML are frequently characterized by cytarabine resistance. A phase 1/2 study evaluated restoration of cytarabine sensitivity in this setting by reversing acquired hypermethylation of gene promoters and subsequent silencing of gene expression which has been implicated in chemoresistance (Borthakur et al. 2010). A total of 34 patients with relapsed and refractory AML and HR-MDS received a combination of AZA and cytarabine in a concomitant fashion. The combination was safe at full doses of AZA and cytarabine, without unexpected toxicities, but it was difficult to deliver more than one cycle of therapy. Minimal antileukemia activity was seen in relapsed/refractory AML, but CR was achieved in two of six minimally pretreated patients. The authors concluded that this combination of AZA and cytarabine was feasible but has limited activity in relapsed/refractory AML.

12.5.4 Other DNMTi Therapy-Based Combinations in HR-MDS

Two published reports described promising responses with a combination of AZA and the CD33-immunoconjugate gemtuzumab ozogamicin in elderly patients with HR-MDS or AML (Bayraktar et al. 2011; Nand et al. 2008), but the withdrawal of gemtuzumab ozogamicin from the US market will complicate confirming these results. In a retrospective analysis of 282 patients with HR-MDS treated with AZA, a subgroup of 32 patients who concomitantly received an erythropoiesis-stimulating agent (ESA) for a median of 5.8 months after AZA onset achieved a significantly higher rate of HI of erythroid lineage, transfusion independence, and median OS than the patients who did not receive an ESA (44 % vs. 29 %, 48 % vs. 20 %, and 19.6 months vs. 11.9 months, respectively) (Itzykson et al. 2012). In this study, the addition of an ESA significantly improved OS independently of AZA schedule and duration,

which provides basis for a prospective study evaluating this question (Itzykson et al. 2012). Another recent study reported an impressive 72 % ORR to a combination of AZA with the tumor necrosis factor inhibitor etanercept in patients with intermediate- and high-risk MDS and CMML, with the median response duration not reached at 2 years (Scott et al. 2010).

Conclusions

The first decade of the twenty-first century witnessed major advances in the management of HR-MDS. Since the majority of patients with HR-MDS are not candidates for alloHSCT, many such patients were treated only with supportive care prior to the wide use of DNMTi therapy. The first decade of the twenty-first century saw the incorporation of DNMTi therapy, especially with AZA, as a first-line standard of care for patients with HR-MDS. While DNMTi therapy is not curative, it resulted in improvements in blood counts with reduced transfusion needs, delayed leukemic progression, and extended survival in many of these patients. Our understanding of DNMTi and their ideal use for HR-MDS continues to be a process in evolution with many questions still waiting to be answered. More data is needed about the best schedules, doses, possible combinations and sequencing with other drugs, biomarkers to predict response, and duration of therapy with DNMTi. Although recent data showed that several cycles of DNMTi therapy are needed before responses are typically seen, the optimal number of cycles and the role of maintenance therapy after achieving best response need further exploration. It has been shown that improved survival with AZA in HR-MDS extends to any hematological response, but it is not clear yet if such benefit exists for patients whose best response to AZA is stable disease. As more data emerges on the mechanism of action of DNMTi and their effects on methylation, immune system, hematopoietic stem cells, and synergism with other agents, the deployment of these agents will likely result in better outcomes in HR-MDS.

Patients with HR-MDS who lose response or are refractory to DNMTi therapy have dismal prognosis and very limited therapeutic options. The development of novel agents or ways to restore or prevent emergence of resistance is a high-priority research area in HR-MDS. Achieving a breakthrough in the management of DNMTi-refractory HR-MDS will likely require a better understanding of the molecular mechanisms and signaling pathway alterations that contribute to the emergence of these resistant clones. Understanding the mechanisms of resistance to DNMTi therapy may allow for the rational design of drugs that would prevent development of such resistance or restore sensitivity to DNMTi therapy.

References

Ades L, Boehrer S, Prebet T, Beyne-Rauzy O, Legros L, Ravoet C, Dreyfus F, Stamatoullas A, Chaury MP, Delaunay J, Laurent G, Vey N, Burcheri S, Mbida RM, Hoarau N, Gardin C, Fenaux P (2009) Efficacy and safety of lenalidomide in intermediate-2 or high-risk myelodysplastic syndromes with 5q deletion: results of a phase 2 study. Blood 113:3947–3952

Bayraktar UD, Domingo GC, Schmit J, Pereira D (2011) Azacitidine combined with gemtuzumab ozogamicin in patients with relapsed/refractory acute myeloid leukemia. Leuk Lymphoma 52:913–915

Bello C, Yu D, Komrokji RS, Zhu W, Wetzstein GA, List AF, Lancet JE (2011) Outcomes after induction chemotherapy in patients with acute myeloid leukemia arising from myelodysplastic syndrome. Cancer 117:1463–1469

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C (1982) Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 51:189–199

Beran M, Shen Y, Kantarjian H, O'Brien S, Koller CA, Giles FJ, Cortes J, Thomas DA, Faderl S, Despa S, Estey EH (2001) High-dose chemotherapy in high-risk myelodysplastic syndrome: covariate-adjusted comparison of five regimens. Cancer 92:1999–2015

Blum W, Garzon R, Klisovic RB, Schwind S, Walker A, Geyer S, Liu S, Havelange V, Becker H, Schaaf L, Mickle J, Devine H, Kefauver C, Devine SM, Chan KK, Heerema NA, Bloomfield CD, Grever MR, Byrd JC, Villalona-Calero M, Croce CM, Marcucci G (2010) Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine. Proc Natl Acad Sci USA 107:7473–7478

Bolanos-Meade J, Smith BD, Gore SD, McDevitt MA, Luznik L, Fuchs EJ, Jones RJ (2011) 5-azacytidine as salvage treatment in relapsed myeloid tumors after allogeneic bone marrow transplantation. Biol Blood Marrow Transplant 17:754–758

Borthakur G, Ahdab SE, Ravandi F, Faderl S, Ferrajoli A, Newman B, Issa JP, Kantarjian H (2008) Activity of decitabine in patients with myelodysplastic syndrome previously treated with azacitidine. Leuk Lymphoma 49:690–695

Borthakur G, Huang X, Kantarjian H, Faderl S, Ravandi F, Ferrajoli A, Torma R, Morris G, Berry D, Issa JP (2010) Report of a phase 1/2 study of a combination of azacitidine and cytarabine in acute myelogenous leukemia and high-risk myelodysplastic syndromes. Leuk Lymphoma 51:73–78

Boultwood J, Wainscoat JS (2001) Clonality in the myelodysplastic syndromes. Int J Hematol 73:411–415

Breccia M, Loglisci G, Cannella L, Finsinger P, Mancini M, Serrao A, Santopietro M, Salaroli A, Alimena G (2012) 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 53:985–986

Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nat Genet 21:103–107

Cogle CR, Imanirad I, Wiggins LE, Hsu J, Brown R, Scornik JC, Wingard JR (2010) Hypomethylating agent induction therapy followed by hematopoietic cell transplantation is feasible in patients with myelodysplastic syndromes. Clin Adv Hematol Oncol 8: 40–46

Cutler CS, Lee SJ, Greenberg P, Deeg HJ, Perez WS, Anasetti C, Bolwell BJ, Cairo MS, Gale RP, Klein JP, Lazarus HM, Liesveld JL, McCarthy PL, Milone GA, Rizzo JD, Schultz KR, Trigg ME, Keating A, Weisdorf DJ, Antin JH, Horowitz MM (2004) A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. Blood 104:579–585

Czibere A, Bruns I, Kroger N, Platzbecker U, Lind J, Zohren F, Fenk R, Germing U, Schroder T, Graf T, Haas R, Kobbe G (2010) 5-azacytidine for the treatment of patients with acute myeloid leukemia or myelodysplastic syndrome who relapse after allo-SCT: a retrospective analysis. Bone Marrow Transplant 45:872–876

de Lima M, Giralt S, Thall PF, de Padua Silva L, Jones RB, Komanduri K, Braun TM, Nguyen HQ, Champlin R, Garcia-Manero G (2010) Maintenance therapy with low-dose azacitidine after allogeneic hematopoietic stem cell transplantation for recurrent acute myelogenous leukemia or myelodysplastic syndrome: a dose and schedule finding study. Cancer 116: 5420–5431

Faderl S, Garcia-Manero G, Estrov Z, Ravandi F, Borthakur G, Cortes JE, O'Brien S, Gandhi V, Plunkett W, Byrd A, Kwari M, Kantarjian HM (2010) Oral clofarabine in the treatment of patients with higher-risk myelodysplastic syndrome. J Clin Oncol 28:2755–2760

Faderl S, Garcia-Manero G, Jabbour E, Ravandi F, Borthakur G, Estrov Z, Gandhi V, Byrd AL, Kwari M, Cortes J, Kantarjian HM (2012) A randomized study of 2 dose levels of intravenous clofarabine in the treatment of patients with higher-risk myelodysplastic syndrome. Cancer 118:722–728

Fandy TE, Herman JG, Kerns P, Jiemjit A, Sugar EA, Choi SH, Yang AS, Aucott T, Dauses T, Odchimar-Reissig R, Licht J, McConnell MJ, Nasrallah C, Kim MK, Zhang W, Sun Y, Murgo A, Espinoza-Delgado I, Oteiza K, Owoeye I, Silverman LR, Gore SD, Carraway HE (2009) Early epigenetic changes and DNA damage do not predict clinical response in an overlapping schedule of 5-azacytidine and entinostat in patients with myeloid malignancies. Blood 114:2764–2773

Fehniger TA, Uy GL, Trinkaus K, Nelson AD, Demland J, Abboud CN, Cashen AF, Stockerl-Goldstein KE, Westervelt P, DiPersio JF, Vij R (2011) A phase 2 study of high-dose lenalidomide as initial therapy for older patients with acute myeloid leukemia. Blood 117:1828–1833

Feldman EJ, Cortes J, DeAngelo DJ, Holyoake T, Simonsson B, O'Brien SG, Reiffers J, Turner AR, Roboz GJ, Lipton JH, Maloisel F, Colombat P, Martinelli G, Nielsen JL, Petersdorf S, Guilhot F, Barker J, Kirschmeier P, Frank E, Statkevich P, Zhu Y, Loechner S, List A (2008) On the use of lonafarnib in myelodysplastic syndrome and chronic myelomonocytic leukemia. Leukemia 22:1707–1711

Fenaux P, Raza A, Mufti GJ, Aul C, Germing U, Kantarjian H, Cripe L, Kerstens R, De Porre P, Kurzrock R (2007) A multicenter phase 2 study of the farnesyltransferase inhibitor tipifarnib in intermediate- to high-risk myelodysplastic syndrome. Blood 109:4158–4163

Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, Schoch R, Gattermann N, Sanz G, List A, Gore SD, Seymour JF, Bennett JM, Byrd J, Backstrom J, Zimmerman L, McKenzie D, Beach C, Silverman LR, International Vidaza High-Risk MDS Survival Study Group (2009) 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 10:223–232

Fenaux P, Bowen D, Gattermann N, Hellstrom-Lindberg E, Hofmann WK, Pfeilstocker M, Sanz G, Santini V (2010a) Practical use of azacitidine in higher-risk myelodysplastic syndromes: an expert panel opinion. Leuk Res 34:1410–1416

Fenaux P, Gattermann N, Seymour JF, Hellstrom-Lindberg E, Mufti GJ, Duehrsen U, Gore SD, Ramos F, Beyne-Rauzy O, List A, McKenzie D, Backstrom J, Beach

CL (2010b) Prolonged survival with improved tolerability in higher-risk myelodysplastic syndromes: azacitidine compared with low dose ara-C. Br J Haematol 149:244–249

Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Gattermann N, Germing U, Sanz G, List AF, Gore S, Seymour JF, Dombret H, Backstrom J, Zimmerman L, McKenzie D, Beach CL, Silverman LR (2010c) Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. J Clin Oncol 28:562–569

Fenaux P, Giagounidis A, Selleslag D, Beyne-Rauzy O, Mufti G, Mittelman M, Muus P, Te Boekhorst P, Sanz G, Del Canizo C, Guerci-Bresler A, Nilsson L, Platzbecker U, Lubbert M, Quesnel B, Cazzola M, Ganser A, Bowen D, Schlegelberger B, Aul C, Knight R, Francis J, Fu T, Hellstrom-Lindberg E, MDS-004 Lenalidomide del5q Study Group (2011) A randomized phase 3 study of lenalidomide versus placebo in RBC transfusion-dependent patients with low-/Intermediate-1-risk myelodysplastic syndromes with del5q. Blood 118:3765–3776

Field T, Perkins J, Huang Y, Kharfan-Dabaja MA, Alsina M, Ayala E, Fernandez HF, Janssen W, Lancet J, Perez L, Sullivan D, List A, Anasetti C (2010) 5-azacitidine for myelodysplasia before allogeneic hematopoietic cell transplantation. Bone Marrow Transplant 45: 255–260

Garcia-Manero G (2011) Myelodysplastic syndromes: 2011 update on diagnosis, risk-stratification, and management. Am J Hematol 86:490–498

Garcia-Manero G, Fenaux P (2011) Hypomethylating agents and other novel strategies in myelodysplastic syndromes. J Clin Oncol 29:516–523

Garcia-Manero G, Kantarjian HM, Sanchez-Gonzalez B, Yang H, Rosner G, Verstovsek S, Rytting M, Wierda WG, Ravandi F, Koller C, Xiao L, Faderl S, Estrov Z, Cortes J, O'Brien S, Estey E, Bueso-Ramos C, Fiorentino J, Jabbour E, Issa JP (2006) Phase 1/2 study of the combination of 5-aza-2′-deoxycytidine with valproic acid in patients with leukemia. Blood 108:3271–3279

Garcia-Manero G, Gore SD, Cogle C, Ward R, Shi T, Macbeth KJ, Laille E, Giordano H, Sakoian S, Jabbour E, Kantarjian H, Skikne B (2011) Phase I study of oral azacitidine in myelodysplastic syndromes, chronic myelomonocytic leukemia, and acute myeloid leukemia. J Clin Oncol 29:2521–2527

Germing U, Gattermann N, Strupp C, Aivado M, Aul C (2000) Validation of the WHO proposals for a new classification of primary myelodysplastic syndromes: a retrospective analysis of 1600 patients. Leuk Res 24:983–992

Ghanem H, Jabbour E, Faderl S, Ghandhi V, Plunkett W, Kantarjian H (2010) Clofarabine in leukemia. Expert Rev Hematol 3:15–22

Goodyear O, Agathanggelou A, Novitzky-Basso I, Siddique S, McSkeane T, Ryan G, Vyas P, Cavenagh J, Stankovic T, Moss P, Craddock C (2010) Induction of a CD8+ T-cell response to the MAGE cancer testis antigen by combined treatment with azacitidine and sodium valproate in patients with acute myeloid leukemia and myelodysplasia. Blood 116:1908–1918

Gore SD (2011) New ways to use DNA methyltransferase inhibitors for the treatment of myelodysplastic syndrome. Hematology Am Soc Hematol Educ Program 2011:550–555

Gore SD, Weng LJ, Figg WD, Zhai S, Donehower RC, Dover G, Grever MR, Griffin C, Grochow LB, Hawkins A, Burks K, Zabelena Y, Miller CB (2002) Impact of prolonged infusions of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia. Clin Cancer Res 8:963–970

Gore SD, Baylin S, Sugar E, Carraway H, Miller CB, Carducci M, Grever M, Galm O, Dauses T, Karp JE, Rudek MA, Zhao M, Smith BD, Manning J, Jiemjit A, Dover G, Mays A, Zwiebel J, Murgo A, Weng LJ, Herman JG (2006) Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. Cancer Res 66:6361–6369

Gore SD, Fenaux P, Santini V et al (2010) Time-dependent decision analysis: stable disease in azacitidine-treated patients with higher-risk MDS [abstract]. Proc Am Soc Clin Oncol 28:15s

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 (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89:2079–2088

Greenberg PL, Attar E, Bennett JM, Bloomfield CD, De Castro CM, Deeg HJ, Foran JM, Gaensler K, Garcia-Manero G, Gore SD, Head D, Komrokji R, Maness LJ, Millenson M, Nimer SD, O'Donnell MR, Schroeder MA, Shami PJ, Stone RM, Thompson JE, Westervelt P, National Comprehensive Cancer Network (2011) NCCN clinical practice guidelines in oncology: myelodysplastic syndromes. J Natl Compr Canc Netw 9:30–56

Griffiths EA, Gore SD (2008a) DNA methyltransferase inhibitors: class effect or unique agents? Leuk Lymphoma 49:650–651

Griffiths EA, Gore SD (2008b) DNA methyltransferase and histone deacetylase inhibitors in the treatment of myelodysplastic syndromes. Semin Hematol 45:23–30

Grovdal M, Khan R, Aggerholm A, Antunovic P, Astermark J, Bernell P, Engstrom LM, Kjeldsen L, Linder O, Nilsson L, Olsson A, Wallvik J, Tangen JM, Oberg G, Jacobsen SE, Hokland P, Porwit A, Hellstrom-Lindberg E (2007) Negative effect of DNA hypermethylation on the outcome of intensive chemotherapy in older patients with high-risk myelodysplastic syndromes and acute myeloid leukemia following myelodysplastic syndrome. Clin Cancer Res 13:7107–7112

Grovdal M, Karimi M, Khan R, Aggerholm A, Antunovic P, Astermark J, Bernell P, Engstrom LM, Kjeldsen L,

Linder O, Nilsson L, Olsson A, Holm MS, Tangen JM, Wallvik J, Oberg G, Hokland P, Jacobsen SE, Porwit A, Hellstrom-Lindberg E (2010) Maintenance treatment with azacytidine for patients with high-risk myelodysplastic syndromes (MDS) or acute myeloid leukaemia following MDS in complete remission after induction chemotherapy. Br J Haematol 150:293–302

Heise C, Carter T, Schafer P, Chopra R (2010) Pleiotropic mechanisms of action of lenalidomide efficacy in del(5q) myelodysplastic syndromes. Expert Rev Anticancer Ther 10:1663–1672

Itzykson R, Fenaux P (2009) Optimal sequencing of treatments for patients with myelodysplastic syndromes. Curr Opin Hematol 16:77–83

Itzykson R, Fenaux P (2012) Optimizing hypomethylating agents in myelodysplastic syndromes. Curr Opin Hematol 19:65–70

Itzykson R, Kosmider O, Cluzeau T, Mansat-De Mas V, Dreyfus F, Beyne-Rauzy O, Quesnel B, Vey N, Gelsi-Boyer V, Raynaud S, Preudhomme C, Ades L, Fenaux P, Fontenay M, Groupe Francophone des Myelodysplasies (GFM) (2011a) Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia 25:1147–1152

Itzykson R, Thepot S, Quesnel B, Dreyfus F, Beyne-Rauzy O, Turlure P, Vey N, Recher C, Dartigeas C, Legros L, Delaunay J, Salanoubat C, Visanica S, Stamatoullas A, Isnard F, Marfaing-Koka A, de Botton S, Chelghoum Y, Taksin AL, Plantier I, Ame S, Boehrer S, Gardin C, Beach CL, Ades L, Fenaux P, Groupe Francophone des Myelodysplasies (GFM) (2011b) Prognostic factors for response and overall survival in 282 patients with higher-risk myelodysplastic syndromes treated with azacitidine. Blood 117:403–411

