Molecular Pathogenesis of Myelodysplastic Syndromes

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Abstract - Myelodysplastic syndromes (MDS) are a group of clonal hematologic disorders characterized by inefficient hematopoiesis, hypercellular bone marrow, dysplasia of blood cells and cytopenias. Most patients are diagnosed in their late 60s to early 70s. MDS is a risk factor for the development of acute myeloid leukemia which can occur in 10-15% of patients with MDS. A variety of pathophysiologic mechanisms contributes to the genesis and persistence of MDS including immunologic, epigenetic, cytogenetic and genetic factors. The only potential curative option for MDS is hematopoietic cell transplantation which is suitable for only a few patients. Currently approved therapeutic options for MDS, including lenalidomide, decitabine, and 5-azacytidine, are targeted to improve transfusion requirements and quality of life. Moreover, 5-azacytidine has also been demonstrated to improve survival in some patients with higher risk MDS. New ways to predict which patients will better gain benefit from currently available therapeutic agents are the primary challenges in MDS. In the last 10 years, chromosome scanning and high throughput technologies (single nucleotide polymorphism array genotyping, comparative genomic hybridization, and whole genome/ exome sequencing) have tremendously increased our knowledge of MDS pathogenesis. Indeed, the molecular heterogeneity of MDS supports the idea of different therapeutic approaches which will take into account the diverse morphologic and clinical presentations of MDS patients rather than a restricted therapeutic strategy. This review will summarize the molecular abnormalities in key relevant components of the biology and pathogenesis of MDS and will provide an update on the clinical impact and therapeutic response in MDS patients.

Keywords: MDS, pathogenesis, molecular mutations

I. INTRODUCTION OVERVIEW OF MYELODYSPLASTIC SYNDROMES

Myelodysplastic syndromes (MDS) are a group of clonal myeloid disorders which are morphologically characterized by bone marrow (BM) hypercellularity, unilineage dysplasia, and peripheral blood cytopenias. The incidence rate of MDS in the United States for the years 2003-2007 has been estimated at 4.3 per 100,000 people which accounts for 15,000 new cases every year [1,2]. Statistics on incidence rates might be higher due to misdiagnosis of cases with hypocellular BM or misinterpretation of the anemia observed in MDS as a normal condition in the elderly. It is a disease more frequently diagnosed in men with the exception of MDS with 5q- syndrome, which is slightly more frequent in women. Chromosomal defects are usually clonal and recurrent. They are observed in approximately 50% of primary and 80% of cases of therapy-related MDS (t-MDS) [3,4]. The most commonly affected chromosomes are 5, 6, 7, 8, 11, 13 and 20 and the most recurrent chromosomal lesions are partial deletions (5, 7, 11, 13, and 20) and additional copies (6 and 8). Unbalanced translocations are also sometimes seen. Single chromosomal alterations are usually detected in de novo MDS while a complex karyotype, defined as having ≥3 chromosomal abnormalities is usually observed in MDS patients with antecedent exposure to treatments such as chemotherapy or radiotherapy or to toxic chemicals. The latter condition is included in the MDS category called secondary MDS (sMDS) which includes MDS that evolved from a prior hematologic disorder. The impact of having multiple chromosomal anomalies has also been investigated and correlated with unfavorable outcome in patients with sMDS. Cytogenetics has a crucial impact on the outcome of patients with MDS and acute myeloid leukemia (AML) undergoing hematopoietic stem cell transplantation [5]. The accumulation of genetic defects in addition to the primary molecular mutation affecting a stem cell increases the propensity to develop more aggressive diseases like AML. The importance of cytogenetic abnormalities in MDS is also due to the fact that chromosomal regions often contain genes relevant to MDS biology and pathophysiology. Indeed, the presence of chromosomal abnormalities like deletions suggests that haploinsufficiency and/ or loss of tumor suppressor genes (TSGs) are mechanisms important in MDS biology.

Conventional Sanger sequencing, high resolution whole genome scanning technologies (array-based comparative genomic hybridization (aCGH) and single nucleotide polymorphism arrays (SNP-A) genotyping) and high-throughput sequencing technologies (whole genome/ exome sequencing) have brought to light molecular alterations in genes of numerous pathways including methylation, transcriptional factors, signal transduction, histone regulators, and the RNA splicing machinery [6]. The diversity of these lesions and their combinations may reflect the heterogeneity in the morphologic presentations, clinical courses, and potentially response to therapeutic agents. Ultimately, the finding of genes relevant to MDS...
pathophysiology also opens the possibility of targeted therapy.