Itzykson R, Thepot S, Beyne-Rauzy O, Ame S, Isnard F, Dreyfus F, Salanoubat C, Taksin AL, Chelgoum Y, Berthon C, Malfuson JV, Legros L, Vey N, Turlure P, Gardin C, Boehrer S, Ades L, Fenaux P, Groupe Francophone des Myelodysplasies (GFM) (2012) Does addition of erythropoiesis stimulating agents improve the outcome of higher-risk myelodysplastic syndromes treated with azacitidine? Leuk Res 36:397–400

Jabbour E, Garcia-Manero G, Batty N, Shan J, O'Brien S, Cortes J, Ravandi F, Issa JP, Kantarjian H (2010) Outcome of patients with myelodysplastic syndrome after failure of decitabine therapy. Cancer 116:3830–3834

Jabbour E, Kantarjian H, Ravandi F, Garcia-Manero G, Estrov Z, Verstovsek S, O'Brien S, Faderl S, Thomas DA, Wright JJ, Cortes J (2011) A phase 1–2 study of a farnesyltransferase inhibitor, tipifarnib, combined with idarubicin and cytarabine for patients with newly diagnosed acute myeloid leukemia and high-risk myelodysplastic syndrome. Cancer 117:1236–1244

Kadia TM, Jabbour E, Kantarjian H (2011) Failure of hypomethylating agent-based therapy in myelodysplastic syndromes. Semin Oncol 38:682–692

Kantarjian H, Issa JP, Rosenfeld CS, Bennett JM, Albitar M, DiPersio J, Klimek V, Slack J, de Castro C, Ravandi F, Helmer R 3rd, Shen L, Nimer SD, Leavitt R, Raza A, Saba H (2006) Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer 106:1794–1803

Kantarjian H, Oki Y, Garcia-Manero G, Huang X, O'Brien S, Cortes J, Faderl S, Bueso-Ramos C, Ravandi F, Estrov Z, Ferrajoli A, Wierda W, Shan J, Davis J, Giles F, Saba HI, Issa JP (2007) Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. Blood 109:52–57

Kantarjian H, Garcia-Manero G, O'Brien S, Faderl S, Ravandi F, Westwood R, Green SR, Chiao JH, Boone PA, Cortes J, Plunkett W (2010) Phase I clinical and pharmacokinetic study of oral sapacitabine in patients with acute leukemia and myelodysplastic syndrome. J Clin Oncol 28:285–291

Kim DY, Lee JH, Park YH, Lee JH, Kim SD, Choi Y, Lee SB, Lee KH, Ahn SY, Lee YS, Seol M, Kang YA, Jeon M, Jung AR, Lee YJ, Lee KH (2011) Feasibility of hypomethylating agents followed by allogeneic hematopoietic cell transplantation in patients with myelodysplastic syndrome. Bone Marrow Transplant 47:374–379

Knipp S, Hildebrand B, Kundgen A, Giagounidis A, Kobbe G, Haas R, Aul C, Gattermann N, Germing U (2007) Intensive chemotherapy is not recommended for patients aged >60 years who have myelodysplastic syndromes or acute myeloid leukemia with high-risk karyotypes. Cancer 110:345–352

Kuendgen A, Lubbert M (2008) Current status of epigenetic treatment in myelodysplastic syndromes. Ann Hematol 87:601–611

Lavelle D, Chin J, Vaitkus K, Redkar S, Phiasivongsa P, Tang C, Will R, Hankewych M, Roxas B, Singh M, Saunthararajah Y, Desimone J (2007) Oral decitabine reactivates expression of the methylated gamma-globin gene in papio anubis. Am J Hematol 82:981–985

Lee JH, Lee KH, Lee JH, Kim DY, Kim SH, Lim SN, Kim SD, Choi Y, Lee SM, Lee WS, Choi MY, Joo YD (2011) Decreased incidence of febrile episodes with antibiotic prophylaxis in the treatment of decitabine for myelodysplastic syndrome. Leuk Res 35:499–503

Lim Z, Brand R, Martino R, van Biezen A, Finke J, Bacigalupo A, Beelen D, Devergie A, Alessandrino E, Willemze R, Ruutu T, Boogaerts M, Falda M, Jouet JP, Niederwieser D, Kroger N, Mufti GJ, De Witte TM (2010) Allogeneic hematopoietic stem-cell transplantation for patients 50 years or older with myelodysplastic syndromes or secondary acute myeloid leukemia. J Clin Oncol 28:405–411

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 (2006) Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 355:1456–1465

Lubbert M, Bertz H, Wasch R, Marks R, Ruter B, Claus R, Finke J (2010) Efficacy of a 3-day, low-dose treatment with 5-azacytidine followed by donor lymphocyte infusions in older patients with acute myeloid leukemia or chronic myelomonocytic leukemia relapsed after allografting. Bone Marrow Transplant 45:627–632

Lubbert M, Suciu S, Baila L, Ruter BH, Platzbecker U, Giagounidis A, Selleslag D, Labar B, Germing U, Salih HR, Beeldens F, Muus P, Pfluger KH, Coens C, Hagemeijer A, Eckart Schaefer H, Ganser A, Aul C, de Witte T, Wijermans PW (2011) 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 29:1987–1996

Lyons RM, Cosgriff TM, Modi SS, Gersh RH, Hainsworth JD, Cohn AL, McIntyre HJ, Fernando IJ, Backstrom JT, Beach CL (2009) Hematologic response to three alternative dosing schedules of azacitidine in patients with myelodysplastic syndromes. J Clin Oncol 27:1850–1856

Malcovati L, Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, Passamonti F, Arcaini L, Maffioli M, Bernasconi P, Lazzarino M, Cazzola M (2005) Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol 23:7594–7603

Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R, Giagounidis A, Hildebrandt B, Bernasconi P, Knipp S, Strupp C, Lazzarino M, Aul C, Cazzola M (2007) Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 25:3503–3510

Moon JH, Kim SN, Kang BW, Chae YS, Kim JG, Baek JH, Park JH, Song MK, Chung JS, Won JH, Lee SM, Joo YD, Kim YK, Kim HJ, Jo DY, Sohn SK (2010) Predictive value of pretreatment risk group and baseline LDH levels in MDS patients receiving azacitidine treatment. Ann Hematol 89:681–689

Nand S, Godwin J, Smith S, Barton K, Michaelis L, Alkan S, Veerappan R, Rychlik K, Germano E, Stiff P (2008) Hydroxyurea, azacitidine and gemtuzumab ozogamicin therapy in patients with previously untreated non-M3 acute myeloid leukemia and high-risk myelodysplastic syndromes in the elderly: results from a pilot trial. Leuk Lymphoma 49:2141–2147

Oliva EN, Cuzzola M, Nobile F, Ronco F, D'Errigo MG, Lagana C, Morabito F, Galimberti S, Cortelezzi A, Aloe Spiriti MA, Specchia G, Poloni A, Breccia M, Ghio R, Finelli C, Iacopino P, Alimena G, Latagliata R (2010) Changes in RPS14 expression levels during lenalidomide treatment in low- and intermediate-1-risk myelodysplastic syndromes with chromosome 5q deletion. Eur J Haematol 85:231–235

Platzbecker U, Wermke M, Radke J, Oelschlaegel U, Seltmann F, Kiani A, Klut IM, Knoth H, Rollig C, Schetelig J, Mohr B, Graehlert X, Ehninger G, Bornhauser M, Thiede C (2012) Azacitidine for treatment of imminent relapse in MDS or AML patients after allogeneic HSCT: results of the RELAZA trial. Leukemia 26:381–389

Prebet T, Gore SD, Esterni B, Gardin C, Itzykson R, Thepot S, Dreyfus F, Rauzy OB, Recher C, Ades L, Quesnel B, Beach CL, Fenaux P, Vey N (2011) Outcome of high-risk myelodysplastic syndrome after azacitidine treatment failure. J Clin Oncol 29:3322–3327

Qin T, Jelinek J, Si J, Shu J, Issa JP (2009) Mechanisms of resistance to 5-aza-2′-deoxycytidine in human cancer cell lines. Blood 113:659–667

Raza A, Reeves JA, Feldman EJ, Dewald GW, Bennett JM, Deeg HJ, Dreisbach L, Schiffer CA, Stone RM, Greenberg PL, Curtin PT, Klimek VM, Shammo JM, Thomas D, Knight RD, Schmidt M, Wride K, Zeldis JB, List AF (2008) Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and intermediate-1 risk myelodysplastic syndromes with karyotypes other than deletion 5q. Blood 111:86–93

Raza A, Galili N, Smith S, Godwin J, Lancet J, Melchert M, Jones M, Keck JG, Meng L, Brown GL, List A (2009) Phase 1 multicenter dose-escalation study of ezatiostat hydrochloride (TLK199 tablets), a novel glutathione analog prodrug, in patients with myelodysplastic syndrome. Blood 113:6533–6540

Reddy MV, Venkatapuram P, Mallireddigari MR, Pallela VR, Cosenza SC, Robell KA, Akula B, Hoffman BS, Reddy EP (2011) Discovery of a clinical stage multikinase inhibitor sodium (E)-2-{2-methoxy-5-[(2′,4′,6′-trimethoxystyrylsulfonyl)methyl]phenylamino} acetate (ON 01910.na): synthesis, structure-activity relationship, and biological activity. J Med Chem 54:6254–6276

Sanchez-Abarca LI, Gutierrez-Cosio S, Santamaria C, Caballero-Velazquez T, Blanco B, Herrero-Sanchez C, Garcia JL, Carrancio S, Hernandez-Campo P, Gonzalez FJ, Flores T, Ciudad L, Ballestar E, Del Canizo C, San Miguel JF, Perez-Simon JA (2010) Immunomodulatory effect of 5-azacytidine (5-azaC): potential role in the transplantation setting. Blood 115:107–121

Santini V, Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Silverman LR, List A, Gore SD, Seymour JF, Backstrom J, Beach CL (2010) Management and supportive care measures for adverse events in patients with myelodysplastic syndromes treated with azacitidine*. Eur J Haematol 85:130–138

Saunthararajah Y, Hillery CA, Lavelle D, Molokie R, Dorn L, Bressler L, Gavazova S, Chen YH, Hoffman R, DeSimone J (2003) Effects of 5-aza-2′-deoxycytidine on fetal hemoglobin levels, red cell adhesion, and hematopoietic differentiation in patients with sickle cell disease. Blood 102:3865–3870

Schiller GJ, Slack J, Hainsworth JD, Mason J, Saleh M, Rizzieri D, Douer D, List AF (2006) Phase II multicenter study of arsenic trioxide in patients with myelodysplastic syndromes. J Clin Oncol 24:2456–2464

Scott BL, Ramakrishnan A, Storer B, Becker PS, Petersdorf S, Estey EH, Deeg HJ (2010) Prolonged responses in patients with MDS and CMML treated with azacitidine and etanercept. Br J Haematol 148:944–947

Seetharam M, Fan AC, Tran M, Xu L, Renschler JP, Felsher DW, Sridhar K, Wilhelm F, Greenberg PL (2012) Treatment of higher risk myelodysplastic syndrome patients unresponsive to hypomethylating agents with ON 01910.na. Leuk Res 36:98–103

Sekeres MA, Maciejewski JP, Giagounidis AA, Wride K, Knight R, Raza A, List AF (2008) Relationship of treatment-related cytopenias and response to lenalidomide in patients with lower-risk myelodysplastic syndromes. J Clin Oncol 26:5943–5949

Sekeres MA, List AF, Cuthbertson D, Paquette R, Ganetzky R, Latham D, Paulic K, Afable M, Saba HI, Loughran TP Jr, Maciejewski JP (2010) Phase I combination trial of lenalidomide and azacitidine in patients with higher-risk myelodysplastic syndromes. J Clin Oncol 28:2253–2258

Sekeres MA, O'Keefe C, List AF, Paulic K, Afable M 2nd, Englehaupt R, Maciejewski JP (2011a) Demonstration of additional benefit in adding lenalidomide to azacitidine in patients with higher-risk myelodysplastic syndromes. Am J Hematol 86: 102–103

Sekeres MA, Gundacker H, Lancet J, Advani A, Petersdorf S, Liesveld J, Mulford D, Norwood T, Willman CL, Appelbaum FR, List AF (2011b) A phase 2 study of lenalidomide monotherapy in patients with deletion 5q acute myeloid leukemia: Southwest oncology group study S0605. Blood 118:523–528

Shen L, Kantarjian H, Guo Y, Lin E, Shan J, Huang X, Berry D, Ahmed S, Zhu W, Pierce S, Kondo Y, Oki Y, Jelinek J, Saba H, Estey E, Issa JP (2010) DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes. J Clin Oncol 28:605–613

Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, Stone RM, Nelson D, Powell BL, DeCastro CM, Ellerton J, Larson RA, Schiffer CA, Holland JF (2002) Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol 20:2429–2440

Silverman LR, McKenzie DR, Peterson BL, Holland JF, Backstrom JT, Beach CL, Larson RA, Cancer and Leukemia Group B (2006) Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the cancer and leukemia group B. J Clin Oncol 24: 3895–3903

Silverman LR, Fenaux P, Mufti GJ, Santini V, Hellstrom-Lindberg E, Gattermann N, Sanz G, List AF, Gore SD, Seymour JF (2011) Continued azacitidine therapy beyond time of first response improves quality of response in patients with higher-risk myelodysplastic syndromes. Cancer 117:2697–2702

Sloand EM, Olnes MJ, Shenoy A, Weinstein B, Boss C, Loeliger K, Wu CO, More K, Barrett AJ, Scheinberg P, Young NS (2010) Alemtuzumab treatment of intermediate-1 myelodysplasia patients is associated with sustained improvement in blood counts and cytogenetic remissions. J Clin Oncol 28:5166–5173

Soriano AO, Yang H, Faderl S, Estrov Z, Giles F, Ravandi F, Cortes J, Wierda WG, Ouzounian S, Quezada A, Pierce S, Estey EH, Issa JP, Kantarjian HM, Garcia-Manero G (2007) Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-trans retinoic acid in acute myeloid leukemia and myelodysplastic syndrome. Blood 110:2302–2308

Steensma DP, Stone RM (2010) Practical recommendations for hypomethylating agent therapy of patients with myelodysplastic syndromes. Hematol Oncol Clin North Am 24:389–406

Steensma DP, Baer MR, Slack JL, Buckstein R, Godley LA, Garcia-Manero G, Albitar M, Larsen JS, Arora S, Cullen MT, Kantarjian H (2009) Multicenter study of decitabine administered daily for 5 days every 4 weeks to adults with myelodysplastic syndromes: the alternative dosing for outpatient treatment (ADOPT) trial. J Clin Oncol 27:3842–3848

Vey N, Bosly A, Guerci A, Feremans W, Dombret H, Dreyfus F, Bowen D, Burnett A, Dennis M, Ribrag V, Casadevall N, Legros L, Fenaux P (2006) Arsenic trioxide in patients with myelodysplastic syndromes: a phase II multicenter study. J Clin Oncol 24: 2465–2471

Wattel E, De Botton S, Luc Lai J, Preudhomme C, Lepelley P, Bauters F, Fenaux P (1997) Long-term follow-up of de novo myelodysplastic syndromes treated with intensive chemotherapy: incidence of long-term survivors and outcome of partial responders. Br J Haematol 98:983–991

Yang H, Hoshino K, Sanchez-Gonzalez B, Kantarjian H, Garcia-Manero G (2005) Antileukemia activity of the combination of 5-aza-2′-deoxycytidine with valproic acid. Leuk Res 29:739–748

Ye Y, McDevitt MA, Guo M, Zhang W, Galm O, Gore SD, Karp JE, Maciejewski JP, Kowalski J, Tsai HL, Gondek LP, Tsai HC, Wang X, Hooker C, Smith BD, Carraway HE, Herman JG (2009) Progressive chromatin repression and promoter methylation of CTNNA1 associated with advanced myeloid malignancies. Cancer Res 69:8482–8490

Hematopoietic Cell Transplantation (HCT)

13

H. Joachim Deeg

13.1 Introduction

The availability of drugs such as azacitidine, decitabine, and lenalidomide offers treatment options for patients with MDS that can alter the disease course. Randomized trials have shown that azacitidine, for example, extends the life expectancy by about 9 months, but eventually the disease will progress. Aside from rare patients with MDS who may have been cured by intensive chemotherapy, the only currently available treatment that offers the potential of cure is hematopoietic cell transplantation (HCT). However, HCT is associated with certain risks, related primarily to the "conditioning" regimen administered in preparation for HCT, and the immunologic reaction of donor cells against the patient, known as graft-versus-host disease (GVHD). Furthermore, as the average age of patients with MDS is in the 70s, comorbid conditions are common and may increase the risk of complications of HCT. Finally, HCT does not guarantee eradication of the disease; the probability of relapse correlates with the disease stage at the time of HCT and the disease risk reflected in the patient's karyotype. Thus, consultations with patients regarding HCT involve a comprehensive discussion of timing of HCT, alternative therapy before or instead of HCT, donor availability, and quality of life (QOL) issues, among others.

The pathology of MDS, classic and molecular cytogenetics, pathophysiology, and classification schemes of the disease are discussed in other chapters. We will use that information here as needed to discuss the impact of those parameters on HCT.

13.2 General Considerations for HCT

13.2.1 Why Conditioning?

MDS is a clonal disorder of hematopoietic stem/precursor cells and as such, disregarding contributions of the marrow microenvironment, should be curable by replacing the diseased cells with cells from a healthy donor (see also autologous transplantation, below).

Successful allogeneic HCT requires that the infused cells from the healthy donor establish themselves (engraft) and that the clonal (malignant) cells of the patient's disease are eliminated or inactivated. All patients are prepared with a "conditioning" regimen, consisting of chemotherapy with or without total body irradiation (TBI), antibodies, and possibly radioimmuno-

H.J. Deeg, MD
Division of Clinical Research,
Fred Hutchinson Cancer Research Center,
1100 Fairview Avenue N, D1-100, Seattle,
WA 98109-1024, USA

University of Washington School of Medicine,
Seattle, WA, USA
e-mail: jdeeg@fhcrc.org

This work was supported in part by NIH grants HL036444, HL095999, CA018029, and CA015704, Bethesda, MD.

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_13, © Springer-Verlag Berlin Heidelberg 2013

therapy. The objective is to overcome the immunological barrier, which protects the body against intrusion by foreign cells (Welniak et al. 2007) and to kill the patient's MDS cells . However, many questions arise as to the best strategy: How intensive must the regimen be in order to allow for engraftment and prevent relapse? What intensity will the patient tolerate? Should the conditioning intensity be adjusted to the disease stage (the risk of relapse)? Would it be beneficial to give pre-HCT "debulking" therapy? Is there a place for post-HCT adjuvant or preemptive therapy?

13.2.2 Why Are Not All Patients Being Transplanted?

If treatment-related toxicity and mortality (TRM) were eliminated and GVHD was completely prevented, one could argue that all patients should be offered the option of HCT, and even if post-HCT relapse occurred, the patient might simply be back to the pre-HCT condition, i.e., patients would have nothing to lose. We have so far not arrived at that point, and disease characteristics, prognosis with non-transplant management, and patient age and medical condition remain major factors in the decision making process for HCT. Even in the most favorable risk category, i.e., a young patient with very low marrow blast count without high-risk cytogenetics will have a probability of long-term relapse-free survival (RFS) of maybe 75 %, while 25 % of patients will succumb to various complications.

At the same time, patients in IPSS risk groups low or intermediate-1 or in the revised IPSS (IPSS-R) categories very good or good may have life expectancies in the range of 5–10 years or longer with supportive care or conservative therapy alone (Greenberg et al. 1997, 2012). Weighing the pros and cons of HCT in those patients may lead to the decision to delay HCT until there is evidence of disease progression. Of course, HCT at a more advanced stage of MDS is associated with inferior outcome, primarily due to a higher relapse incidence. This aspect has gained in importance in recent years as growing numbers of patients have come to HCT when no longer responding to hypomethylating therapy, and preliminary analyses indicate that these patients have a lower chance of being transplanted successfully than otherwise expected for a comparable disease stage (Prébet et al. 2011).

The decision for HCT is generally easier for patients with more advanced/higher-risk disease whose life expectancy without HCT may be only a year or less. However, in patients with advanced MDS, pre-HCT debulking therapy may be desirable in an effort to reduce the probability of post-HCT relapse. Such a strategy appears to be beneficial in patients with a large disease burden (high myeloblast count), but may not have a significant impact in patients with high-risk cytogenetics.

13.2.3 What Limits the Success of HCT in Older Patients and Those with Comorbid Conditions?

In addition to the disease stage, major hurdles to success of HCT have been patient age and, more directly, often age-related, comorbid conditions (Sorror et al. 2006, 2011). The development of reduced-intensity conditioning (RIC) regimens along with modern supportive care and complication management has allowed to raise the acceptable patient age into the eighth decade of life. The HCT-specific comorbidity index (HCT-CI) developed by Sorror et al. (2008) (Table 13.1) and the primarily pulmonary function-based risk score generated by Parimon et al. (2006) allow for pre-HCT risk assessment. The current consensus is that these risk scores predict toxicities, non-relapse mortality (NRM), and overall survival after HCT better than does the Karnofsky Performance Score (KPS) (Farina et al. 2009; Kataoka et al. 2010).

13.2.4 Who Should Be the Donor?

About 25 % of patients will have HLA-identical sibling donors (or matched related donors other than a sibling). However, more than 15 million volunteer donors are now registered in donor banks, and an HLA-matched unrelated donor can

be identified for about 50–60 % of Caucasians; the proportion is lower for African Americans and may be as low as 10 % in some ethnic minorities (Rocha and Locatelli 2008; Petersdorf 2009). Importantly, transplant results with unrelated donors who are HLA matched with patients by high-resolution typing are comparable to those with HLA genotypically identical siblings, although the incidence of GVHD tends to be higher. For patients without HLA-matched related or unrelated donors, cord blood cells or cells from HLA-haploidentical related donors may offer alternatives (Brunstein et al. 2011; Luznik et al. 2008) and should make the option of HCT available to almost every patient (Aversa 2008; Luznik et al. 2008; Brunstein et al. 2010). Cord blood has the advantage of immunological "immaturity," allowing to transplant HLA-mismatched cells without a significant increase in GVHD incidence. The drawback of limited numbers of cells is at least in part overcome by the use of two units of cord blood or in vitro "expansion" of one cord blood unit before infusion, which provides a bridge and protection of the patient until full engraftment and hematopoietic function are derived from the non-expanded unit (Dahlberg et al. 2011; Avery et al. 2011; Delaney et al. 2010; Kelly et al. 2009).

Table 13.1 Parameters considered in the HCT-CI

Parameter	Score
Arrhythmia	1
Cardiovascular comorbidity	1
Inflammatory bowel disease	1
Diabetes	1
Cerebro-vascular disease	1
Psychiatric disturbance	1
Hepatic comorbidity	1–3
Obesity	1
Infection	1
Rheumatologic comorbidity	2
Peptic ulcer	2
Renal comorbidity	2
Pulmonary comorbidity	2–3
Prior solid tumor	3
Heart valve disease	3

HCT-CI hematopoietic cell transplantation co-morbidity index (parameters are scored, ranging from 1 to 3). A detailed description and a calculator to determine the total score is available at www.hctci.org

Over the past decade the approach to treating MDS in general and with HCT in particular has undergone major changes. The emphasis with HCT has shifted from high-dose therapy, aimed at maximum tumor cell kill by the conditioning regimen, to low-intensity or RIC regimens, relying more on donor cell-mediated graft versus tumor (GVT) effects to eradicate the disease (Deeg et al. 2006; Welniak et al. 2007; Laport et al. 2008). However, as illustrated in Fig. 13.1, even a cursory review of the literature shows that

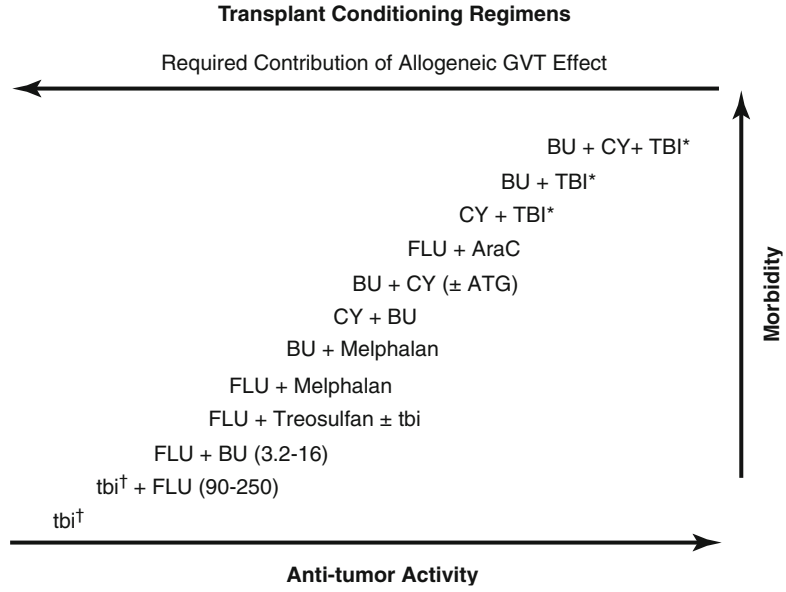

Fig. 13.1 Transplant conditioning regimens

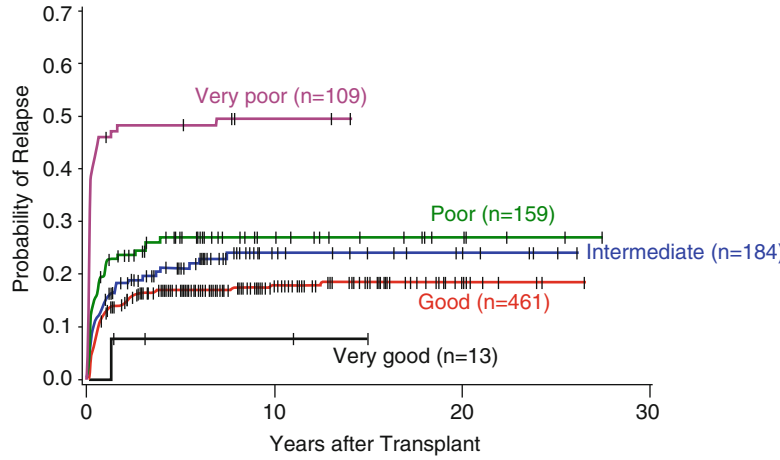

Fig. 13.2 Impact of karyotype on post-transplant relapse. Cumulative incidence (CI) of relapse by 5-group cytogenetic classification

there is a continuum of regimens, spanning the range from low-dose TBI (2 Gy) only to regimens that combine one or two chemotherapy agents with high-dose TBI (e.g., 12 Gy), and any categorization of regimen intensity must remain artificial (Deeg et al. 2006; Deeg and Sandmaier 2010). It appears most appropriate to refer to regimens by their components and dose intensity. The composition of particular regimens may prove to be relevant for subgroups of patients (unpublished data). A major advantage of RIC is the possibility of applying those regimens to patients with comorbid conditions and older patients, and, indeed, patients in the eighth decade of life have been transplanted successfully (Samuelson et al. 2011; Baron and Sandmaier 2005; Valcarcel and Martino 2007). There is evidence, however, that the probability of relapse is higher with RIC (Baron and Sandmaier 2005). Nevertheless, the broad menu of regimens allows to offer "custom-tailored" HCT to subgroups of patients, and transplant-related mortality has steadily declined (Gooley et al. 2010; de Witte et al. 2009).

13.2.5 Pre-HCT Therapy and Relapse

Despite considerable progress, however, post-HCT relapse remains a problem, especially in patients with advanced and high-risk MDS (Warlick et al. 2009; Ramakrishnan and Deeg 2008). This observation has led to efforts at pre-HCT debulking in an attempt to apply HCT to patients with a lower disease burden, thereby increasing the chances of post-HCT RFS. However, no prospective controlled trial has been conducted to document the actual benefit of pre-HCT debulking. Very likely retrospective data reflect a selection bias in so far as patients who did not respond were less likely to come to HCT, either because of the refractoriness of their disease or because of complications they suffered related to pre-HCT therapy. Importantly, a risk factor even more powerful than the myeloblast count is the patient's karyotype due to the profound impact on relapse (Oliansky et al. 2009; Alessandrino et al. 2008). An analysis by Armand et al. suggests that MDS patients can be separated into two groups, good/intermediate versus poor-risk cytogenetics, with significantly differing impacts on post-HCT course (Armand et al. 2010), although a recent study suggests that a new 5-group classification (Schanz et al. 2012) further distinguishes patients with the highest and lowest relapse risk (Deeg et al. 2012) (Fig. 13.2). This is important to remember as the karyotype may also determine the response to chemotherapy, and as a result, pre-HCT therapy would select for "sensitive" patients.

Other strategies aimed at reducing the frequency of post-HCT relapse include *post*-HCT preemptive therapy with hypomethylating agents

(de Lima et al. 2010) and vaccination efforts (Ma et al. 2010).

13.3 Timing of HCT

Determining the optimal timing of HCT for MDS has proven difficult, although all available data indicate that patients transplanted at an early stage of their disease have superior outcomes. The probability of relapse increases progressively with increasing IPSS or WPSS scores (Deeg et al. 2002; Alessandrino et al. 2008). An earlier Markov analysis by Cutler et al. (in patients with HLA-identical related donors) showed that patients with high or intermediate-2 risk by IPSS did benefit from early HCT, while patients with low or intermediate-1 risk may have a longer life expectancy if HCT is delayed until evidence of disease progression (Cutler et al. 2004). A recent study by Koreth et al. (2011) in patients older than 60 years and prepared with RIC regimens shows, similarly, that those with low-risk MDS are unlikely to gain extended survival by HCT, whereas high-risk patients may gain life years, although the benefit became apparent only with several years of follow-up after HCT. Nevertheless, it is wise to advise patients in the lower-risk categories individually. For example, a patient may be categorized as low risk by established criteria but may be at considerable risk, e.g., of bleeding or infection, on the basis of thrombocytopenia and severe neutropenia, respectively. Also, a patient may not meet any of the hematologic risk parameters, but may have a higher-risk karyotype predisposing the patient to the risk of relapse while still being categorized as intermediate-1 risk by IPSS. Those parameters weigh more heavily in the revised IPSS (IPSS-R) (Greenberg et al. 2012).

13.4 Pretransplant Therapy

13.4.1 Who Should Receive Which Therapy?

To improve chances for post-HCT success, patients with marrow myeloblast counts of greater than 5 or 10 % often will receive pre-HCT therapy with a hypomethylating agent or with more classical induction-type chemotherapy (Warlick et al. 2009; Oliansky et al. 2009; Kindwall-Keller and Isola 2009; Yakoub-Agha et al. 2000; Nakai et al. 2005; de Lima et al. 2004). The choice of therapy typically depends upon the tumor burden, patient age, and overall health status. Currently, no results from randomized prospective trials are available. Retrospective data indicate that patients whose disease has *responded to pre-HCT therapy* have superior post-HCT survival (Yakoub-Agha et al. 2000; Scott et al. 2005; de Lima et al. 2004). However, the clinical impression is that pre-HCT therapy may select for chemosensitive patients, and, in fact, the cohorts of patients who did not respond had a poorer outcome than patients who were untreated at the time of HCT. The impact of the depth of response on post-HCT outcome is illustrated in Fig. 13.3 (de Lima et al. 2004). Whether pre-HCT therapy improves the post-HCT prognosis in patients with high-risk cytogenetics (independent from the disease burden) is doubtful, at least with currently used modalities.

13.4.2 Hypomethylating Agents and HCT

As discussed in the respective chapters, hypomethylating agents are now considered standard of care for many patients with MDS, not necessarily dependent upon the myeloblast count. Several trials have shown that patients gain, on average, 9–10 months in life expectancy. Should HCT be carried out *after* patients have failed hypomethylating therapy or, rather, *while still responding* to that treatment? No controlled trials are available, but a recent meta-analysis by Prébet et al. (2011) showed the following: patients in whom treatment with 5-azacitidine was discontinued for various reasons had a median life expectancy of 5–6 months. If HCT was carried out after treatment was stopped because of intolerance of the drug or because no response was achieved, about 40 % of patients survived in remission. In contrast, among patients who had progressed on 5-azacitidine, the median survival was 14 months,

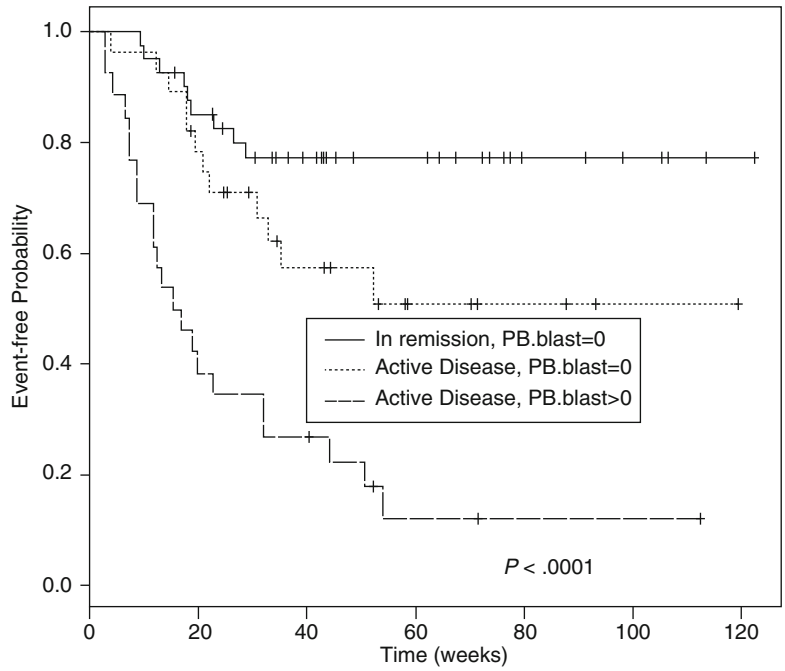

Fig. 13.3 Relapse-free survival by disease status and circulating blasts. HCT results in 74 patients with AML and 22 patients with MDS. Patients had high-risk disease or were given pre-HCT chemotherapy or both. At HCT, patients were either in complete remission without blasts in peripheral blood (*PB*) or had active disease in the marrow without or with circulating blasts (This research was originally published in *Blood*. © the American Society of Hematology. Reused with permission from de Lima et al. 2004)

and no plateau was reached. There may be various reasons for such a pattern. It is not unlikely that evolution of the disease under therapy, possibly with new clones arising, accounts for the difference. Patients must be informed about these considerations; some might choose to "buy the maximum time with good quality of life" before being exposed to the potential risks of HCT; others might decide to "go for the cure." The latter are more likely to be younger and in good clinical condition, the former more likely to be older or suffer from other medical ailments.

In a retrospective analysis, overall survival, RFS, and cumulative incidence of relapse at 1 year were 47, 41, and 20 % , respectively, for patients with MDS (and CML) receiving 5-azacitidine, compared to 60, 51, and 32 % for patients who were not treated (Pidala et al. 2009b). In a small study including 17 patients with MDS, treatment with decitabine did not negatively affect toxicity after HCT, and disease downstaging improved HCT outcome (De Padua et al. 2009). Clearly, however, the available data are not conclusive.

To what extent other therapies given before HCT, such as lenalidomide and agents discussed in Chap. 12, impact transplant outcome is currently not known.

13.5 Conditioning Regimens

Basically two strategies have been pursued to minimize toxicity and optimize efficacy: (1) a drastic reduction of cytotoxic intensity with a shift to rely heavily on the immunological GVT effect of donor cells and (2) a stepwise remodeling of more conventional regimens. There is no one-size-fits-all conditioning regimen (Oliansky et al. 2009). Instead, conditioning should be tailored to diagnosis, disease stage, patient age, prior therapy, comorbidities, and other parameters of HCT, such as donor and stem cell source (Ramakrishnan and Deeg 2008; Scott and Deeg 2006; Deeg et al. 2006). Some regimens used widely for transplant conditioning of patients with MDS are summarized in Fig. 13.1.

High-dose conditioning tends to be associated with a lower relapse risk than RIC regimens (Warlick et al. 2009), but toxicity may render those regimens unsuitable for older patients and those with comorbidities (Scott and Deeg 2006).

Currently, patients age 60 or 65 years and older, and patients of younger age with comorbidities that result in a score of 3 and higher on the HCT-CI scale, are typically offered HCT using RIC regimens. The higher risk of relapse is offset by lower TRM (Alyea et al. 2006; Martino et al. 2006; Oliansky et al. 2009; Warlick et al. 2009; Scott et al. 2006). However, retrospective data must be interpreted with caution, because of selection bias (Martino et al. 2006; Sorror et al. 2004, 2005). A prospective randomized phase III trial comparing conditioning regimens of different intensities in patients with MDS or AML is currently underway in the USA (CTN # 0901). Results are expected to provide some answers, but it is clear that further refinements and steps of "individualization" for a given target group of patients will be necessary in order to optimize results.

13.6 Selection of Donor and Stem Cell Source

13.6.1 Donor Options

The standard approach at most centers is to search for HLA-identical siblings and try to identify HLA-matched (by high-resolution typing) unrelated donors if no suitably matched siblings are available. Some recent investigations have compared results with cord blood as a source of stem cells to those with unrelated donors, reporting similar outcomes for certain diagnoses, but only limited data in patients with MDS are available (Harrison et al. 2006; Ooi et al. 2003; Sato et al. 2011). Finally, based on results reported initially from the Perugia and the Johns Hopkins teams, HLA-haploidentical transplants from related donors represent an area of intensive research with promising early results, particularly in patients with lymphoid malignancies (Chen et al. 2010; Luznik et al. 2008; Aversa 2008; O'Donnell et al. 2010).

13.6.2 Sources of Stem Cells

Hematopoietic stem cells can be aspirated directly from the marrow, can be mobilized into peripheral blood by granulocyte colony-stimulating factor (G-CSF) or the CXCR4 blocking molecule plerixafor, and can be harvested by leukapheresis (PBPC) or, as indicated already, can be collected from umbilical cord blood. These cell populations express different characteristics, in regard to kinetics of engraftment, GvHD, and GVT effects (Welniak et al. 2007). PBPC allow for more rapid engraftment than seen with marrow but are also associated with a higher incidence of chronic GVHD (Anasetti et al. 2012), an observation that may lead to more frequent use of bone marrow, particularly in patients with good-risk disease. The use of cord blood is typically associated with more delayed engraftment, but the incidence of GVHD may be low, despite HLA mismatching.