II. LOW AND HIGH RESOLUTION TECHNOLOGIES

The World Health Organization (WHO) has classified MDS into several distinct disease groups differentiated from others by BM and peripheral blood (PB) morphology (blasts, ring sideroblasts, dysplasia, cytopenias) and cytogenetic changes. Different risk stratification scoring systems have been created and revised for de

novod MDS. Metaphase cytogenetics (MC) still serves as an important weighted parameter in the risk stratification assessment (Figure 1). Classically, MC is the gold standard in the determination of cytogenetic abnormalities in myeloid cancers. The advantages of MC include the simplicity of the method, the detection of unbalanced/balanced chromosomal defects, and the feasibility in discriminating single cellular clones. However, MC reaches a sensitivity of only 10% and it is informative in only 46-59% of patients with MDS due to the limitation in achieving results when cells are unable to grow or in case of non-informative karyotypes [7,8]. Further, even if one-half of MDS patients have a normal karyotype by MC, this does not necessarily exclude the presence of latent genetic defects not clinically dominant which escape detection due to the low resolution of MC.

A combination of chromosome painting and multi-color fluorescence called spectral karyotyping (SKY) has complemented the photographic images of condensed chromosomes by G-banding and fluorescence in situ hybridization (FISH) for specific chromosome (5, 7, and 20) and improved the detection of chromosomal rearrangements and minimal monosomies leading to a better picture of the karyotype [9].

Advances in high resolution genome scanning technologies like aCGH and SNP-A have improved the detection rate of genomic lesions in MDS and other related disorders and have clarified many aspects of MDS biology [10]. aCGH is a technology that identifies differences in copy number between DNA of patients and

![Microscopy, Immunohistochemistry, Cytogenetics, Flow cytometry](image)

![Figure 1. Schema of the diagnostic tests, classification systems, and prognostic scoring systems in Myelodysplastic syndromes. Microscopy, immunohistochemistry, G-banding, and sometimes flow cytometry are all useful diagnostic tests in Myelodysplastic syndromes (MDS). Prognostic markers include: bone marrow and peripheral blood morphology, numbers of blasts, morphologic features (ring sideroblasts), dysplasia, cytopenias and cytogenetic changes. Classification systems include (FAB, 2002 and 2008 WHO). Risk stratification scoring systems have been created for de novo MDS with and without account for cytogenetic findings.](image)

**Abbreviations.** BM, bone marrow; LDH, lactate dehydrogenase; ALIP, abnormal localization of immature precursors; MC, metaphase cytogenetics; FAB, French American British Classification; IPSS, International prognostic scoring system; WPSS, WHO classification-based Prognostic Scoring System; IPSS-R: International prognostic scoring system-Revised.
healthy subjects on the basis of fluorescent hybridization signals. SNP-A has combined two techniques commonly used in the DNA microarray technologies, DNA hybridization and fluorescence. The use of SNP-A has overcome a limitation of the aCGH technology and provides the ability to detect a cryptic cytogenetic defect called acquired somatic uniparental disomy (AS-UPD) which is recurrent in 20% of patients with MDS. Several studies have reported the clinical importance of both aCGH and SNP-A on the survival settings of MDS [11-14]. However, efforts have been made towards the combination of all these approaches with MC and several multicenter studies have highlighted the worse survival outcomes of patients with MDS and other disorders carrying defects detected by MC/ SNP-A in addition to several other studies where the number of defects by SNP-A predict worse outcomes [15-18].

More recently, high-throughput sequencing, such as whole genome/ exome and deep sequencing, has been instrumental in discovering germ-line and somatic variants in a number of solid tumors and blood cancers including MDS. These technologies are able to generate a comprehensive sequence database of an individual (whole genome), a targeted genome re-sequencing which allows for the generation of specific sequences (whole exome) or a sequence of the entire genome with higher “depth” indicating that the process of the sequencing is performed more than one time for any one region of the genome (deep sequencing). This latest approach has markedly improved the accuracy of sequencing, reducing the error output.

III. BIOLOGICAL AND CLINICAL IMPACT OF MOLECULAR MARKERS IN MDS

More than a decade ago, cytogenetic defects were the foremost determinant of MDS biology. The advancement in high resolution genomic technologies helped in the identification of regions of the genome highly suspected to contain molecular mutations. This was the case for the original discoveries of TET2, EZH2, CBL, IDH1/ IDH2, ASXL1, DNMT3A, UTX, TP53, SF3B1, U2AF1, and SETBP1 (Table 1).

We will discuss the role of several of these genes and pathways for which the biological and clinical impact has been studied and associated with MDS pathophysiology.