13.6.3 Donor and Patient Characteristics Affecting the Selection of Stem Cell Source

Several factors in addition to HLA matching determine the choice of stem cells. One is the size of the donor: leukapheresis is difficult to carry out in children. Another is the ratio of patient to donor size: for a large patient it may simply not be possible to identify cord blood units of sufficient size to assure prompt engraftment. The use of PBPC may convey a more potent GVT effect than marrow and, therefore, may be preferred for patients with high-risk disease. Several studies showed that transplantation of PBPC from related donors in patients with MDS was associated with lower relapse rates than the use of marrow (Guardiola et al. 2002; Deeg et al. 2002). More recent data from a Markov decision analysis of results in 1,111 patients transplanted from HLA-identical siblings (Pidala et al. 2009a) and conditioned with high-dose regimens showed significantly higher survival and better quality of life with PBPC than with marrow, despite a higher incidence of GvHD, primarily due to lower relapse incidence.

With the use of cord blood (Brunstein and Weisdorf 2009; Rocha and Gluckman 2009; Ooi 2006), the degree of HLA match combined with cell numbers determines the success of HCT (Barker et al. 2010). As stated above, the use of two cord blood units (Rocha and Gluckman 2009; Avery et al. 2011; Delaney et al. 2010) and in vitro expansion of cord blood have improved results (Dahlberg et al. 2011; Rocha and Gluckman 2009; Avery et al. 2011; Delaney et al. 2010).

13.7 Results of Allogeneic HCT

Several recent reviews have summarized currently achievable results (Harrison et al. 2006; Oliansky et al. 2009; Bartenstein and Deeg 2010).

13.7.1 "Conventional" Regimens

Traditionally, high-dose chemotherapy has been used to eradicate clonal stem cells. The FHCRC team reported results for 109 patients with MDS conditioned with oral BU (16 mg/kg), with dose adjustments to maintain plasma concentrations at steady state (Css) of 800–900 ng/mL (targeted BU [tBU]), and cyclophosphamide (CY) (120 mg/kg) (oral tBU/CY). Patients were 6–66 (median 46) years old. The BU Css levels reached were 635–1,140 (median, 883) ng/mL. Pre-HCT marrow myeloblast percentage and IPSS score were the most significant predictors of RFS. NRM at 100 days and 3 years was 16 and 31 %, respectively. The 3-year RFS was 56 % with related and 59 % with unrelated donors. The cumulative incidence of acute GVHD was 64 % with HLA-matched related and 68 % for HLA-matched unrelated donors. The regimen was most effective in patients with early stage disease. In a subsequent trial, CY was replaced by fludarabine (Flu) (120 mg/m^2) followed by oral BU (16 mg/kg) targeted to Css levels of 900 ± 100 ng/mL (Flu/oral tBU). Forty-two patients, including 38 with high-risk MDS, were enrolled. Engraftment was achieved in all patients, and the day-100 NRM was 7 %. Overall survival, NRM, and RFS at 18 months were 42, 24, and 35 %, respectively. The use of Flu instead of CY appeared to permit higher average BU exposure without increasing the toxicity. It is of note, however, that a recent analysis indicates that Flu administration concurrently with BU results in decreased BU clearance (Yeh et al. 2012). Also, in the FHCRC experience, long-term observation failed to show an advantage of the Flu/Bu regimen (Deeg et al. 2012).

The MD Anderson Cancer Center team used a slightly different Flu/BU regimen consisting of Flu, 40 mg/m^2/day, and IV BU, 130 mg/m^2/day, on days −6 to −3 (total doses Flu 160 mg/m^2, BU 520 mg/m^2) in 96 patients with MDS or AML, 19–66 (median 45) years of age. One-year overall survival was 65 %, NRM 3 %, and RFS 52 %. Thus, NRM was lower than observed with the Flu/*oral* tBU (3 % vs.15 %, respectively). The Bu Css was 836.6 ng/mL, only slightly lower than the 908 ng/mL in the Flu/oral tBU regimen. It is likely, therefore, that BU given IV offers an advantage. The same investigators reported results with an RIC regimen consisting of Flu and melphalan (Mel). One hundred twelve patients with AML or MDS, 22–74 (median 55) years of age, were conditioned with Flu 100–150 mg/m^2 and Mel 100–180 mg/m^2. With a median follow-up of 2.5 years, there were no differences in survival or risk of progression between patients given Mel 140 mg/m^2 vs. Mel 180 mg/m^2. The cumulative incidence of day-100 and 2-year NRM was 0 and 20 %, respectively. The estimated 2-year survival was 66 % for patients in remission at HCT and 40 % for patients with active disease but without circulating blasts.

13.7.2 Low Intensity/RIC Regimens

A RIC regimen consisting of Flu + low-dose BU was first reported by Slavin et al. Twenty-six patients with various disorders were conditioned with Flu, 180 mg/m^2, and oral BU, 8 mg/kg. Overall survival at 8 months was 85 %, and 81 % of patients were disease-free. A regimen of Flu (150 mg/m^2), oral BU (8 mg/kg), and anti-CD52 antibody alemtuzumab (100 mg) was used in 62 patients, 22–70 (median 53) years of age, with MDS (all WHO categories), chronic myelomonocytic leukemia, or AML developing from MDS. The 1-year overall survival was 74 %, NRM

15 %, and RFS 62 %. Importantly, however, many of these patients required donor lymphocyte infusion (DLI) to achieve complete chimerism and remissions post-HCT (Ho et al. 2004).

In a study of 84 patients conditioned with high-dose or RIC regimens, respectively, and transplanted with marrow from HLA-related or unrelated donors or cord blood (Warlick et al. 2009), 1 (5)-year overall survival was 48 % (31 %), relapse incidence 23 % (25 %), and RFS 38 % (29 %). Transplant-related mortality at 1 year was 39 %. The incidence of acute GvHD grades II–IV was 43 % and of chronic GvHD 15 %. RFS did not differ significantly by graft source or conditioning intensity. The incidence of relapse was 18 % for patients with ≤5 % myeloblasts and 35 % for patients with ≥5 % blasts. While, among patients with less than 5 % blasts, high-dose conditioning was associated with a relapse incidence of 9 %, compared to 31 % in patients with RIC, no significant difference was observed among patients with higher myeloblast counts. The reason is not immediately apparent, but may lie in treatments given pre-HCT to patients with higher blast counts (see also discussion about selection of treatment-sensitive patients).

In an EBMT registry study of 374 patients with refractory anemia (RA) or RA with ringed sideroblasts (RARS) receiving HLA-matched HCT after various conditioning regimens (de Witte et al. 2009), the 4-year overall survival was 52 %, RFS 48 %, relapse 15 %, and NRM 37 %. The risk of relapse was higher after RIC compared to high-dose conditioning (hazard ratio [HR] 2.8) (Lim et al. 2010). However, overall survival and RFS did not differ significantly as NRM was significantly lower after RIC (HR 0.8). The relapse incidence was lower with unrelated than with related donors (HR 0.6), presumably due to a greater GVT effect, but NRM was higher (HR 1.4), and overall survival was comparable to that with related donors. T-cell depletion was associated with increased NRM. Older patient age and delay of HCT by more than 12 months after diagnosis adversely affected outcome.

Martino et al. (2006) analyzed HCT results in 836 patients with MDS transplanted from HLA-identical siblings following RIC ($n=215$) or high-dose conditioning ($n=621$) and found no significant difference for overall survival (45 % vs. 41 %, respectively). Lack of remission or progression to AML before HCT, poor-risk karyotype , and age older than 50 years negatively impacted RFS.

In agreement with other reports (Casper et al. 2010; Kroger et al. 2006b; Cutting et al. 2008; Beelen et al. 2005), we showed that a conditioning regimen composed of Flu and treosulfan was associated with an NRM of less than 10 % (Nemecek et al. 2011), and among patients with AML or MDS without high-risk cytogenetics, the 2-year RFS was 80 % (Fig. 13.4). This regimen may be considered to be of intermediate intensity and may point to new strategies for successful HCT in patients with MDS.

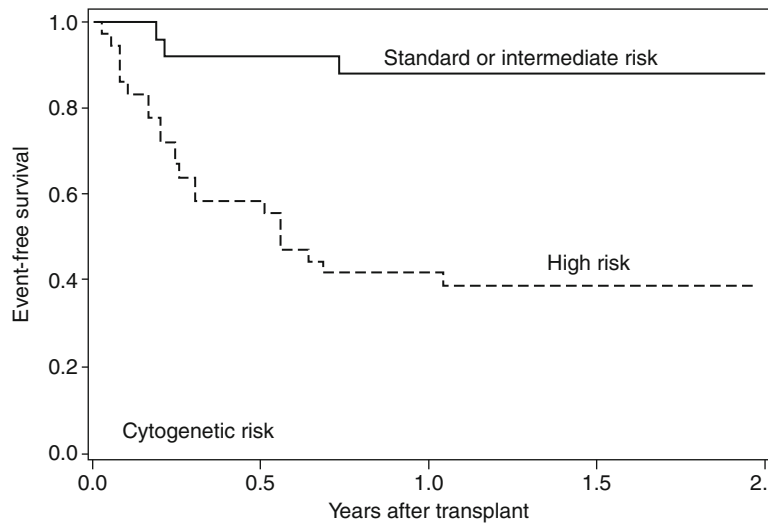

Fig. 13.4 Relapse-free survival in patients with MDS or AML conditioned with fludarabine plus treosulfan and transplanted from HLA-matched related or unrelated donors. Shown are the results based on cytogenetic risk (per IPSS criteria for MDS and per cooperative group criteria for AML) (Reprinted from Nemecek et al. (2011). With permission from Elsevier and the author (H.J. Deeg))

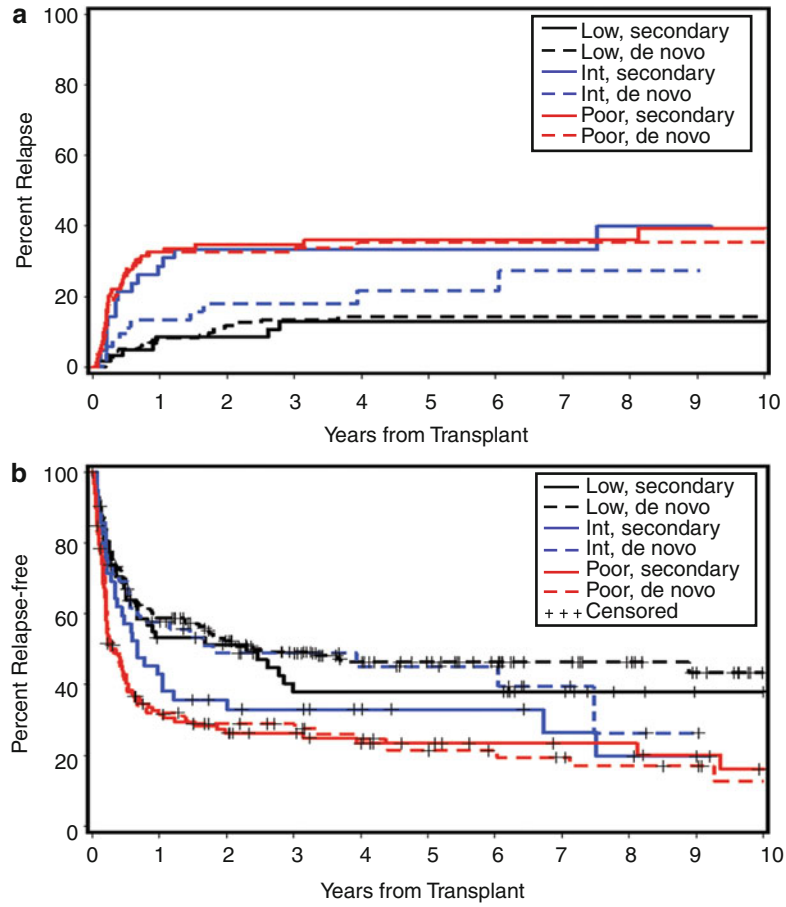

Fig. 13.5 Impact of cytogenetic risk category on HCT outcome in patients with de novo and with secondary MDS/tAML. (**a**) Probability of relapse; (**b**) probability of relapse-free survival, based on cytogenetic risk as defined by IPSS (Greenberg et al. 1997) (This research was originally published in *Blood*. © the American Society of Hematology. Reprinted from Chang et al. 2007)

Very encouraging results were also achieved with regimens that combine Flu and low-dose TBI (2 Gy) (Laport et al. 2008) with radioimmunotherapy (Pagel et al. 2009).

These latter regimens do not easily fit into what has been contrasted as "myeloablative" and "non-myeloablative" HCT – the *objective of all regimens* is to ablate the patient's disease, but to do so with the least toxicity possible.

13.7.3 Transplantation for Secondary MDS

The central importance of cytogenetics was underscored in an analysis of results in 257 patients with *secondary* MDS (including patients who had progressed to AML). The 5-year incidence of relapse was 33 % for tAML, 36 % for RAEB, and 12 % for RA/RARS. The 5-year RFS was 29 % overall, 19 % for tAML, 25 % for RAEB, and 41 % for RA/RARS. Outcomes were compared to results in 339 patients with de novo MDS/tAML. After adjusting for cytogenetic risk, there were no significant differences between the two cohorts (Fig. 13.5). Relapse probability and RFS significantly correlated with disease stage ($p < .001$) and karyotype ($p < .001$). Patients receiving unrelated donor transplants ($n = 122$) had a lower risk of relapse ($p = .003$) and higher RFS ($p = .02$) compared to those receiving grafts from related donors. Conditioning with tBU plus CY ($n = 93$) was associated with the highest RFS (43 %) and lowest NRM (28 %). In principle these results were confirmed in a more recent analysis of CIBMTR data by Litzow

et al. (2010) and by an update of the FHCRC data (Deeg et al. 2012).

13.8 Managing Relapse After HCT

While considerable progress has been made in regard to transplant-related toxicity and mortality, post-HCT relapse, particularly in patients with high-risk MDS (by IPSS, WHO, or WPSS criteria), has remained a major challenge in all studies, particularly after RIC. Marrow cyto- and histomorphology, cytogenetic monitoring, PCR assessment of molecular markers, assessment of donor-host chimerism, and immunophenotyping have all been applied (Bacher et al. 2008) in attempts to detect persistent or recurrent MDS early and thereby improve the chances of eradicating the disease. Post-HCT decline in donor CD34+ cell chimerism is a harbinger of relapse, and close monitoring and early intervention with 5-azacitidine appears to restore complete chimerism and prevent relapse at least in a proportion of patients (Platzbecker et al. 2012). Data supportive of such a strategy have also been presented by de Lima et al. (2010).

Further, minimal residual disease, as determined by multiparameter flow cytometric analysis, significantly impacts outcome after HCT (Scott et al. 2008; Walter et al. 2011; Diez-Campelo et al. 2009). Patients with low or no flow cytometric aberrancy had lower relapse rates and superior survival than patients with more severely aberrant flow parameters. However, since most patients with MDS have aberrant marrow cell phenotypes by flow cytometry and may not be in remission as classically defined for patients with AML, the definition of minimal residual disease at the time of HCT is a matter of debate.

DLI in patients with hematologic relapse has shown only limited efficacy, as has withdrawal of immunosuppressive therapy (Warlick et al. 2008; Campregher et al. 2007; Rizzieri et al. 2007; Kang et al. 2008; Lim et al. 2007; Mielcarek et al. 2007). Intensive chemotherapy has generally been disappointing, and second HCT in adults have yielded low success rates (Shaw et al. 2008). Results may be superior with the use of RIC regimens for second HCT.

13.9 Autologous HCT

The role of autologous HCT has been investigated mostly in Europe. Among 100 out of 184 MDS patients who achieved complete remissions with induction chemotherapy, based on donor availability (de Witte et al. 2001), 28 received allogeneic and 36 autologous HCT while in first remission; 4-year RFS was 31 and 27 %, respectively (de Witte et al. 2001). Another report showed a relapse incidence of 58 % and TRM of 2 % among 65 autologous HCT recipients (Kroger et al. 2006a). De Witte et al. (2010) studied 264 patients with MDS and 77 with secondary AML and observed significantly superior RFS among intermediate- and poor-risk patients who had an allogeneic donor.

An analysis of results in 593 patients showed 3-year overall survival rates of 50 % among those transplanted from unrelated donors while in first remission, 42 % with autologous HCT in first remission, and 40 % among patients not in remission and transplanted from unrelated donors ($p=0.01$). The relapse rates were 62 % after autologous HCT, 24 % with HCT from HLA-matched unrelated donors while in remission, and 30 % among those who had not received prior chemotherapy (Al Ali et al. 2007).

Thus, the data indicate that autologous HCT is feasible in a small proportion of patients with MDS, but with the expansion of HCT to cord blood and HLA-haploidentical donors, autologous HCT for MDS currently plays no significant role.

13.10 MDS in Children

See Chap. 14.

13.11 Additional Risk Factors

In addition to the widely accepted risk factors recognized in formal classification systems, some other parameters may also impact outcome of HCT:

1. *Immunophenotypic aberrancy*. Myeloblast aberrancies as determined by flow cytometry

affect HCT outcome, specifically relapse. Even among patients with less than 5 % myeloblasts in the marrow, the presence of severe flow aberrancies is a associated with a threefold higher relapse rate than seen in patients with no or minimal aberrancies (Scott et al. 2008). Westers et al. recently provided a standardization of flow cytometric criteria for MDS (Westers et al. 2012), and Cutler et al. described a correlation of a flow cytometric severity score with cytogenetic abnormalities (Cutler et al. 2011).

2. *Marrow fibrosis*. Marrow fibrosis is associated with faster disease progression and shorter survival, and fibrosis is listed in a footnote to the IPSS-R (Greenberg et al. 2012) Therefore, one may want to consider earlier HCT in these patients, particularly since HCT studies show that results with HCT in patients with MDS and marrow fibrosis was inferior when HCT was carried out for advanced disease by established criteria (Scott et al. 2007; Kröger et al. 2011).
3. *Transfusion dependency*. Transfusion dependence has been recognized as a prognostic risk factor (Malcovati et al. 2007), and the severity of anemia is considered in the IPSS-R. It has been less clear whether the negative impact on transplant outcome is related primarily to the underlying biology that results in transfusion dependence or the transfusion-related effects such as allosensitization and iron overload (Platzbecker et al. 2008; Armand et al. 2007). Whether chelation therapy pre-HCT will have a positive impact on HCT outcome has not been determined.
4. *Patient age*. Patient age has long been a major limitation for the application of HCT. Again, this is acknowledged in the IPSS-R. Our current understanding is that biologic rather than chronologic age is the more relevant factor, and, indeed, patients in their 70s have been transplanted successfully; it is primarily the presence of comorbidities that negatively impact HCT outcome. As discussed elsewhere (Deeg and Sandmaier 2010; Sorror et al. 2011), age by itself should not be the only factor when arguing for or against HCT; however, consideration must be given to the patient's outlook based on other parameters, to the morbidity that may occur following HCT, and to the quality of life in general. Not everybody may be fit for transplantation.
5. *Molecular defects*. A wealth of data on DNA mutations and other molecular markers in patients with MDS has been generated in recent years (Li et al. 2010; Bejar et al. 2011a). The life expectancy is shortened in the presence of most of the identified mutations even if classic cytogenetics are normal (Bejar et al. 2011b). Conversely, It has been suggested that, for example, the *absence* of a mutation of *TET 2* was associated with inferior outcome (Mittelman et al. 2010). The relevance for the success of HCT remains to be investigated.