IV. COMMONLY AFFECTED PATHWAYS:
METHYLATION PATHWAY: TET2, DNMT3A, IDH1/2

Aberrant DNA methylation has been observed in MDS. Methylation of promoters at CpG loci is one of the mechanisms that can regulate and silence TSGs. The identification of distinct methylation patterns, hypermethylation of key genes important in differentiation, and the success of using DNA-methyltransferase inhibitors (5-aza-2’-deoxycytidine and 5-azacytidine) in some MDS patients provides the rationale supporting the key role of methylation as a crucial regulatory mechanism in MDS. Indeed, several studies have demonstrated that the increase in aberrant DNA methylation and chromosomal anomalies is directly proportional to the malignant transition of MDS to AML [19,20].

| Gene Name | Frequency MDS | Frequency MDS/ MPN | Frequency AML |
|-----------|---------------|--------------------|---------------|
| TET2*     | 12–14         | 37–46              | 9–43          |
| IDH1/2*   | 1–5           | 9                  | 5–10          |
| DNMT3A*   | 8             | 12                 | 25            |
| ASXL1*    | 21            | 17–46              | 17            |
| EZH2      | 2             | 4–12               | 1             |
| CBL       | 1–2           | 5                  | 9             |
| SF3B1*    | 4–10          | 1–5                | 6             |
| U2AF1*    | 6–12          | 8–17               | 1–10          |
| SF3B2*    | 6–12          | 28–47              | 1–7           |
| TP53*     | 8             | 7                  | 56–78         |
| NRAS/KRAS | 4/1           | 7/4                | 9–40/5–17     |
| RUNX1*    | 9             | 13                 | 13            |
| SETBP1*   | 4–1           | 9–44               | 0.9–9.1*      |

*Indicates the genes discussed in the text. †68% in refractory anemia with ring sideroblasts. ‡81% on refractory anemia with ring sideroblasts associated with thrombocytosis. §frequency for de novo and secondary AML, respectively.

Abbreviations. MDS, Myelodysplastic syndromes; MDS/ MPN, Myelodysplastic syndromes/ Myeloproliferative neoplasms; AML, Acute myeloid leukemia.

Genes that act as regulators of the DNA methylation have been found to be frequently mutated in MDS. SNP-A identified a region of loss of heterozygosity on chromosome 4q24 which contains a gene called TET2 which is frequently mutated in MDS. TET2 was mutated in 20-25% of MDS patients [21,22]. TET2 is a dioxygenase that catalyzes the conversion of the modified base 5-methylcytosine (5-MC) to 5-hydroxymethylcytosine (5-hMC) by oxidizing 5-MC. The conversion of 5-MC to 5hMC represents the first step in cytosine demethylation. Indeed, methylation at the C5 position of cytosine appears to be an epigenetic modification which plays an important role in transcriptional regulation. TET2 mutations are restricted to the C-terminus of the protein, resulting in loss of function which correlates with a decrease of 5-hMC levels and an increase of 5-MC leading to DNA hypermethylation and gene silencing [23,24]. Even though TET2 mutations are correlated with low 5-hMC levels in myeloid disorders, a fraction of patients with wild-type TET2 also have low 5-hMC levels. A recent report correlated a low level of 5-hMC to the over-expression of the ancestral CXXC domain of TET2 which encodes for a specific gene called IDAX [25]. In addition, several reports have described that
low 5-hmC levels are associated with DNA hypomethylation in patients with myeloid malignancies while others have described DNA hypermethylation in AML [23]. A mouse model of TET2 loss of function shows increased proliferation of myeloid cells without manifesting any dysplastic changes resembling MDS [26]. The clinical impact of TET2 mutations has been widely studied by many groups and somehow is still under debate. (Table 2) TET2 mutations do not seem to predict an increased risk for transformation to AML. Most studies looking at the prognostic value of TET2 in MDS did not show a difference in survival outcomes between mutant and wild type cases. The efficacy and effectiveness of hypomethylating therapy in specific subsets of MDS patients and the presence of molecular mutations in genes relevant to methylation also justifies the efforts in genotyping large cohorts of patients with MDS receiving hypomethylating drugs for these specific molecular changes. Itzykson and colleagues showed that high-risk MDS patients carrying TET2 mutations have better response rate to 5-azacytidine without any improvement in OS [27]. We demonstrated in a cohort of 92 MDS patients that the presence of TET2 and DNMT3A mutations confers better response to both azacitidine and decitabine [28].

Whole genome sequencing was instrumental in the identification of a somatic mutation in a DNA methyltransferase gene called DNMT3A located on chromosome 2 p23.3. DNMT3A catalyzes the addition of a methyl group to the cytosine of the CpG dinucleotides. The amino terminus of the human DNMT3A gene contains various motifs that recruit transcriptional repressors. The role of DNMT3A is extremely important since this enzyme acts as a check-point of DNA replication by keeping de novo methylation mechanisms partially methylated. The methylation of the CpG islands induces repression of downstream genes. The first genetic alteration identified in DNMT3A was a frameshift mutation found in a patient with de novo AML [29]. DNMT3A has been found to be mutated in 8% of MDS cases [30,31]. The interplay between TET2 and DNMT3A has captured the attention of many groups, especially for their role in altering the cytosine methylation and for the fact that current treatments for MDS heavily rely on hypomethylating therapy. In vitro murine embryonic stem cells deficient for dnmt3a have a competitive advantage compared to cells expressing normal DNMT3A, indicating that DNMT3A mutations might be clonally dominant [32]. The role of DNMT3A in somatic cells is still unknown. Data from mouse models has also suggested a role for DNMT3A in epigenetic silencing. Hematopoietic stem cells deficient for DNMT3A show differential methylation and up-regulation of multipotency genes. The clinical impact of DNMT3A has been correlated with worse OS [33]. Moreover, MDS is usually associated with a hypermethylation and DNMT-inhibitors are associated with increased survival in MDS.

In 2008, parallel massive sequencing identified mutations in the isocitrate dehydrogenase 1 (IDH1) gene in gliomas. IDH1 mutations alter the catalytic activity of the enzyme producing a toxic oncometabolite called R-2-hydroxylutarate which affecting the redox state of the cells, blocking the production of NADPH. The modality of how IDH1 promotes the transformation of the normal cells is still unclear. Several studies have postulated that mutations (either by loss of the normal allele or as a result of a dominant-negative effect) might alter the mitochondrial function and lead the cells to use glycolysis. On the other hand, mutations lead to a hypermethylation phenotype with a change in the oxidative metabolism. Mutations in two isoforms of this metabolic enzyme (IDH1 and IDH2) have been found in many solid and blood cancers, including in MDS at a low frequency. This finding suggests that malignancies can be caused by dysfunction in cellular metabolism. A small proportion of late stage MDS cases (5-10%) have IDH1/2 mutations. In addition, a slightly higher frequency of mutations in this gene is found in sAML patients that evolved from a prior MDS (10-20%). The clinical impact of these mutations is still controversial and varies in different cancers [34]. The higher frequency in patients with higher risk MDS suggests a predisposition to develop AML. (Table 2) It has recently reported that serum measurement of R-2-hydroxylutarate can predict clinical outcome in IDH1/2 mutant patients [35]. Several studies have indicated an increased risk for relapse in AML patients carrying IDH1/2 mutations while others have not found any statistical correlation. IDH1/2 proteins are interconnected with TET2 since TET2 seem to be a target of R-2-hydroxylutarate [36]. In addition, TET2 and IDH1/2 mutations are mutually exclusive.

V. HISTONE MODIFICATION: ASXL1, RUNX1

ASXL1 (Additional Sex Combs Like 1) is a gene located in chromosome 20q11.21. This mutation appears to be independent of the deletion of 20q found in patients with MDS, as ASXL1 mutations were found in 28% of patients carrying isolated del(20q). ASXL1 is a member of the Polycomb family which includes genes implicated in chromatin remodeling and gene repression. Indeed, several structural similarities have been identified in genes of the Drosophilidae where ASXL1 interacts with chromosomal elements. The function of the ASXL1 protein is required for the activation and repression of homeotic loci. The protein is believed to disrupt chromatin in localized areas of certain genes while repressing the transcription of other genes. Indeed, mutations lead to the disruption of activating domains (PDH, RARalpha, and SRC1). Monoallelic mutations in ASXL1 have been found in 16% of high-risk MDS patients and in ~30% of patients with AML and a prior MDS diagnosis. ASXL1 mutations have been associated with a worse OS in MDS [37]. (Table 2) Mutational analysis has also revealed the co-occurrence of ASXL1 mutations with TET2, IDH1/2, and EZH2 mutations, indicating that the epigenetic mechanisms in total are highly dominant in
MDS pathophysiology [38]. The effect of ASXL1 mutations is still unclear with some studies pointing towards a gain of function and dominant negative effect rather than loss of function.

RUNX1 (Runt related transcription factor), also known as AML1, maps to chromosome 21q22 and belongs to a family of DNA core binding factors. RUNX1 regulates the transcription of genes important in hematopoietic stem cell formation during embryogenesis. Indeed, runx1 knock-out mice die few days after birth without achieving complete hematopoiesis. RUNX1 translocations produce chimeric proteins, and RUNX1 is usually highly mutated in AML (M0, M2, and M1 types), while RUNX1 mutations are found in 20% of MDS cases, mainly t-MDS and radiation exposure [39]. RUNX1 mutations occur near DNA-binding domains altering the