13.12 Summary and Conclusions

HCT is currently the only treatment with proven curative potential in patients with MDS. With careful selection, patients in the seventh and even eighth decades of life can be transplanted successfully. Results of transplants from unrelated donors, selected on the basis of HLA identity at the DNA level, are comparable to those with HLA-identical siblings. The availability of cord blood as a source of stem cells and progress made with transplantation from HLA-haploidentical donors allow to offer HCT to almost any patient. With progressive modification of conditioning regimens, the day-100 NRM has been reduced to less than 10 % or even less than 5 %. Dependent upon disease stage and characteristics, some 25–75 % of patients with MDS undergoing HCT will be cured, with follow-up now extending to more than 25 years. However, as many as 60 % of patients may experience chronic GVHD, and 20–25 % may suffer from chronic medical problems often related to GVHD. While more than 70 % report their quality of life as being "good to excellent" by 2 years after HCT, disease relapse and GVHD remain

major hurdles. Great hopes are placed on immunotherapy with restricted activity against the disease and avoidance of the undesired manifestations of GVHD. With the availability of approved drugs for the non-HCT treatment of MDS, ongoing studies are exploring the incorporation of those agents into the overall transplant approach with the goal of further improving long-term RFS.

Acknowledgments We thank Helen Crawford and Bonnie Larson for the help with manuscript preparation.

References

Al Ali HK, Brand R, van Biezen A, Finke J, Boogaerts M, Fauser AA, Egeler M, Cahn JY, Arnold R, Biersack H, Niederwieser D, de Witte T (2007) A retrospective comparison of autologous and unrelated donor hematopoietic cell transplantation in myelodysplastic syndrome and secondary acute myeloid leukemia: a report on behalf of the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Leukemia 21: 1945–1951

Alessandrino EP, Della Porta MG, Bacigalupo A, van Lint MT, Falda M, Onida F, Bernardi M, Iori AP, Rambaldi A, Cerretti R, Marenco P, Pioltelli P, Malcovati L, Pascutto C, Oneto R, Fanin R, Bosi A (2008) WHO classification and WPSS predict posttransplantation outcome in patients with myelodysplastic syndrome: a study from the Gruppo Italiano Trapianto di Midollo Osseo (GITMO). Blood 112:895–902

Alyea EP, Kim HT, Ho V, Cutler C, DeAngelo DJ, Stone R, Ritz J, Antin JH, Soiffer RJ (2006) Impact of conditioning regimen intensity on outcome of allogeneic hematopoietic cell transplantation for advanced acute myelogenous leukemia and myelodysplastic syndrome. Biol Blood Marrow Transplant 12: 1047–1055

Anasetti C, Logan BR, Lee SJ, Waller EK, Weisdorf DJ, Wingard JR, Cutler CS, Westervelt P, Woolfrey A, Couban S, Ehninger G, Johnston L, Maziarz RT, Pulsipher MA, Porter DL, Mineishi S, McCarty JM, Khan SP, Anderlini P, Bensinger WI, Leitman SF, Rowley SD, Bredeson C, Carter SL, Horowitz MM, Confer DL (2012) Peripheral-blood stem cells versus bone marrow from unrelated donors. N Engl J Med 367:1487–1496

Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Alyea EP, Soiffer RJ, Antin JH (2007) Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation. Blood 109:4586–4588

Armand P, Deeg HJ, Kim HT, Lee H, Armistead P, de Lima M, Gupta V, Soiffer RJ (2010) Multicenter validation study of a transplantation-specific cytogenetics grouping scheme for patients with myelodysplastic syndromes. Bone Marrow Transplant 45: 877–885

Aversa F (2008) Haploidentical haematopoietic stem cell transplantation for acute leukaemia in adults: experience in Europe and the United States (Review). Bone Marrow Transplant 41:473–481

Avery S, Shi W, Lubin M, Gonzales AM, Heller G, Castro-Malaspina H, Giralt S, Kernan NA, Scaradavou A, Barker JN (2011) Influence of infused cell dose and HLA match on engraftment after double-unit cord blood allografts. Blood 117:3277–3285

Bacher U, Zander AR, Haferlach T, Schnittger S, Fehse B, Kroger N (2008) Minimal residual disease diagnostics in myeloid malignancies in the post transplant period (Review). Bone Marrow Transplant 42:145–157

Barker JN, Scaradavou A, Stevens CE (2010) Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. Blood 115:1843–1849

Baron F, Sandmaier BM (2005) Current status of hematopoietic stem cell transplantation after nonmyeloablative conditioning. Curr Opin Hematol 12:435–443

Bartenstein M, Deeg HJ (2010) Hematopoietic stem cell transplantation for MDS. Hematol Oncol Clin North Am 24:407–422

Beelen DW, Trenschel R, Casper J, Freund M, Hilger RA, Scheulen ME, Basara N, Fauser AA, Hertenstein B, Mylius HA, Baumgart J, Pichlmeier U, Hahn JR, Holler E (2005) Dose-escalated treosulphan in combination with cyclophosphamide as a new preparative regimen for allogeneic haematopoietic stem cell transplantation in patients with an increased risk for regimen-related complications. Bone Marrow Transplant 35:233–241

Bejar R, LeVine R, Ebert BL (2011a) Unraveling the molecular pathophysiology of myelodysplastic syndromes (Review). J Clin Oncol 29:504–515

Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, Kantarjian H, Raza A, Levine RL, Neuberg D, Ebert BL (2011b) Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 364:2496–2506

Brunstein CG, Weisdorf DJ (2009) Future of cord blood for oncology uses (Review). Bone Marrow Transplant 44:699–707

Brunstein CG, Gutman JA, Weisdorf DJ, Woolfrey AE, Defor TE, Gooley TA, Verneris MR, Appelbaum FR, Wagner JE, Delaney C (2010) Allogeneic hematopoietic cell transplantation for hematological malignancy: relative risks and benefits of double umbilical cord blood. Blood 116:4693–4699

Brunstein CG, Fuchs EJ, Carter SL, Karanes C, Costa LJ, Wu J, Devine SM, Wingard JR, Aljitawi OS, Cutler CS, Jagasia MH, Ballen KK, Eapen M, O'Donnell PV

(2011) Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated umbilical cord blood grafts. Blood 118:282–288

Campregher PV, Gooley T, Scott BL, Moravec C, Sandmaier B, Martin PJ, Deeg HJ, Warren EH, Flowers MED (2007) Results of donor lymphocyte infusions for relapsed myelodysplastic syndrome after hematopoietic cell transplantation. Bone Marrow Transplant 40:965–971

Casper J, Wolff D, Knauf W, Blau IW, Ruutu T, Volin L, Wandt H, Schafer-Eckart K, Holowiecki J, Giebel S, Aschan J, Zander AR, Kroger N, Hilgendorf I, Baumgart J, Mylius HA, Pichlmeier U, Freund M (2010) Allogeneic hematopoietic stem-cell transplantation in patients with hematologic malignancies after dose-escalated treosulfan/fludarabine conditioning. J Clin Oncol 28:3344–3351

Chang C, Storer BE, Scott BL, Bryant EM, Shulman HM, Flowers ME, Sandmaier BM, Witherspoon RP, Nash RA, Sanders JE, Bedalov A, Hansen JA, Clurman BE, Storb R, Appelbaum FR, Deeg HJ (2007) Hematopoietic cell transplantation in patients with myelodysplastic syndrome or acute myeloid leukemia arising from myelodysplastic syndrome: similar outcomes in patients with de novo disease and disease following prior therapy or antecedent hematologic disorders. Blood 110:1379–1387

Chen Y, Liu K, Xu L, Chen H, Liu D, Zhang X, Shi H, Han W, Wang Y, Zhao T, Wang J, Wang J, Huang X (2010) HLA-mismatched hematopoietic SCT without in vitro T-cell depletion for myelodysplastic syndrome. Bone Marrow Transplant 45:1333–1339

Cutler CS, Lee SJ, Greenberg P, Deeg HJ, Pérez WS, Anasetti C, Bolwell BJ, Cairo MS, Gale RP, Klein JP, Lazarus HM, Liesveld JL, McCarthy PL, Milone GA, Rizzo JD, Schultz KR, Trigg ME, Keating A, Weisdorf DJ, Antin JH, Horowitz MM (2004) A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. Blood 104:579–585

Cutler JA, Wells DA, van de Loosdrecht AA, de Baca ME, Kalnoski MH, Zehentner BK, Eidenschink L, Ghirardelli KM, Biggerstaff JS, Loken MR (2011) Phenotypic abnormalities strongly reflect genotype in patients with unexplained cytopenias. Cytometry B Clin Cytom 80:150–157

Cutting R, Mirelman A, Vora A (2008) Treosulphan as an alternative to busulphan for myeloablative conditioning in paediatric allogeneic transplantation. Br J Haematol 143:748–751

Dahlberg A, Delaney C, Bernstein ID (2011) Ex vivo expansion of human hemtopoietic stem and progenitor cells. Blood 117:6083–6090

de Lima M, Couriel D, Thall PF, Wang X, Madden T, Jones R, Shpall EJ, Shahjahan M, Pierre B, Giralt S, Korbling M, Russell JA, Champlin RE, Andersson BS (2004) Once-daily intravenous buslfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. Blood 104:857–864

de Lima M, Giralt S, Thall PF, De Padua SL, Jones RB, Komanduri K, Braun TM, Nguyen HQ, Champlin R, Garcia-Manero G (2010) Maintenance therapy with low-dose azacitidine after allogeneic hematopoietic stem cell transplantation for recurrent acute myelogenous leukemia or myelodysplastic syndrome: a dose and schedule finding study. Cancer 116:5420–5431

De Padua SL, de Lima M, Kantarjian H, Faderl S, Kebriaei P, Giralt S, Davisson J, Garcia-Manero G, Champlin R, Issa JP, Ravandi F (2009) Feasibility of allo-SCT after hypomethylating therapy with decitabine for myelodysplastic syndrome. Bone Marrow Transplant 43:839–843

de Witte T, Suciu S, Verhoef G, Labar B, Archimbaud E, Aul C, Selleslag D, Ferrant A, Wijermans P, Mandelli F, Amadori S, Jehn U, Muus P, Boogaerts M, Zittoun R, Gratwohl A, Zwierzina H, Hagemeijer A, Willemze R (2001) Intensive chemotherapy followed by allogeneic or autologous stem cell transplantation for patients with myelodysplastic syndromes (MDSs) and acute myeloid leukemia following MDS. Blood 98: 2326–2331

de Witte T, Brand R, van Biezen A, Mufti G, Ruutu T, Finke J, von dem Borne P, Vitek A, Delforge M, Alessandrino P, Harlahakis N, Russell N, Martino R, Verdonck L, Kröger N, Niederwieser D (2009) Allogeneic stem cell transplantation for patients with refractory anaemia with matched related and unrelated donors: delay of the transplant is associated with inferior survival. Br J Haematol 146:627–636

de Witte T, Hagemeijer A, Suciu S, Belhabri A, Delforge M, Kobbe G, Selleslag D, Schouten HC, Ferrant A, Biersack H, Amadori S, Muus P, Jansen JH, Hellstrom-Lindberg E, Kovacsovics T, Wijermans P, Ossenkoppele G, Gratwohl A, Marie JP, Willemze R (2010) Value of allogeneic versus autologous stem cell transplantation and chemotherapy in patients with myelodysplastic syndromes and secondary acute myeloid leukemia. Final results of a prospective randomized European Intergroup Trial. Haematologica 95:1754–1761

Deeg HJ, Sandmaier BM (2010) Who is fit for allogeneic transplantation? Blood 116:4762–4770

Deeg HJ, Storer B, Slattery JT, Anasetti C, Doney KC, Hansen JA, Kiem H-P, Martin PJ, Petersdorf E, Radich JP, Sanders JE, Shulman HM, Warren EH, Witherspoon RP, Bryant EM, Chauncey TR, Getzendaner L, Storb R, Appelbaum FR (2002) Conditioning with targeted busulfan and cyclophosphamide for hemopoietic stem cell transplantation from related and unrelated donors in patients with myelodysplastic syndrome. Blood 100:1201–1207

Deeg HJ, Maris MB, Scott BL, Warren EH (2006) Optimization of allogeneic transplant conditioning: not the time for dogma. Leukemia 20:1701–1705

Deeg HJ, Scott BL, Fang M, Shulman HM, Gyurkocza B, Myerson D, Pagel JM, Platzbecker U, Ramakrishnan

A, Radich JP, Sandmaier BM, Sorror M, Stirewalt DL, Wilson WA, Storb R, Appelbaum FR, Gooley T (2012) Five-group cytogenetic risk classification, monosomal karyotype, and outcome after hematopoietic cell transplantation for MDS or acute leukemia evolving from MDS. Blood 120:1398–1408

Delaney C, Ratajczak MZ, Laughlin MJ (2010) Strategies to enhance umbilical cord blood stem cell engraftment in adult patients (Review). Expert Rev Hematol 3:273–283

Diez-Campelo M, Perez-Simon JA, Perez J, Alcoceba M, Richtmon J, Vidriales B, San Miguel J (2009) Minimal residual disease monitoring after allogeneic transplantation may help to individualize post-transplant therapeutic strategies in acute myeloid malignancies. Am J Hematol 84:149–152

Farina L, Bruno B, Patriarca F, Spina F, Sorasio R, Morelli M, Fanin R, Boccadoro M, Corradini P (2009) The hematopoietic cell transplantation comorbidity index (HCT-CI) predicts clinical outcomes in lymphoma and myeloma patients after reduced-intensity or non-myeloablative allogeneic stem cell transplantation. Leukemia 23:1131–1138

Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M, Martin PJ, Sandmaier BM, Marr KA, Appelbaum FR, Storb R, McDonald GB (2010) Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med 363:2091–2101

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 (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89:2079–2088 [erratum appears in Blood 1998; 91(3):1100]

Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Sole 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, Luebbert M, Maciejewski J, Magalhaes SM, Miyazaki Y, Pfeilstocker M, Sekeres M, Sperr WR, Stauder R, Tauro S, Valent P, Vallespi T, van de Loosdrecht AA, Germing U, Haase D (2012) Revised international prognostic scoring system for myelodysplastic syndromes. Blood 120:2454–2465

Guardiola P, Runde V, Bacigalupo A, Ruutu T, Locatelli F, Boogaerts MA, Pagliuca A, Cornelissen JJ, Schouten HC, Carreras E, Finke J, van Biezen A, Brand R, Niederwieser D, Gluckman E, de Witte TM, Subcommittee for Myelodysplastic Syndromes of the Chronic Leukaemia Working Group of the European Blood and Marrow Transplantation Group (2002) Retrospective comparison of bone marrow and granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells for allogeneic stem cell transplantation using HLA identical sibling donors in myelodysplastic syndromes. Blood 99:4370–4378

Harrison SJ, Cook G, Nibbs RJ, Prince HM (2006) Immunotherapy of multiple myeloma: the start of a long and tortuous journey (Review). Expert Rev Anticancer Ther 6:1769–1785

Ho AYL, Pagliuca A, Kenyon M, Parker JE, Mijovic A, Devereux S, Mufti GJ (2004) Reduced-intensity allogeneic hematopoietic stem cell transplantation for myelodysplastic syndrome and acute myeloid leukemia with multilineage dysplasia using fludarabine, busulphan and alemtuzumab (FBC) conditioning. Blood 104:1616–1623

Kang Y, Chao NJ, Aversa F (2008) Unmanipulated or CD34 selected haplotype mismatched transplants (Review). Curr Opin Hematol 15:561–567

Kataoka K, Nannya Y, Ueda K, Kumano K, Takahashi T, Kurokawa M (2010) Differential prognostic impact of pretransplant comorbidity on transplant outcomes by disease status and time from transplant: a single Japanese transplant centre study. Bone Marrow Transplant 45:513–520

Kelly SS, Sola CBS, de Lima M, Shpall E (2009) *Ex vivo* expansion of cord blood (Review). Bone Marrow Transplant 44:673–681

Kindwall-Keller T, Isola LM (2009) The evolution of hematopoietic SCT in myelodysplastic syndrome (Review). Bone Marrow Transplant 43:597–609

Koreth J, Pidala J, Perez WS, Deeg HJ, Garcia-Manero G, Malcovati L, Cazzola M, Park S, Itzykson R, Ades L, Fenaux P, Jadersten M, Hellstrom-Lindberg E, Gale RP, Beach CL, Greenberg PL, Tallman MS, DiPersio JF, Bunjes D, Weisdorf DJ, Cutler CS (2011) A decision analysis of reduced-intensity conditioning allogeneic hematopoietic stem cell transplantation for older patients with de-novo myelodysplastic syndrome (MDS): early transplantation offers survival benefit in higher-risk MDS. Blood 118:Abstract #115 (Abstract)

Kroger N, Brand R, van Biezen A, Cahn JY, Slavin S, Blaise D, Sierra J, Zander A, Niederwieser D, de Witte T (2006a) Autologous stem cell transplantation for therapy-related acute myeloid leukemia and myelodysplastic syndrome. Bone Marrow Transplant 37:183–189

Kroger N, Shimoni A, Zabelina T, Schieder H, Panse J, Ayuk F, Wolschke C, Renges H, Dahlke J, Atanackovic D, Nagler A, Zander A (2006b) Reduced-toxicity conditioning with treosulfan, fludarabine and ATG as preparative regimen for allogeneic stem cell transplantation (alloSCT) in elderly patients with secondary acute myeloid leukemia (sAML) or myelodysplastic syndrome (MDS). Bone Marrow Transplant 37:339–344

Kröger N, Zabelina T, van Biezen A, Brand R, Niederwieser D, Martino R, Lim ZY, Onida F, Schmid C, Garderet L, Robin M, van Gelder M, Marks R, Symeonidis A, Kobbe G, de Witte T (2011) Allogeneic stem cell transplantation for myelodysplastic syndromes with bone marrow fibrosis. Haematologica 96:291–297

Laport GG, Sandmaier BM, Storer BE, Scott BL, Stuart MJ, Lange T, Maris MB, Agura ED, Chauncey TR, Wong RM, Forman SJ, Petersen FB, Wade JC, Epner E, Bruno B, Bethge WA, Curtin PT, Maloney DG,

Blume KG, Storb RF (2008) Reduced-intensity conditioning followed by allogeneic hematopoietic cell transplantation for adult patients with myelodysplastic syndrome and myeloproliferative disorders. Biol Blood Marrow Transplant 14:246–255

Li X, Marcondes AM, Gooley TA, Deeg HJ (2010) The helix-loop-helix transcription factor TWIST is dysregulated in myelodysplastic syndromes. Blood 116:2304–2314

Lim ZY, Pearce L, Ho AY, Barber L, Ingram W, Usai M, Tobal K, Devereux S, Pagliuca A, Mufti GJ (2007) Delayed attainment of full donor chimaerism following alemtuzumab-based reduced-intensity conditioning haematopoietic stem cell transplantation for acute myeloid leukaemia and myelodysplastic syndromes is associated with improved outcomes. Br J Haematol 138:517–526

Lim ZY, Ingram W, Brand R, Ho A, Kenyon M, Devereux S, Marsh J, Mufti GJ, Pagliuca A (2010) Impact of pretransplant comorbidities on alemtuzumab-based reduced-intensity conditioning allogeneic hematopoietic SCT for patients with high-risk myelodysplastic syndrome and AML. Bone Marrow Transplant 45:633–639

Litzow MR, Tarima S, Perez WS, Bolwell BJ, Cairo MS, Camitta BM, Cutler CS, de Lima M, DiPersio JF, Gale RP, Keating A, Lazarus HM, Luger S, Marks DI, Maziarz RT, McCarthy PL, Pasquini MC, Phillips GL, Rizzo JD, Sierra J, Tallman MS, Weisdorf DJ (2010) Allogeneic transplantation for therapy-related myelodysplastic syndrome and acute myeloid leukemia. Blood 115:1850–1857