| Gene | Studies | Disease Group | Overall Survival | AML Transformation |
|------|---------|---------------|-----------------|-------------------|
| TET2 | Kao HW et al. 52nd ASH Meeting Abstract 4019 | MDS (N=161) | no difference | increased risk |
|      | Jankowska A et al. Blood 2011 6;118(14):3932-41 | CMML (N=52), CMML to AML (N=20) | no difference | - |
|      | Bejar R et al. N Engl J Med. 2011. 30;364(26):2496-506 | MDS=439 | no difference | - |
|      | Izykson R et al. Leukemia. 2011;25(7):1147-52 | MDS and AML with 20-30% blast (N=86); Tx with 5-aza | no difference | - |
|      | Walter MJ et al. Leukemia. 2011;25(7):1153-8 | MDS (N=150) | worse | more rapid progression |
| DNMT3A | Jankowska A et al. Blood. 2011 6;118(14):3932-41 | CMML (N=52), CMML to AML (N=20) | no difference | - |
|      | Lin J et al. PloSOne. 2011;6(10):e26906. Epub 2011 Oct 31. | AML (N=182), MDS (N=51) | worse in MDS | - |
| IDH1/2 | Jankowska A et al. Blood. 2011 6;118(14):3932-41 | CMML (N=52), CMML to AML (N=20) | no difference | - |
|      | Bejar R et al. N Engl J Med. 2011. 30;364(26):2496-506 | MDS=439 | no difference | - |
|      | Gelsi-Boyer V et al. Br J Haematol. 2010;151(4):365-75 | CMML (N=53) | worse | increased risk |
| ASXL1 | Kulasekararaj A et al. 52nd ASH Meeting Abstract 125 | MDS (N=45); AML from MDS (N=9), t-AML/MDS (N=7), CMML (N=2) | better if analyzed with EZH2 | - |
|      | Jankowska A et al. Blood. 2011 6;118(14):3932-41 | CMML (N=52), CMML to AML (N=20) | no difference | - |
|      | Tho F et al. J Clin Oncol. 2011 20;29(18):2499-506 | MDS (N=193) | worse | decreased time to AML progression |
|      | Bejar R et al. N Engl J Med. 2011. 30;364(26):2496-506 | MDS=439 | worse | - |

**Abbreviations.** MDS, Myelodysplastic syndromes; MDS/MPN, Myelodysplastic syndromes/Myeloproliferative neoplasms; AML, Acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; RARS, refractory anemia with ring sideroblasts; RARS-T, refractory anemia with ring sideroblasts associated with thrombocytosis; LFS, leukemia free survival; - Indicates not available leukemia; Tx, treatment.
### TABLE II. EFFECTS OF MOLECULAR MUTATIONS ON OVERALL SURVIVAL AND AML TRANSFORMATION IN MYELODYSPLASTIC SYNDROMES AND OTHER MYELOID MALIGNANCIES (CONTINUED)

| Gene | Studies | Disease Group | Overall Survival | AML Transformation |
|------|---------|---------------|------------------|--------------------|
| **SF3B1** | Visconte V et al. Leukemia. 2012 Mar26(3):542-5 Epub 2011 Sep 2 | MDS MDS/MPN (N=56) | no difference | - |
| | Papaemmanuil E et al. NEJM. 2011 Oct 13;365(15):1384-95. Epub 2011 Sep 26 | MDS (N=354) MDS (N=354) | better | lower risk |
| | Malcovati L et al. Blood. 2011 Dec 8;118(24):6239-46 | MDS (N=583) MDS/MPN (N=83) | better | lower risk |
| | Damm F et al. 2012 May;26(5):1137-40 | MDS (N=317) clinical data available from 253 patients | no difference | no difference |
| | Papaemmanuil MM et al. Blood 2012 Jan 12;119(2):569-72 and Am J Hematol. 2013 Mar88(3):201-6 | MDS-RS (N=107) | univariate analysis better but not an independent factor | - |
| | Visconte V et al. Blood 2012 Oct 18;120(16):3173-86 | MDS and MDS/MPN (N=456) | better for MDS and MDS/MPN and within the subset of RARS and RARS-T | - |
| **U2AF1** | Yoshida et al. Nature 2011;478: 64-69 | MDS MDS/MPN AML (N=582) | - | - |
| | Makishima et al. 2012;119:3203-3210 | MDS MDS/MPN AML (N=350) | - | - |
| | Graubert et al. Nat Genet. 2012;44: 53-57. | de novo MDS MDS/sAML | no difference | Increased risk |
| | Yoshida et al. Nature 2011;478: 64-69 | MDS, MDS/MPN, AML (N=582) | - | - |
| | Patnaik MM et al. Am J Hematol. 2013 Mar88(3):201-6 | CMML (N=226) | no difference | no difference in LFS |
| | Kar SA et al. Haematologica. 2013 Jan;98(1):107-13 | CMML (N=87) | - | - |
| | Wu SH et al. Blood. 2012 Oct 11;120(15):3106-11 | MDS (N=230) | worse | no impact of the disease evolution |
| **SETBP1** | Makishima et al. Nat Genet. 2013 Jul 7. In press | MDS, MDS/ MPN, MPN, AML (N=727) | worse | association with myeloid transformation |
| | Thol F et al. Leukemia. 2013 May. In press | MDS and AML, (N=944) | no difference | - |

**Abbreviations.** MDS, Myelodysplastic syndromes; MDS/MPN, Myelodysplastic syndromes/Myeloproliferative neoplasms; AML, Acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; RARS, refractory anemia with ring sideroblasts; RARS-T, refractory anemia with ring sideroblasts associated with thrombocytosis; LFS, leukemia free survival; - Indicates not available leukemia; Tx, treatment.

DNA core-binding capacity and appear to be independent prognostic factors of worse OS. The mutations induce loss of trans-activation. An elegant study conducted in mice reported that mice transplanted with human cells carrying a mutant RUNX1 allele develop dysplasia in the erythroid lineage and MDS features.

VI. RNA SPlicINC MACHINERY: SF3B1, U2AF1, SRSF2

RNA splicing is an evolutionary mechanism important in maintaining genomic variability. Splicing mutations lead to hereditary diseases, such as X-linked disorders of copper metabolism and retinitis pigmentosa [40]. Somatic mutations in components of the spliceosome machinery were discovered by several groups using whole
genome/exon sequencing in MDS with ring sideroblasts (RS) and MDS/myeloproliferative overlap syndromes (MDS/MPN) and later in lymphoid malignancies like chronic lymphocytic leukemia (CLL). SF3B1 mutations range in frequency, occurring in 68-75% of cases of refractory anemia with ring sideroblasts (RARS) and 81% in RARS associated with thrombocytosis (RARS-T) [41-43] while being rare in other diseases [44]. SF3B1 has been linked to the pathogenesis of RARS and RARS-T. We associated SF3B1 mutations to the presence of RS [45,46]. In terms of clinical significance, SF3B1 mutations have been associated with better OS (P=0.1), leukemia-free survival (P=.05), event free survival (P=.008) and with a lower risk of progression to AML [42]. Better OS was noted in RARS/ RARS-T carrying SF3B1 mutations [45] (Table 2). No difference in OS and AML transformation was found in a cohort of 317 patients with MDS. Patnaik et al. determined that the prognostic value of SF3B1 mutations was completely accounted for the WHO morphologic grouping [47].

Pharmacologic inhibitors of the spliceosome machinery are currently available [48,49]. We described functional data on Meayamycin, which specifically targets the splicing factor 3b (SF3b) complex [50]. Other pharmacologic compounds including spliceostatin, FR901464, E7107, pladienolide and Sudemycins are currently being investigated. Interestingly, the clinical significance of SF3B1 in MDS is different from that observed in CLL. The other disease where SF3B1 is highly mutated is CLL (15%) [51]. It has been reported that SF3B1 mutations predict worse outcome in CLL patients, and they are commonly associated with deletion of long arm of the chromosome 11, an abnormality present in 5-10% of CLL patients. These findings suggest that different targets might be involved and also that the clonal nature of the two diseases is regulated by different drivers. Our laboratory is actively working in investigating the molecular mechanisms responsible for the good outcomes of patients carrying SF3B1 mutations focusing attention on DNA damage differences, iron accumulation, mitochondrial targets, and in vitro response to hypomethylating agents. Our preliminary data suggests that the presence of SF3B1 mutations confer better response rate to erthropoiesis stimulating agents and hypomethylating agents [52].

Mammalian U2 small nuclear ribonucleoprotein auxiliary factor (U2AF) is a heterodimer composed of a 65-kDa subunit (U2AF65) and a 35-kDa subunit (U2AF35). U2AF65 contacts the pyrimidine tract while U2AF35 interacts with the AG splice acceptor dinucleotide of the target intron at the 3′ splice site. U2AF65 is also known as U2AF1. Recurrent somatic mutations (S34 and Q157) in U2AF1 were found in 8.7% of patients with primary MDS and were associated with progression to sAML [53,54]. U2AF1 mutations result in a gain of function. Indeed, a significant increase in exon skipping was observed when the mutant p.Ser34Phe cDNA was transiently expressed in vitro [53]. Pattern of splicing for U2AF1 has recently been elucidated [55].

SF3B1 encodes a member of the serine/arginine (SR)-rich family of pre-mRNA splicing factors with an RNA recognition motif (RRM) and a RS domain. SF3B1 is believed to be a key element in the acetylation/phosphorylation network and an important regulator of the DNA stability. The first report describing SF3B1 mutations in CMML found mutational frequencies ranging between 28.4-47% [56]. The most common alteration occurs at amino acid position Prolin95 between the RRM motif and the RS domain. SF3B1 has been indicated as a new diagnostic target in CMML. The presence of SF3B1 mutations correlate with higher age, anemia, and normal karyotyping and do not seem to impact survival. Other studies associated SF3B1 mutations with poor outcomes (Table 2). The impact of baseline spliceosomal mutations were investigated in a cohort of patients that underwent allogeneic hematopoietic cell transplantation, finding that SF3B1 mutants had similar outcomes to wild type patients, suggesting that transplant can compensate for the adverse impact of this gene [57].