Luznik L, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M, Gooley TA, Piantadosi S, Kaup M, Ambinder RF, Huff CA, Matsui W, Bolaños-Meade J, Borrello I, Powell JD, Harrington E, Warnock S, Flowers M, Brodsky RA, Sandmaier BM, Storb RF, Jones RJ, Fuchs EJ (2008) HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, post-transplantation cyclophosphamide. Biol Blood Marrow Transplant 14:641–650

Ma Q, Wang C, Jones D, Quintanilla KE, Li D, Wang Y, Wieder ED, Clise-Dwyer K, Alatrash G, Mj Y, Munsell MF, Lu S, Qazilbash MH, Molldrem JJ (2010) Adoptive transfer of PR1 cytotoxic T lymphocytes associated with reduced leukemia burden in a mouse acute myeloid leukemia xenograft model. Cytotherapy 12:1056–1062

Malcovati L, Germing U, Kuendgen A, Della Porta MG, Pascutto C, Invernizzi R, Giagounidis A, Hildebrandt B, Bernasconi P, Knipp S, Strupp C, Lazzarino M, Aul C, Cazzola M (2007) Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 25:3503–3510

Martino R, Iacobelli S, Brand R, Jansen T, van Biezen A, Finke J, Bacigalupo A, Beelen D, Reiffers J, Devergie A, Alessandrino E, Mufti GJ, Barge R, Sierra J, Ruutu T, Boogaerts M, Falda M, Jouet JP, Niederwieser D, de Witte T (2006) Retrospective comparison of reduced-intensity conditioning and conventional high-dose conditioning for allogeneic hematopoietic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes. Blood 108: 836–846

Mielcarek M, Storer BE, Flowers MED, Storb R, Sandmaier BM, Martin PJ (2007) Outcomes among patients with recurrent high-risk hematologic malignancies after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 13:1160–1168

Mittelman M, Oster HS, Hoffman M, Neumann D (2010) The lower risk MDS patient at risk of rapid progression (Review). Leuk Res 34:1551–1555

Nakai K, Kanda Y, Fukuhara S, Sakamaki H, Okamoto S, Kodera Y, Tanosaki R, Takahashi S, Matsushima T, Atsuta Y, Hamajima N, Kasai M, Kato S (2005) Value of chemotherapy before allogeneic hematopoietic stem cell transplantation from an HLA-identical sibling donor for myelodysplastic syndrome. Leukemia 19:396–401

Nemecek ER, Guthrie KA, Sorror ML, Wood BL, Doney KC, Hilger RA, Scott BL, Kovacsovics TJ, Maziarz RT, Woolfrey AE, Bedalov A, Sanders JE, Pagel JM, Sickle EJ, Witherspoon R, Flowers ME, Appelbaum FR, Deeg HJ (2011) Conditioning with treosulfan and fludarabine followed by allogeneic hematopoietic cell transplantation for high-risk hematologic malignancies. Biol Blood Marrow Transplant 17:341–350

O'Donnell PV, Harrington E, Gooley TA, Pagel JM, Pereira SE, Flowers ME, Hansen JA, Burroughs LM, Sandmaier BM, Storb RF, Luznik L, Jones RJ, Symons HJ, Kasamon YL, Fuchs EJ (2010) Cyclophosphamide-induced tolerance following bone marrow transplantation from haploidentical donors. Haematologica Edición Española 95(Extra 1):266–271

Oliansky DM, Antin JH, Bennett JM, Deeg HJ, Engelhardt C, Heptinstall KV, de Lima M, Gore SD, Potts RG, Silverman LR, Jones RB, McCarthy PL Jr, Hahn T (2009) The role of cytotoxic therapy with hematopoietic stem cell transplantation in the therapy of myelodysplastic syndromes: an evidence-based review. Biol Blood Marrow Transplant 15:137–172

Ooi J (2006) The efficacy of unrelated cord blood transplantation for adult myelodysplastic syndrome. Leuk Lymphoma 47:599–602

Ooi J, Iseki T, Takahashi S, Tomonari A, Ishii K, Takasugi K, Shimohakamada Y, Ohno N, Uchimaru K, Nagamura F, Tojo A, Asano S (2003) Unrelated cord blood transplantation for adult patients with advanced myelodysplastic syndrome. Blood 101:4711–4713

Pagel JM, Gooley TA, Rajendran J, Fisher DR, Wilson WA, Sandmaier BM, Matthews DC, Deeg HJ, Gopal AK, Martin PJ, Storb R, Press OW, Appelbaum FR (2009) Allogeneic hematopoietic cell transplantation after conditioning with 131I-anti-CD45 antibody plus fludarabine and low-dose total body irradiation for elderly patients with advanced acute myeloid leukemia or high-risk myelodysplastic syndrome. Blood 114:5444–5453

Parimon T, Au DH, Martin PJ, Chien JW (2006) A risk score for mortality after allogeneic hematopoietic cell transplantation. Ann Intern Med 144:407–414

Petersdorf EW (2009) Hematopoietic cell transplantation from unrelated donors. In: Appelbaum FR, Forman SJ, Negrin RS, Blume KG (eds) Thomas' hematopoietic cell transplantation. Wiley-Blackwell, Oxford, p 675

Pidala J, Anasetti C, Kharfan-Dabaja MA, Cutler C, Sheldon A, Djulbegovic B (2009a) Decision analysis of peripheral blood versus bone marrow hematopoietic stem cells for allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 15:1415–1421

Pidala J, Kim J, Field T, McBride A, Kharfan-Dabaja M, Perkins J, Fernandez H, Perez L, Ayala E, Anasetti C (2009b) Infliximab for managing steroid-refractory acute graft-versus-host disease. Biol Blood Marrow Transplant 15:1116–1121

Platzbecker U, Bornhäuser M, Germing U, Stumpf J, Scott BL, Kröger N, Schwerdtfeger R, Böhm A, Kobbe G, Theuser C, Rabitsch W, Valent P, Sorror ML, Ehninger G, Deeg HJ (2008) 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 14:1217–1225

Platzbecker U, Wermke M, Radke J, Oelschlaegel U, Seltmann F, Kiani A, Klut IM, Knoth H, Röllig C, Schetelig J, Mohr B, Graehlert X, Ehninger G, Bornhäuser M, Thiede C (2012) Azacitidine for treatment of imminent relapse in MDS or AML patients after allogeneic HSCT: results of the RELAZA trial. Leukemia 26:381–389

Prébet T, Gore SD, Esterni B, Gardin C, Itzykson R, Thepot S, Dreyfus F, Rauzy OB, Recher C, Ades L, Quesnel B, Beach CL, Fenaux P, Vey N (2011) Outcome of high-risk myelodysplastic syndrome after azacitidine treatment failure. J Clin Oncol 29: 3322–3327

Ramakrishnan A, Deeg HJ (2008) Allogeneic hematopoietic cell transplantation for patients with myelodysplastic syndrome and myeloproliferative disorders. In: Soiffer RJ (ed) Hematopoietic stem cell transplantation. Humana Press, Totowa, p 167

Rizzieri DA, Koh LP, Long GD, Gasparetto C, Sullivan KM, Horwitz M, Chute J, Smith C, Gong JZ, Lagoo A, Niedzwiecki D, Dowell JM, Waters-Pick B, Liu C, Marshall D, Vredenburgh JJ, Gockerman J, DeCastro C, Moore J, Chao NJ (2007) Partially matched, nonmyeloablative allogeneic transplantation: clinical outcomes and immune reconstitution. J Clin Oncol 25:690–697

Rocha V, Gluckman E (2009) Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. Br J Haematol 147:262–274

Rocha V, Locatelli F (2008) Searching for alternative hematopoietic stem cell donors for pediatric patients (Review). Bone Marrow Transplant 41: 207–214

Samuelson S, Sandmaier BM, Heslop HE, Popat U, Carrum G, Champlin RE, Storb R, Prchal JT, Gooley TA, Deeg HJ (2011) Allogeneic haematopoeietic cell transplantation for myelofibrosis in 30 patients 60–78 years of age. Br J Haematol 153:76–82

Sato A, Ooi J, Takahashi S, Tsukada N, Kato S, Kawakita T, Yagyu T, Nagamura F, Iseki T, Tojo A, Asano S (2011) Unrelated cord blood transplantation after myeloablative conditioning in adults with advanced myelodysplastic syndromes. Bone Marrow Transplant 46:257–261

Schanz J, Tuchler H, Sole F, Mallo M, Luno E, Cervera J, Granada I, Hildebrandt B, Slovak ML, Ohyashiki K, Steidl C, Fonatsch C, Pfeilstocker M, Nosslinger T, Valent P, Giagounidis A, Aul C, Lubbert M, Stauder R, Krieger O, Garcia-Manero G, Faderl S, Pierce S, Le Beau MM, Bennett JM, Greenberg P, Germing U, Haase D (2012) 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 30:820–829

Scott B, Deeg HJ (2006) Hemopoietic cell transplantation as curative therapy of myelodysplastic syndromes and myeloproliferative disorders. Best Pract Res Clin Haematol 19:519–522

Scott BL, Storer B, Loken M, Storb R, Appelbaum FR, Deeg HJ (2005) Pretransplantation induction chemotherapy and posttransplantation relapse in patients with advanced myelodysplastic syndrome. Biol Blood Marrow Transplant 11:65–73

Scott BL, Sandmaier BM, Storer B, Maris MB, Sorror ML, Maloney DG, Chauncey TR, Storb R, Deeg HJ (2006) Myeloablative vs nonmyeloablative allogeneic transplantation for patients with myelodysplastic syndrome or acute myelogenous leukemia with multilineage dysplasia: a retrospective analysis. Leukemia 20:128–135

Scott BL, Storer BE, Greene JE, Hackman RC, Appelbaum FR, Deeg HJ (2007) Marrow fibrosis as a risk factor for post-transplant outcome in patients with advanced MDS or AML with multilineage dysplasia. Biol Blood Marrow Transplant 13:345–354

Scott BL, Wells DA, Loken MR, Myerson D, Leisenring WM, Deeg HJ (2008) Validation of a flow cytometric scoring system as a prognostic indicator for posttransplantation outcome in patients with myelodysplastic syndrome. Blood 112:2681–2686

Shaw BE, Mufti GJ, Mackinnon S, Cavenagh JD, Pearce RM, Towlson KE, Apperley JF, Chakraverty R, Craddock CF, Kazmi MA, Littlewood TJ, Milligan DW, Pagliuca A, Thomson KJ, Marks DI, Russell NH (2008) Outcome of second allogeneic transplants using reduced-intensity conditioning following relapse of haematological malignancy after an initial allogeneic transplant. Bone Marrow Transplant 42:783–789

Sorror M, Maris M, Baron F, Sandmaier B, Maloney D, Storb R, Storer B (2004) A modified hematopoietic cell transplantation (HCT)-specific co-morbidity index. Blood 104(Part 1):324a–325a, Abstract #1146

Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, Storer B (2005) Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood 106:2912–2919

Sorror ML, Giralt S, Sandmaier B, Maris M, Maloney D, Deeg HJ, Appelbaum F, Storer B, Storb R (2006) Validation of the predictive power of the hematopoietic cell transplantation-comorbidity index (HCT-CI) for non-relapse mortality (NRM) and survival after allogeneic HCT. Biol Blood Marrow Transplant 12(suppl 1):8–9, Abstract #17

Sorror M, Storer B, Sandmaier BM, Maloney DG, Chauncey TR, Langston A, Maziarz RT, Pulsipher M, McSweeney PA, Storb R (2008) Hematopoietic cell transplantation-comorbidity index and Karnofsky performance status are independent predictors of morbidity and mortality after allogeneic nonmyeloablative hematopoietic cell transplantation. Cancer 112: 1992–2001

Sorror ML, Sandmaier BM, Storer BE, Franke GN, Laport GG, Chauncey TR, Agura E, Maziarz RT, Langston A, Hari P, Pulsipher MA, Bethge W, Sahebi F, Bruno B, Maris MB, Yeager A, Petersen FB, Vindeløv L, McSweeney PA, Hübel K, Mielcarek M, Georges GE, Niederwieser D, Blume KG, Maloney DG, Storb R (2011) Long-term outcomes among older patients following nonmyeloablative conditioning and allogeneic hematopoietic cell transplantation for advanced hematologic malignancies. J Am Med Assoc 306:1874–1883

Valcarcel D, Martino R (2007) Reduced intensity conditioning for allogeneic hematopoietic stem cell transplantation in myelodysplastic syndromes and acute myelogenous leukemia (Review). Curr Opin Oncol 19:660–666

Walter RB, Gooley TA, Wood BL, Milano F, Fang M, Sorror ML, Estey EH, Salter AI, Lansverk E, Chien JW, Gopal AK, Appelbaum FR, Pagel JM (2011) Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. J Clin Oncol 29:1190–1197

Warlick ED, O'Donnell PV, Borowitz M, Grupka N, Decloe L, Garrett-Mayer E, Borrello I, Brodsky R, Fuchs E, Huff CA, Luznik L, Matsui W, Ambinder R, Jones RJ, Douglas SB (2008) Myeloablative allogeneic bone marrow transplant using T cell depleted allografts followed by post-transplant GM-CSF in high-risk myelodysplastic syndromes. Leuk Res 32:1439–1447

Warlick ED, Cioc A, DeFor T, Dolan M, Weisdorf D (2009) Allogeneic stem cell transplantation for adults with myelodysplastic syndromes: importance of pre-transplant disease burden. Biol Blood Marrow Transplant 15:30–38

Welniak LA, Blazar BR, Murphy WJ (2007) Immunobiology of allogeneic hematopoietic stem cell transplantation (Review). Annu Rev Immunol 25:139–170

Westers TM, Ireland R, Kern W, Alhan C, Balleisen JS, Bettelheim P, Burbury K, Cullen M, Cutler JA, Della Porta MG, Drager AM, Feuillard J, Font P, Germing U, Haase D, Johansson U, Kordasti S, Loken MR, Malcovati L, te Marvelde JG, Matarraz S, Milne T, Moshaver B, Mufti GJ, Ogata K, Orfao A, Porwit A, Psarra K, Richards SJ, Subira D, Tindell V, Vallespi T, Valent P, van der Velden VH, de Witte TM, Wells DA, Zettl F, Bene MC, van de Loosdrecht AA (2012) Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European LeukemiaNet Working Group. Leukemia 26:1730–1741

Yakoub-Agha I, de La Salmonière P, Ribaud P, Sutton L, Wattel E, Kuentz M, Jouet JP, Marit G, Milpied N, Deconinck E, Gratecos N, Leporrier M, Chabbert I, Caillot D, Damaj G, Dauriac C, Dreyfus F, François S, Molina L, Tanguy ML, Chevret S, Gluckman E (2000) Allogeneic bone marrow transplantation for therapy-related myelodsyplastic syndrome and acute myeloid leukemia: a long-term study of 70 patients-report of the French Society of bone marrow transplantation. J Clin Oncol 18:963–971

Yeh RF, Pawlikowski MA, Blough DK, McDonald GB, O'Donnell PV, Rezvani A, Deeg HJ, McCune JS (2012) Accurate targeting of daily intravenous busulfan with 8-hour blood sampling in hospitalized adult hemtopoietic cell transplant recipients. Biol Blood Marrow Transplant 18:265–272

Part IV

MDS in Children

Myelodysplastic Syndrome in Children

14

Charlotte Niemeyer

14.1 Introduction

Myelodysplastic Syndrome (MDS) is much rarer in children than in adults, accounting for less than 5 % of hematopoietic neoplasia in childhood. Population-based data suggest an annual incidence of 1–2/million (Hasle et al. 1995, 1999b; Passmore et al. 2003). There are significant differences in presentation, underlying molecular and cytogenetic abnormalities and classification between MDS in children and adults. The therapeutic aim in children with MDS is primarily a cure, and therapeutic efforts concentrate on hematopoietic stem cell transplantation (HSCT).

14.2 Classification of Childhood MDS

MDS in childhood is a very heterogeneous group of disorders associated with a variety of different clinical conditions. This diversity hampered a generally accepted classification in the past. Following an International consensus myelodysplastic and myelodysplastic/myeloproliferative disorders in children are separated into three main groups: juvenile myelomonocytic leukemia (JMML), MDS, and myeloid proliferations related to Down syndrome (Table 14.1) (Hasle et al. 2003). JMML is a unique disorder of infancy characterized by a hyperactive RAS signalling pathway due to molecular aberrations in the genes encoding for SHP-2, NRAS, KRAS, CBL, or neurofibromatosis type 1. MDS in Down syndrome in the first 5 years in life is not biologically different from AML in these patients. The unifying term "myeloid leukemia associated with Down syndrome" encompasses both MDS and AML (Baumann et al. 2008a), and patients are no longer included in MDS series.

MDS in childhood displays many of the morphologic and genetic features observed in adulthood forms. There are, however, significant differences reported. For instance, in childhood MDS, refractory cytopenia with ringed

Table 14.1 Classification of MDS and myelodysplastic/myeloproliferative disorders of childhood

I. Myelodysplastic/myeloproliferative disease
Juvenile myelomonocytic leukemia (JMML)
Chronic myelomonocytic leukemia (CMML) (secondary only)
II. Down syndrome disease
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia of Down syndrome
III. Myelodysplastic Syndrome (MDS)
Refractory cytopenia of childhood (RCC) (PB blasts <2 % and BM blasts <5 %)
Refractory anemia with excess blasts (RAEB) (PB blasts 2–19 % or BM blasts 5–19 %)
RAEB in transformation (RAEB-T) (PB or BM blasts 20–29 %)

C. Niemeyer, MD
Division of Pediatric Hematology and Oncology,
Department of Pediatrics and Adolescent Medicine,
University Hospital of Freiburg,
Mathildenstrasse 1, Freiburg 79106, Germany
e-mail: charlotte.niemeyer@uniklinik-freiburg.de

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3_14,

sideroblasts is infrequently found, the unique 5q– syndrome has not been described, and the importance of multilineage dysplasia in refractory cytopenia is unknown. To account for these differences, the most recent WHO classification on hematopoietic and lymphoid neoplasia incorporated a chapter on MDS in children (Baumann et al. 2008b).

For children with MDS and 2–19 % blasts in the peripheral blood (PB) or 5–19 % blasts in the bone marrow (BM), the same criteria utilized for adults with refractory anemia with excess blasts (RAEB) are applied. Children diagnosed of RAEB generally have stable disease for weeks or months. A similar clinical course with lack of features of acute leukemia can be observed in some cases with 20–30 % blasts in PB or BM who are considered as RAEB in transformation (RAEB-T) in the French-American British cooperative classification (Baumann et al. 2008b; Hasle et al. 2003). It should be emphasized, however, that the blast count is insufficient to differentiate MDS from de novo AML. Disease with rapid increase in marrow blasts, organ infiltration, or cytogenetic abnormalities like t(8;21)(q22;q22), inv(16)(p13;1q22), t(16;16)(p13.1;q22), or t(15;17)(q22;q12) should be considered AML which is by far the more common disorder.

Childhood MDS characterized by persistent cytopenia with <5 % blasts in the BM and <2 % blasts in the PB is classified as refractory cytopenia of childhood (RCC), a provisional entity of the current WHO classification (Groupe Francais de Cytogenetique Hematologique 1997; Kardos et al. 2003). Because many patients with RCC have a hypocellular BM, a trephine biopsy is indispensable (Baumann et al. 2012). Increased and left-shifted erythropoiesis with sparse maturation and normocellular or decreased granulopoiesis is considered characteristic for RCC. The presence of micro-megakaryocytes is a strong indicator of RCC but megakaryocytes can be low in number or absent (Baumann et al. 2012).