VII. INFREQUENT MOLECULAR MUTATIONS

We have discussed the genes for which the clinical impact has been intensively studied and correlated with the patient phenotypes. Several of the above genes are relevant because they can serve as therapeutic targets in MDS, like hypomethylating agents and histone deacetylase. On the other hand, there are numerous other genes with lower mutational frequencies reported in MDS and other related malignancies in the same biological pathways discussed above. Mutations in other genes regulating histone function and members of the polycomb group are less frequent in MDS. EZH2 acts as histone methyltransferase that normally works in maintaining the repressed status of TSGs and has been found mutated in 2-6% of patients with MDS [58]. UTX, a demethylase gene located in the X chromosome, has been actively interrogated for mutations but they have only been found in MDS/MPN patients at a frequency of 6%. Mutations in c-CBL, CBL-b, and CBL-c are extremely rare in MDS (1%), while present in a much higher frequency in patients with MDS/MPN (8.1%) [59]. Infrequent mutations in other spliceosomal genes, such as U2AF65, ZRSR2, SF1, SRSF1, SF3A1, PRF40B, PRPF8, and U2AF1, were also detected [54]. Mutations in N/KRAS, encoding proteins homologous to RAS, were reported at frequencies of 2-6%. Several other genes have been found but are even less commonly dysregulated and/or mutated in MDS like IER3, a sentinel of cellular stress, FLT3, a class III receptor tyrosine kinase which determines proliferative advantage if mutated in AML, and CDC25c, a gene in the cell division pathway that interacts with MAP kinase family members for which the frequency of mutations is unavailable.
VII. BRIEF DESCRIPTION OF GENES ASSOCIATED WITH SPECIFIC CHROMOSOMAL ABNORMALITIES: TP53 AND RPS14

Gene mutations can be associated with specific classes of MDS patients carrying distinct chromosomal anomalies. Mutations in TP53, a TSG, have been correlated with anomalies of chromosome 5 (isolated del5q, -5/5q-), 17p- and complex karyotypes, suggesting different biological and driving mechanisms. Mutations were detected early in the MDS presentation, suggesting a primary event in the manifestation of the disease. Associations of TP53 mutations and t-MDS have been reported. Moreover, mutations have also been correlated with response to therapy (5-azacytidine) and seemed to disappear upon response [60].

The discovery of the haploinsufficiency of RPS14 and its somatic deletion in patients with 5q-syndrome associated MDS to other ribosomopathies and correlated this ribosomal protein to the pathogenesis of 5q- patients. In addition, RPS14 seems to be a major player in the anemia of patients with 5q- and a marker of good prognosis [61].

IX. NEWLY DISCOVERED MOLECULAR MUTATIONS

Whole exome sequencing aided in the discovery of mutations in SETBP1 with a higher frequency in atypical CML (aCML). Germline alterations in SETBP1 are characteristic of the Schinzel-Giedion midface retraction syndrome. Heterozygous variants, commonly found in a germline configuration, are found to be somatic in aCML [62]. Direct sequencing of SETBP1 in different subsets of MDS, MDS/ MPN, and other diseases found a lower frequency in MDS (2.2%) [63]. SETBP1 mutations have been correlated with leukemogenesis [64] and found to be associated with the presence of other two genes which correlate with a negative prognosis (ASXL1 and EZH2) and with two deleterious chromosomal abnormalities (iso17(q) and -7).

X. CONCLUSION

High-resolution genomic technologies have discovered a myriad of somatic alterations in different genes with a variety of functions in MDS and other hematologic malignancies. Even in the era of genomic technologies, MC still represents the gold standard in the identification of chromosomal abnormalities in MDS and still holds a fundamental role in the prognostic scoring systems. Molecular mutations represent a new parameter to take into account in the risk stratifications models. Whole genome/exome sequencing represents a platform of discovery for new markers of MDS, but the role and the mechanisms explaining the heterogeneity of the clinical/morphologic manifestations of MDS is still unknown. On the other hand, even with the advent of all these sophisticated technologies conventional Sanger sequencing still represents the most reliable and cost affordable technique. Pitfalls including artefacts/ false negatives are also commonly faced using the next generation technologies as mentioned above. Lower-frequency molecular mutations are also important in MDS, but harder to study in terms of prognostic and predictive significance. Nonetheless, they may provide important insight in disease biology unique to the specific individual carrying the rare mutation. Recurrence of somatic alterations is a pre-requisite to define a specific gene as a possible candidate in the pathogenesis of a disease subtype. We have interrogated hundreds of possible candidates selected after performing whole exome sequencing in MDS and other related disorders, finding sporadic somatic alterations with no detectable frequency in large cohort of same disease type. Germ-line sources (lymphocytes and/or buccal swabs when possible), scoring of the genetic variants based on an acceptable coverage, stringent bioinformatics algorithms, searching in public database for SNPs exclusion, absence of variants in healthy individuals, and further confirmation of the eventual alterations with bidirectional sequencing are all useful tasks to generate valid results.