MDS after prior chemo- or radiation therapy, prior acquired aplastic anemia, and in inherited bone marrow failure disorders are classified as secondary MDS (Hasle et al. 2003). All other cases are named primary MDS, although it is reasonable to assume that most if not all of these disorders are secondary to some genetic predisposition. These underlying genetic changes can also give rise to subtle phenotypic abnormalities observed in many children with primary MDS.

14.3 Cytogenetic

The frequency of an abnormal karyotype in hematopoietic cells varies among subtypes of MDS in childhood (Table 14.2). In advanced primary MDS, about 60 % of children have a chromosomal aberration. In contrast to AML, numerical abnormalities dominate; structural abnormalities are frequently part of a complex karyotype with numeric abnormalities. Monosomy 7 is the most common cytogenetic abnormality being identified in approximately 25 % of cases of advanced MDS (Gohring et al. 2010; Groupe Francais de Cytogenetique Hematologique 1997; Luna-Fineman et al. 1999; Sasaki et al. 2001). In the absence of standard banding cytogenetics, in situ hybridization (FISH) for identification in monosomy 7 is helpful (Ketterling et al. 2002), although the importance of small clones of monosomy 7 cells remains unknown. In most instances, monosomy 7 is a sole aberration; outcome of children with monosomy 7 and clonal evolution or monosomy 7 and other aberrations (excluding those with structurally complex karyotypes) is similar to those with sole monosomy 7 (Gohring et al. 2010). Acquired trisomy 8 and trisomy 21 are the most common numerical abnormalities after monosomy 7 in advanced primary MDS. Constitutional trisomy 8 mosaicism may remain unrecognized and should be tested for when trisomy 8 is found in the BM (Hasle et al. 1995). In MDS following chemo- or radiation therapy, structurally complex karyotypes defined as more than or equal to 3 chromosomal aberrations, including at least one structural aberration, are common (Table 14.2).

Monosomy 7 is associated with a shorter time to progression in RCC (Kardos et al. 2003). In advanced MDS, monosomy 7 as the sole cytogenetic aberration has not been an unfavorable feature in most studies (Hasle et al. 1999a; Strahm et al. 2011; Woods et al. 2002). In contrast, the presence of a structurally complex karyotype is the strongest prognostic factor in advanced primary and secondary childhood MDS (Gohring et al. 2010).

Table 14.2 Cytogenetic subgroups in primary MDS and MDS secondary to chemo- or radiation therapy

Karyotype	Primary MDS (%)			MDS secondary to chemo- or radiation therapy (%)
	All ($N=492$)	RCC ($N=311$)	RAEB/RAEB-T ($N=181$)	($N=110$)
Normal	62	77	38	20
−7 (± other aberrations)	21	13	33	35
Structurally complex	2	1	4	14
Other aberrations	15	9	25	31

Results of Study EWOG-MDS 98 and interim analysis of EWOG-MDS 2006

Favorable cytogenetic aberrations, −Y, 20q−, and 5q−, have been reported in adults, but these aberrations are so infrequent in children that they are of no practical importance.

14.4 Molecular Aberrations

In line with a multi-hit model, the development of myeloid malignancies may require mutations leading to increased proliferation (type I) and impaired differentiation (type II) of leukemic cells. A recent study of 107 children with primary or secondary MDS screened for a broad spectrum of type 1 and 2 gene aberrations indicated that these aberrations occur less frequently in pediatric compared to adult MDS (de Vries et al. 2012). *RUNX1* and *CEBPA* mutations were the most frequent changes observed in this pediatric study, while mutations in the TET2, ASXL1, IDH1, IDH2, and DNMT3A genes were absent. Similarly, spliceosome mutations are exceedingly rare in children with MDS (Hirabayashi et al. 2012). Taken together, these differences in frequency of common molecular aberrations between childhood and adult MDS suggest different pathogenetic mechanisms. One might speculate that the pathogenesis in children is related to genetic determinants acquired prenatally and less to accumulation of somatic events during life.

14.5 Primary MDS

In primary MDS the main diagnostic challenges are to differentiate low-grade MDS from aplastic anemia or inherited bone marrow failure syndrome and high-grade MDS from AML.

14.5.1 Refractory Cytopenia of Childhood

MDS with less than 5 % blasts in the bone marrow is particularly difficult to diagnose, because dysplasia of hematopoietic cells is frequently observed in children with a variety of disease states. RCC is the most common subtype of childhood MDS accounting for about half of the cases (Passmore et al. 2003). The majority of patients with RCC have a decreased marrow cell content (Kardos et al. 2003) (Fig. 14.1), down to 5–10 % of the normal age-matched value. The morphological picture of RCC with hypocellular bone marrow is similar to that observed in cases with normo- or hypercellular bone marrow and characterized by island of immature erythroid precursors. Two biopsies are recommended to facilitate the detection of representative bone marrow spaces containing diagnostic foci of erythropoiesis. In contrast to RCC, acquired severe aplastic anemia reveals aplasia of all 3 hematological cell lineages. Following immunosuppressive therapy, the histological pattern can, however, no longer be separated from that observed in RCC.

Inherited bone marrow failure disorders such as Fanconi anemia, dyskeratosis congenita, Shwachman-Diamond syndrome, amegakaryocytic thrombocytopenia, or pancytopenia with radioulnar synostosis disorders show overlapping morphological features with RCC and cannot be separated from RCC by histomorphology only. They have to be excluded by medical and family history, careful physical examination, and the appropriate laboratory/molecular studies before a definite diagnosis of RCC can be made. In a recent study Fanconi anemia is found in about 15 % of patients with a morphological picture

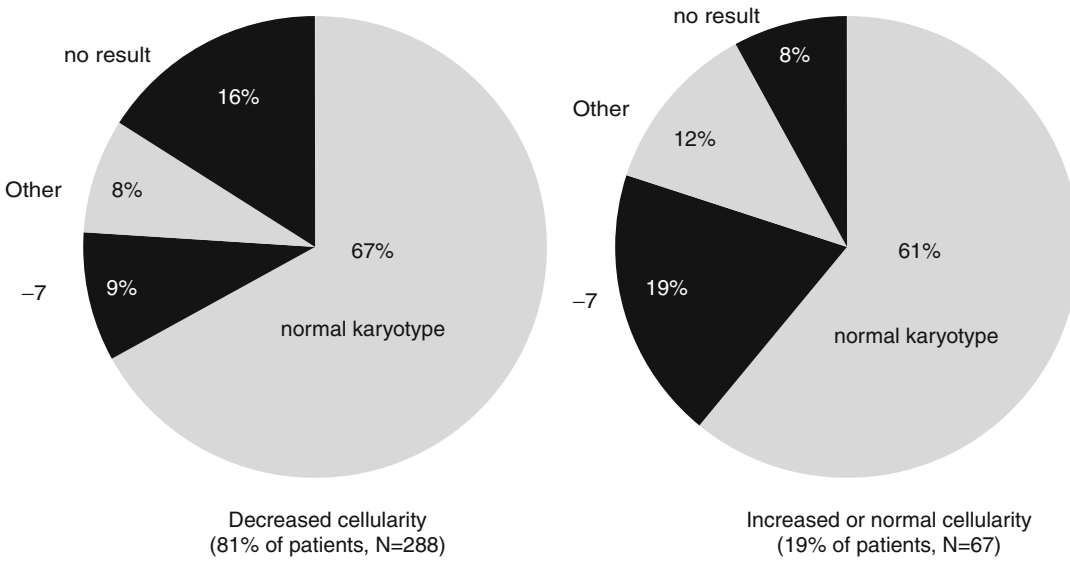

Fig. 14.1 Cellularity and karyotype in 355 children with refractory cytopenia of childhood. Results of study EWOG-MDS 98 and interim EWOG-MDS 2006 (date May 2011)

consistent with RCC; thus, Fanconi anemia needs to be excluded by a chromosomal breakage assay or other methods before a final diagnosis of RCC can be made (Yoshimi et al. 2013). In addition, dyskeratosis congenita (DC) can present with cytopenia preceding the appearance of the characteristic mucocutaneous triad of nail dystrophy, mucosal leukoplakia, and abnormal skin pigmentation or pulmonary symptoms. In different series of patients diagnosed of RCC or SAA, about 5–10 % of patients were found to have specific mutations in components of telomerase or the shelterin complex (Ortmann et al. 2006; Walne et al. 2008; Yamaguchi et al. 2005). Screening for telomere length of mononuclear cells is generally recommended to select patients for molecular analysis of the involved genes (Du et al. 2008).

Karyotype is the most important factor for the progression of RCC to advanced MDS (Kardos et al. 2003) (Fig. 14.2). The median time to progression for children with RCC and monosomy 7 is less than 2 years. In contrast to monosomy 7, patients with trisomy 8 and other karyotypes may experience a long stable course of their disease. Spontaneous disappearance of RCC with monosomy 7 has been reported in some infants but remains a rare event (Mantadakis et al. 1999; Parker et al. 2008).

HSCT from an HLA-compatible related or unrelated donor is the treatment of choice for patients with RCC and monosomy 7 early in the course of their disease (Anderson et al. 1996; Kardos et al. 2003). For children with a normal karyotype or chromosomal abnormalities other than monosomy 7 and absence of transfusion dependency or neutropenia, a watch-and-wait strategy can be appropriate. If cytopenia necessitates treatment, current therapy options include HSCT with either myeloablative or reduced intensity preparative therapies. The use of a reduced intensity conditioning regimen (RIC) represents an effective strategy to reduce the toxicity of transplantation (Strahm et al. 2007). In children with hypocellular RCC, normal karyotype, and a sibling or unrelated 9/10 or 10/10 HLA loci-matched donor, the overall survival following RIC transplantation is greater than 80 % (Strahm et al. 2007). Infections were the predominant complications.

Because early BM failure can at least in part be mediated by T-cell immunosuppression of hematopoiesis (de Vries et al. 2008), immunosuppressive therapy has been effectively applied in children with RCC and reduced cellularity (Hasegawa et al. 2009; Yoshimi et al. 2007). Response rates are, however, less favorable than

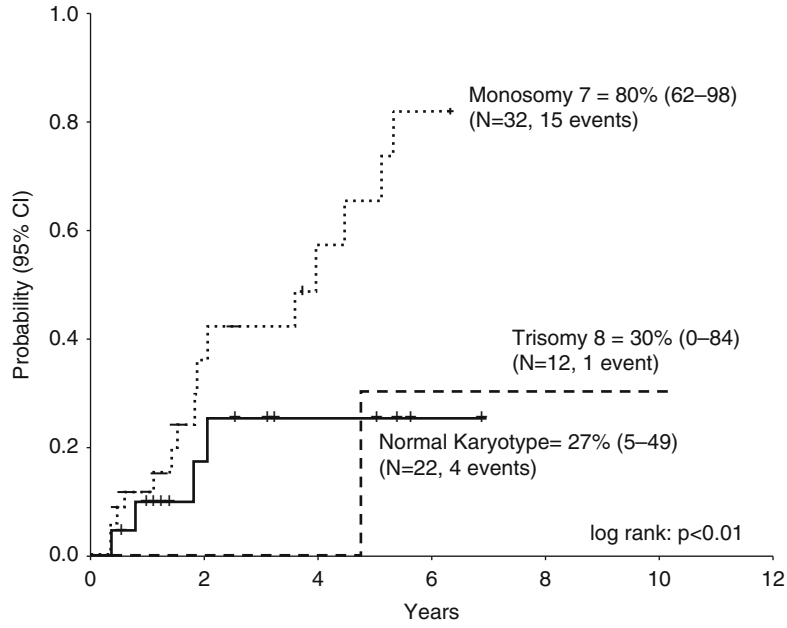

Fig. 14.2 Cumulative incidence of progression to advanced MDS for patients with refractory cytopenia and either monosomy 7, trisomy 8, or normal karyotype at the time of diagnosis. Patients who underwent stem cell transplantation were censored at the time of transplantation (Adapted from Kardos et al. 2003)

those observed in SAA (Yoshimi et al. 2007). Overall and failure-free survival rates at 5 years are in the order of 90 and 40 %, respectively (Yoshimi-Nöllke et al. 2011). Therapies like hematopoietic growth factors, differentiating agents, or hypomethylating agents are generally felt not to be indicated in children and adolescents with RCC, because none of these approaches has been shown to prolong survival.

14.5.2 Primary MDS with Increased Blasts (RAEB and RAEB-T)

There is consensus that the relationship between MDS and de novo AML is better defined by biological and clinical behavior than by blast count. Consequently, myeloid disease with low blast count and cytogenetic abnormalities typically associated with de novo AML is classified as AML. To allow for the interface between MDS and de novo AML, RAEB-T was retained in the pediatric classification (Baumann et al. 2008b; Hasle et al. 2003).

The therapy of patient with primary RAEB, RAEB-T, and AML evolving from MDS (MDR-AML) remains a major challenge. Therapy of these disorders has been associated with intensive therapy-related toxicities and a high risk of relapse. Conventional AML-type chemotherapy without HSCT resulted in survival rates below 30 % (Sasaki et al. 2001; Woods et al. 2002) and is inferior to outcome following HSCT (Kikuchi et al. 2012; Munoz et al. 2009; Strahm et al. 2011; Woodard et al. 2011). The European Working Group of MDS in Childhood (EWOG-MDS) recently reported on a cohort of 97 children with advanced MDS transplanted following a preparative regimen with busulfan, cyclophosphamide, and melphalan (Strahm et al. 2011). With a median follow-up of 3.9 years, the 5-year probability of overall survival was 63 %, while the 5-year cumulative incidence of transplantation-related mortality (TRM) and relapse was 21 % each. Outcomes are comparable for children with RAEB and RAEB-T, whereas patients with MDR-AML have an increased risk of relapse (Kikuchi et al. 2012; Strahm et al. 2011). While recent studies indicate a similar outcome following sibling or matched unrelated donor HSCT, transplantation from insufficiently matched unrelated donors are associated with a lower probability of survival (Strahm et al. 2011; Woodard et al. 2011). In the absence of a matched unrelated donor, umbilical cord blood (UCB) transplantation is an appropriate alternative and may result in outcomes similar

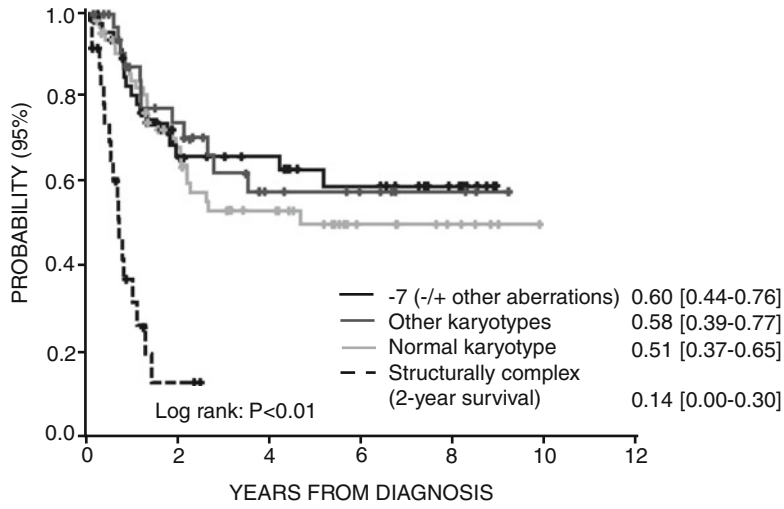

Fig. 14.3 Event-free survival of 192 children with advanced primary and secondary MDS according to karyotype (Adjusted from Gohring et al. 2010). Structurally complex karyotype is defined by at least three chromosomal aberrations, including at least one structural aberration, excluding those with clonal evolution of monosomy 7

to that observed for matched BM allografts if offered in centers experienced in this procedure (Madureira et al. 2011; Parikh et al. 2009).

In contrast to what has been reported in adult MDS (Deeg et al. 2012), monosomy 7 is not a high-risk feature in childhood MDS. The presence of a structurally complex karyotype is, however, the strongest predictor of a very unfavorable outcome (Gohring et al. 2010; Kikuchi et al. 2012; Strahm et al. 2011). The role of intensive chemotherapy prior to HSCT remains a matter of debate. While some investigators reported that AML-type induction therapy did not improve survival for patients with RAEB or RAEB-T (Strahm et al. 2011), others generally applied it prior to HSCT (Kikuchi et al. 2012). Like in adult MDS, novel antileukemic strategies including epigenetically active substances will have to be evaluated in well-controlled pediatric series prior to HSCT (Fig. 14.3).

14.6 Secondary MDS

14.6.1 MDS in Inherited Bone Marrow Failure Disorders

Inherited bone marrow failure disorders cannot be separated from RCC by histomorphology only. Consequently, progression to MDS in inherited bone marrow failure can be diagnosed only in the presence of increased marrow cellularity despite continuing cytopenia, evolving chromosomal abnormalities or increased blast percentage. Because abnormal clones can potentially regress, careful follow-up of children with inherited bone marrow failure and suspected MDS is recommended. Management of MDS in these children is largely dependent on the nature of the underlying genetic condition.

Fanconi anemia is the inherited bone marrow failure disorder most frequently followed by hematopoietic neoplasia. The cumulative probability of a Fanconi Anemia patient to develop MDS or AML is 30–40 % by the age of 40 years (Kutler et al. 2003). MDS in Fanconi anemia displays a specific pattern of chromosomal/molecular aberrations which includes 1q+, 3q+, −7/7q, 11q−, and *RUNX1* lesions (Quentin et al. 2011). Gain of chromosomal segment 3q26 is an adverse risk factor for leukemic progression (Tonnies et al. 2003), whereas a sole gain of chromosome 1q may persist for years without development of MDS or AML (Quentin et al. 2011). Changes to the approach to HSCT in Fanconi anemia have recently resulted in reduced regimen-related toxicity and improved survival. In the aplastic state or low-grade MDS, probability for survival following sibling or matched unrelated donor transplants is in the order of 75 and 30–70 %, respectively. For advanced MDS and AML, data are more spaced but indicate that

long-term survival is possible and patients should be candidates of HSCT (MacMillan and Wagner 2010).

About one-third of patients with Shwachman-Diamond syndrome (Alter et al. 2009) may eventually develop MDS by the age of 39 years. Patients with isochromosome 7q [i(7q)] or del(20q) can experience a long stable clinical course. The i(7q) may be related to the SDS gene located on the centromeric region of chromosome 7, because there is a strikingly increased incidence of i(7q) in this disorder.

For patients with severe congenital neutropenia, Rosenberg et al. (2010) reported a cumulative incidence of MDS/AML of 22 % in patients enrolled in the Severe Chronic Neutropenia International Registry (Rosenberg et al. 2010). In a population-based study, the cumulative incidence of myeloid neoplasia was 31 % (Carlsson et al. 2012). Concerns that G-CSF may promote malignant clones had been raised; however, it later appeared that the rate of MDS/AML in severe congenital neutropenia is qualitatively similar to that in Fanconi anemia and dyskeratosis congenita (Rosenberg et al. 2010). In contrast, myeloid neoplasia in Diamond-Blackfan anemia is considerably less common (Vlachos et al. 2012).

14.6.2 MDS After Acquired Aplastic Anemia

MDS develops in 10–15 % of those children with aplastic anemia not treated with HSCT (Kojima et al. 2002; Locasciulli et al. 2001; Tichelli et al. 1988). Most cases of MDS in children have been diagnosed within the first 3 years from presentation with aplastic anemia. The fast progression to MDS raises the question whether at least some of the patients had MDS from the beginning. One might also speculate that there is a biological overlap between aplastic anemia and hypoplastic RCC.

14.6.3 Familial MDS

Rare cases of familial occurrence of MDS, especially with −7/7q−, had been reported in a number of previous case series (Hasle and Olsen 1997; Shannon et al. 1989). Most recently heterozygous germ line mutations in RUNX1 (Song et al. 1999), CEBPA (Smith et al. 2004), and GATA-2 (Hahn et al. 2011) have been found in these cases of familial MDS. It is currently unknown how many cases of primary non-familial MDS in children and adolescents are due to these germ line mutations.

14.6.4 Therapy-Related MDS

New intensive treatment protocols may lead to an increased risk of therapy-related diseases (Aguilera et al. 2009; Barnard et al. 2002; Barnard and Woods 2005). Children with MDS secondary to chemo- or radiation therapy generally have a poor survival (Sasaki et al. 2000). Even if remission can be achieved with AML-type therapy, only very few patients remain disease-free and only HSCT offer cure in about a third of the patients (Woodard et al. 2006).

14.7 Summary

The WHO classification of MDS in children and adolescents facilitates studies on the underlying biological processes. In patients with refractory cytopenia of childhood, a watch-and-wait strategy or immunosuppressive therapy can be appropriate in the absence of monosomy 7. In all other cases, HSCT is the treatment of choice. With the possibility to cure at least half of the children with MDS, there is very little room for palliative therapy approaches.

References

Aguilera DG, Vaklavas C, Tsimberidou AM, Wen S, Medeiros LJ, Corey SJ (2009) Pediatric therapy-related myelodysplastic syndrome/acute myeloid leukemia: the MD Anderson Cancer Center experience. J Pediatr Hematol Oncol 31:803–811

Alter BP, Giri N, Savage SA, Rosenberg PS (2009) Cancer in dyskeratosis congenita. Blood 113:6549–6557

Anderson JE, Appelbaum FR, Schoch G, Gooley T, Anasetti C, Bensinger WI, Bryant E, Buckner CD, Chauncey TR, Clift RA, Doney K, Flowers M, Hansen JA, Martin PJ, Matthews DC, Sanders JE, Shulman H, Sullivan KM, Witherspoon RP, Storb R (1996) Allogeneic marrow transplantation for refractory anemia: a comparison of two preparative regimens and analysis of prognostic factors. Blood 87: 51–58

Barnard DR, Woods WG (2005) Treatment-related myelodysplastic syndrome/acute myeloid leukemia in survivors of childhood cancer – an update. Leuk Lymphoma 46:651–663

Barnard DR, Lange B, Alonzo TA, Buckley J, Kobrinsky JN, Gold S, Neudorf S, Sanders J, Burden L, Woods WG (2002) Acute myeloid leukemia and myelodysplastic syndrome in children treated for cancer: comparison with primary presentation. Blood 100:427–434

Baumann I, Niemeyer CM, Brunning RD, Arber DA, Porwit A (2008a) Myeloid proliferations related to Down syndrome. In: WHO classification of tumours of haematopoietic and lymphoid tissues. International Agency for Research on Cancer (IARC), Lyon, pp 142–144

Baumann I, Niemeyer CM, Benett J, Shannon K (2008b) Childhood myelodysplastic syndrome. In: WHO classification of tumours of haematopoietic and lymphoid tissues. International Agency for Research on Cancer (IARC), Lyon, pp 104–107

Baumann I, Fuhrer M, Behrendt S, Campr V, Csomor J, Furlan I, de Haas V, Kerndrup G, Leguit RJ, De Paepe P, Noellke P, Niemeyer C, Schwarz S (2012) Morphological differentiation of severe aplastic anaemia from hypocellular refractory cytopenia of childhood: reproducibility of histopathological diagnostic criteria. Histopathology 61:10–17

Carlsson G, Fasth A, Berglof E, Lagerstedt-Robinson K, Nordenskjold M, Palmblad J, Henter JI, Fadeel B (2012) Incidence of severe congenital neutropenia in Sweden and risk of evolution to myelodysplastic syndrome/leukaemia. Br J Haematol 158:363–369

de Vries AC, Langerak AW, Verhaaf B, Niemeyer CM, Stary J, Schmiegelow K, van Wering ER, Zwaan CM, Beishuizen A, Pieters R, van den Heuvel-Eibrink MM (2008) T-cell receptor Vbeta CDR3 oligoclonality frequently occurs in childhood refractory cytopenia (MDS-RC) and severe aplastic anemia. Leukemia 22:1170–1174

de Vries ACH, Zwaan CM, Jansen JH, Dworzak M, Hasle H, Locatelli F, De Moerloose B, Arentsen-Peters TJCM, de Haas V, Polychronopoulou S, Stary J, Schmugge-Liner M, Zecca M, Smith O, Català A, Schlegelberger B, Beverloo HB, Pieters R, Niemeyer CM, van den Heuvel-Eibrink MM, and on EWOG-MDS (2012) Molecular aberrations in 107 children with myelodysplastic syndrome (MDS). Haematologica 9:S16

Deeg HJ, Scott BL, Fang M, Shulman HM, Gyurkocza B, Myerson D, Pagel JM, Platzbecker U, Ramakrishnan A, Radich JP, Sandmaier BM, Sorror M, Stirewalt DL, Wilson WA, Storb R, Appelbaum FR, Gooley T (2012) Five-group cytogenetic risk classification, monosomal karyotype, and outcome after hematopoietic cell transplantation for MDS or acute leukemia evolving from MDS. Blood 120:1398–1408

Du HY, Pumbo E, Manley P, Field JJ, Bayliss SJ, Wilson DB, Mason PJ, Bessler M (2008) Complex inheritance pattern of dyskeratosis congenita in two families with 2 different mutations in the telomerase reverse transcriptase gene. Blood 111:1128–1130

Gohring G, Michalova K, Beverloo HB, Betts D, Harbott J, Haas OA, Kerndrup G, Sainati L, Bergstraesser E, Hasle H, Stary J, Trebo M, van den Heuvel-Eibrink MM, Zecca M, van Wering ER, Fischer A, Noellke P, Strahm B, Locatelli F, Niemeyer CM, Schlegelberger B (2010) Complex karyotype newly defined: the strongest prognostic factor in advanced childhood myelodysplastic syndrome. Blood 116:3766–3769

Groupe Francais de Cytogenetique Hematologique (1997) Forty-four cases of childhood myelodysplasia with cytogenetics, documented by the Groupe Francais de Cytogenetique Hematologique. Leukemia 11: 1478–1485

Hahn CN, Chong CE, Carmichael CL, Wilkins EJ, Brautigan PJ, Li XC, Babic M, Lin M, Carmagnac A, Lee YK, Kok CH, Gagliardi L, Friend KL, Ekert PG, Butcher CM, Brown AL, Lewis ID, To LB, Timms AE, Storek J, Moore S, Altree M, Escher R, Bardy PG, Suthers GK, D'Andrea RJ, Horwitz MS, Scott HS (2011) Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. Nat Genet 43:1012–1017

Hasegawa D, Manabe A, Yagasaki H, Ohtsuka Y, Inoue M, Kikuchi A, Ohara A, Tsuchida M, Kojima S, Nakahata T (2009) Treatment of children with refractory anemia: The Japanese Childhood MDS Study Group trial (MDS99). Pediatr Blood Cancer 53:1011–1015

Hasle H, Olsen JH (1997) Cancer in relatives of children with myelodysplastic syndrome, acute and chronic myeloid leukaemia. Br J Haematol 97:127–131

Hasle H, Kerndrup G, Jacobsen BB (1995) Childhood myelodysplastic syndrome in Denmark: incidence and predisposing conditions. Leukemia 9:1569–1572

Hasle H, Arico M, Basso G, Biondi A, Cantù-Rajnoldi A, Creutzig U, Fenu S, Fonatsch C, Haas OA, Harbott J, Kardos G, Kerndrup G, Mann G, Niemeyer CM, Ptoszkova H, Ritter J, Slater R, Stary J, Stollmann B, Testi A, van Wering ER, Zimmermann M (1999a) Myelodysplastic syndrome and acute myeloid leukemia associated with complete or partial monosomy 7. Leukemia 13:376–385

Hasle H, Wadsworth LD, Massing BG, McBride M, Schultz KR (1999b) A population-based study of childhood myelodysplastic syndrome in British Columbia, Canada. Br J Haematol 106:1027–1032

Hasle H, Niemeyer CM, Chessells JM, Baumann I, Bennett JM, Kerndrup G, Head DR (2003) A pediatric approach to the WHO classification of myelodysplas-

tic and myeloproliferative diseases. Leukemia 17:277–282

Hirabayashi S, Flotho C, Moetter J, Heuser M, Hasle H, Gruhn B, Klingebiel T, Thol F, Schlegelberger B, Baumann I, Strahm B, Stary J, Locatelli F, Zecca M, Bergstraesser E, Dworzak M, van den Heuvel-Eibrink MM, De Moerloose B, Ogawa S, Niemeyer CM, Wlodarski MW (2012) Spliceosomal gene aberrations are rare, coexist with oncogenic mutations, and are unlikely to exert a driver effect in childhood MDS and JMML. Blood 119:e96–e99

Kardos G, Baumann I, Passmore SJ, Locatelli F, Hasle H, Schultz KR, Stary J, Schmitt-Graeff A, Fischer A, Harbott J, Chessells JM, Hann I, Fenu S, Rajnoldi AC, Kerndrup G, van Wering E, Rogge T, Nollke P, Niemeyer CM (2003) Refractory anemia in childhood: a retrospective analysis of 67 patients with particular reference to monosomy 7. Blood 102: 1997–2003

Ketterling RP, Wyatt WA, VanWier SA, Law M, Hodnefield JM, Hanson CA, Dewald GW (2002) Primary myelodysplastic syndrome with normal cytogenetics: utility of 'FISH panel testing' and M-FISH. Leuk Res 26:235–240

Kikuchi A, Hasegawa D, Ohtsuka Y, Hamamoto K, Kojima S, Okamura J, Nakahata T, Manabe A (2012) Outcome of children with refractory anaemia with excess of blast (RAEB) and RAEB in transformation (RAEB-T) in the Japanese MDS99 study. Br J Haematol 158:657–661

Kojima S, Ohara A, Tsuchida M, Kudoh T, Hanada R, Okimoto Y, Kaneko T, Takano T, Ikuta K, Tsukimoto I (2002) Risk factors for evolution of acquired aplastic anemia into myelodysplastic syndrome and acute myeloid leukemia after immunosuppressive therapy in children. Blood 100:786–790

Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF, Hanenberg H, Auerbach AD (2003) A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood 101:1249–1256

Locasciulli A, Arcese W, Locatelli F, Di Bona E, Bacigalupo A (2001) Treatment of aplastic anaemia with granulocyte-colony stimulating factor and risk of malignancy. Italian Aplastic Anaemia Study Group. Lancet 357:43–44

Luna-Fineman S, Shannon KM, Atwater SK, Davis J, Masterson M, Ortega J, Sanders J, Steinherz P, Weinberg V, Lange BJ (1999) Myelodysplastic and myeloproliferative disorders of childhood: a study of 167 patients. Blood 93:459–466

MacMillan ML, Wagner JE (2010) Haematopoietic cell transplantation for Fanconi anaemia – when and how? Br J Haematol 149:14–21

Madureira AB, Eapen M, Locatelli F, Teira P, Zhang MJ, Davies SM, Picardi A, Woolfrey A, Chan KW, Socie G, Vora A, Bertrand Y, Sales-Bonfim CM, Gluckman E, Niemeyer C, Rocha V (2011) Analysis of risk factors influencing outcome in children with myelodysplastic syndrome after unrelated cord blood transplantation. Leukemia 25:449–454

Mantadakis E, Shannon KM, Singer DA, Finklestein J, Chan KW, Hilden JM, Sandler ES (1999) Transient monosomy 7: a case series in children and review of the literature. Cancer 85:2655–2661

Munoz A, Diaz-Heredia C, Badell I, Bureo E, Gomez P, Martinez A, Verdeguer A, Perez-Hurtado JM, Fernandez-Delgado R, Gonzalez-Vicent M, Maldonado MS (2009) Allogeneic stem cell transplantation for myelodysplastic syndromes in children: a report from the Spanish Working Party for Blood and Marrow Transplantation in Children (GETMON). Pediatr Hematol Oncol 26:345–355

Ortmann CA, Niemeyer CM, Wawer A, Ebell W, Baumann I, Kratz CP (2006) TERC mutations in children with refractory cytopenia. Haematologica 91:707–708

Parikh SH, Mendizabal A, Martin PL, Prasad VK, Szabolcs P, Driscoll TA, Kurtzberg J (2009) Unrelated donor umbilical cord blood transplantation in pediatric myelodysplastic syndrome: a single-center experience. Biol Blood Marrow Transplant 15:948–955

Parker TM, Klaassen RJ, Johnston DL (2008) Spontaneous remission of myelodysplastic syndrome with monosomy 7 in a young boy. Cancer Genet Cytogenet 182:122–125

Passmore SJ, Chessells JM, Kempski H, Hann IM, Brownbill PA, Stiller CA (2003) Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia in the UK: a population-based study of incidence and survival. Br J Haematol 121:758–767

Quentin S, Cuccuini W, Ceccaldi R, Nibourel O, Pondarre C, Pages MP, Vasquez N, Dubois d'Enghien C, Larghero J, Peffault de Latour L, Rocha V, Dalle JH, Schneider P, Michallet M, Michel G, Baruchel A, Sigaux F, Gluckman E, Leblanc T, Stoppa-Lyonnet D, Preudhomme C, Socie G, Soulier J (2011) Myelodysplasia and leukemia of Fanconi anemia are associated with a specific pattern of genomic abnormalities that includes cryptic RUNX1/AML1 lesions. Blood 117:e161–e170

Rosenberg PS, Zeidler C, Bolyard AA, Alter BP, Bonilla MA, Boxer LA, Dror Y, Kinsey S, Link DC, Newburger PE, Shimamura A, Welte K, Dale DC (2010) Stable long-term risk of leukaemia in patients with severe congenital neutropenia maintained on G-CSF therapy. Br J Haematol 150:196–199

Sasaki H, Manabe A, Kojima S, Tsuchida M, Hayashi Y, Ikuta K, Okamura J, Koike K, Ohara A, Ishii E, Komada Y, Hibi S, Nakahata T (2000) Myelodysplastic syndromes (MDS) in childhood: a retrospective study in Japan. Leukemia 14:968

Sasaki H, Manabe A, Kojima S, Tsuchida M, Hayashi Y, Ikuta K, Okamura I, Koike K, Ohara A, Ishii E, Komada Y, Hibi S, Nakahata T (2001) Myelodysplastic syndrome in childhood: a retrospective study of 189 patients in Japan. Leukemia 15:1713–1720

Shannon KM, Turhan A, Chang SS, Bowcock AM, Rogers PC, Carroll WL, Cowan MJ, Glader BE, Eaves CJ, Eaves AC (1989) Familial bone marrow monosomy 7: evidence that the predisposing locus is not on the long arm of chromosome 7. J Clin Invest 84:984–989

Smith ML, Cavenagh JD, Lister TA, Fitzgibbon J (2004) Mutation of CEBPA in familial acute myeloid leukemia. N Engl J Med 351:2403–2407

Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, Ratajczak J, Resende IC, Haworth C, Hock R, Loh M, Felix C, Roy DC, Busque L, Kurnit D, Willman C, Gewirtz AM, Speck NA, Bushweller JH, Li FP, Gardiner K, Poncz M, Maris JM, Gilliland DG (1999) Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat Genet 23:166–175

Strahm B, Locatelli F, Bader P, Ehlert K, Kremens B, Zintl F, Fuhrer M, Stachel D, Sykora KW, Sedlacek P, Baumann I, Niemeyer CM (2007) Reduced intensity conditioning in unrelated donor transplantation for refractory cytopenia in childhood. Bone Marrow Transplant 40:329–333

Strahm B, Nollke P, Zecca M, Korthof ET, Bierings M, Furlan I, Sedlacek P, Chybicka A, Schmugge M, Bordon V, Peters C, O'Marcaigh A, de Heredia CD, Bergstraesser E, Moerloose BD, van den Heuvel-Eibrink MM, Stary J, Trebo M, Wojcik D, Niemeyer CM, Locatelli F (2011) Hematopoietic stem cell transplantation for advanced myelodysplastic syndrome in children: results of the EWOG-MDS 98 study. Leukemia 25:455–462

Tichelli A, Gratwohl A, Wursch A, Nissen C, Speck B (1988) Late haematological complications in severe aplastic anaemia. Br J Haematol 69:413–418

Tonnies H, Huber S, Kuhl JS, Gerlach A, Ebell W, Neitzel H (2003) Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients: gains of the chromosomal segment 3q26q29 as an adverse risk factor. Blood 101:3872–3874

Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM (2012) Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. Blood 119:3815–3819

Walne AJ, Vulliamy T, Beswick R, Kirwan M, Dokal I (2008) TINF2 mutations result in very short telomeres: analysis of a large cohort of patients with dyskeratosis congenita and related bone marrow failure syndromes. Blood 112:3594–3600

Woodard P, Barfield R, Hale G, Horwitz E, Leung W, Ribeiro R, Rubnitz J, Srivistava DK, Tong X, Yusuf U, Raimondi S, Pui CH, Handgretinger R, Cunningham JM (2006) Outcome of hematopoietic stem cell transplantation for pediatric patients with therapy-related acute myeloid leukemia or myelodysplastic syndrome. Pediatr Blood Cancer 47:931–935

Woodard P, Carpenter PA, Davies SM, Gross TG, He W, Zhang MJ, Horn BN, Margolis DA, Perentesis JP, Sanders JE, Schultz KR, Seber A, Woods WG, Eapen M (2011) Unrelated donor bone marrow transplantation for myelodysplastic syndrome in children. Biol Blood Marrow Transplant 17:723–728

Woods WG, Barnard DR, Alonzo TA, Buckley JD, Kobrinsky N, Arthur DC, Sanders J, Neudorf S, Gold S, Lange BJ (2002) Prospective study of 90 children requiring treatment for juvenile myelomonocytic leukemia or myelodysplastic syndrome: a report from the Children's Cancer Group. J Clin Oncol 20: 434–440

Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, Lansdorp PM, Young NS (2005) Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. N Engl J Med 352:1413–1424

Yoshimi A, Baumann I, Fuhrer M, Bergstrasser E, Gobel U, Sykora KW, Klingebiel T, Gross-Wieltsch U, van den Heuvel-Eibrink MM, Fischer A, Nollke P, Niemeyer C (2007) Immunosuppressive therapy with anti-thymocyte globulin and cyclosporine A in selected children with hypoplastic refractory cytopenia. Haematologica 92:397–400

Yoshimi A, Niemeyer C, Baumann I, Schwarz-Furlan S, Schindler D, Ebell W, Strahm B (2013) High incidence of Fanconi anaemia in patients with a morphological picture consistent with refractory cytopenia of childhood. Br J Haematol 160(1):109–111

Yoshimi-Nöllke A, van den Heuvel-Eibrink MM, Baumann I, Niemeyer CM (2011) Immunosuppressive therapy with anti-thymocyte globulin and cyclosporine A in patients with refractory cytopenia in childhood. Bone Marrow Transplant 46:S369

Index

H.J. Deeg et al., *Myelodysplastic Syndromes*, Hematologic Malignancies,
DOI 10.1007/978-3-642-36229-3, © Springer-Verlag Berlin Heidelberg 2013