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REFERENCES

[1] Ma X, Does M, Raza A, Mayne ST. Myelodysplastic syndromes: incidence and survival in the United States. Cancer 2007;109: 1536-1542.
[2] Sekeres MA. Epidemiology, natural history, and practice patterns of patients with myelodysplastic syndromes in 2010. J Natl Compr Canc Netw 2011;9: 57-63.
[3] Solé F, Espinet B, Sanz GF, Cervera J, Calasaniz MJ, Luño E, Prieto F, Granada I, Hernández JM, Cigudosa JC, Diez JL, Bureo E, Marqués ML, Arranz E, Ríos R, Martínez Climent JA, Vallespi T, Florensa L, Woessner S. Incidence, characterization and prognostic significance of chromosomal abnormalities in 640 patients with primary

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myelodysplastic syndromes. Grupo Cooperativo Espanol de Citogenetica Hematologica. Br J Haematol 2000;108: 346-356.

[4] Le Beau MM, Albain KS, Larson RA, Vardiman JW, Davis EM, Blough RR, Golomb HM, Rowley JD. 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 1986;4: 325-345.

[5] Deeg HJ, Scott BL, Fang M, Shulman HM, Gyurkocza B, Myerson D, Pagel JM, Platzbecker U, Ramakrishnan A, Radich JP, Sandmaier BM, Soror M, Stirewalt DL, Wilson WA, Storb R, Appelbaum FR, Gooley T. Five-gene cytogenetic risk classification, monosomal karyotype, and outcome after hematopoietic cell transplantation for MDS or acute leukemia evolving from MDS. Blood 2012:120: 1398-1408.

[6] Graubert T, Walter MJ. Genetics of myelodysplastic syndromes: new insights. Hematology Am Soc Hematol Educ Program 2011 2011: 543-549.

[7] Tiu RV, Visconte V, Traina F, Schwandt A, Maciejewski JP. Updates in cytogenetics and molecular markers in MDS. Curr Hematol Malig Rep 2011;6: 126-135.

[8] Schlegelberger B, Gohring G, Thol F, Heuser M. Update on cytogenetic and molecular changes in myelodysplastic syndromes. Leuk Lymphoma 2012;62: 13-17.

[9] Ahmad A, Iqbal MA. Significance of genome-wide analysis of copy number alterations and UPD in myelodysplastic syndromes using combined CGH - SNP arrays. Curr Med Chem 2012;19: 3739-3747.

[10] Tiu RV, Gondek LP, O’Keefe CL, Elson P, Huh J, Mohamedali A, Kulasekararaj A, Advani AS, Paquette R, List AF, Sekeres MA, McDevitt MA, Mufti GJ, Maciejewski JP. Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies. Blood 2011;117: 4525-4560.

[11] Starczynowski DT, Vercauteren S, Telenius A, Sung S, Toyhama K, Brooks-Wilson A, Spinelli JJ, Eaves CJ, Eaves AC, Horsman DE, Lam WL, Karsan A. High-resolution whole genome tiling path array CGH analysis of CD34+ cells from patients with low-risk myelodysplastic syndromes reveals cryptic copy number alterations and predicts overall and leukemia-free survival. Blood 2008;112: 3412-3424.

[12] Gondek LP, Dunbar AJ, Szpurka H, McDevitt MA, Maciejewski JP. SNP array karyotyping allows for the detection of uniparental disomy and cryptic chromosomal abnormalities in MDS/MPD-U and MPD. PLoS One 2007;2: e1225.

[13] Jerez A, Gondek LP, Jankowska AM, Makishima H, Przychodzen B, Tiu RV, O’Keefe CL, Mohamedali AM, Batista D, Sekeres MA, McDevitt MA, Mufti GJ, Maciejewski JP. Topography, clinical, and genomic correlates of 5q myeloid malignancies revisited. J Clin Oncol 2012;30: 1343-1349.

[14] Maciejewski JP, Batista D, Przychodzen B. Characterization of SNP arrays in MDS, MDS/MPD, and MDS-derived AML. Blood 2008;111: 1534-1542.

[15] Maciejewski JP, Tiu R, Haddad AS, O’Keefe CL, Sekeres MA, Theil KS, Maciejewski JP. Single nucleotide polymorphism arrays complement metaphase cytogenetics in detection of new chromosomal lesions in MDS. Leukemia 2007;21: 2058-2061.

[16] Mohamedali A, Gäken J, Twine NA, Ingram W, Westwood N, Lea NC, Hayden J, Donaldson N, Aul C, Gattermann N, Giagounidis A, Germing U, List AF, Mufti GJ. Prevalence and prognostic significance of allelic imbalance by single-nucleotide polymorphism analysis in low-risk myelodysplastic syndromes. Blood 2007;110: 3365-3373.

[17] Yih JH, Huh J, Kim HJ, Kim SH, Kim KH, Do YR, Mun YC, Kim H, Kim MK, Kim HJ, Kim T, Kim DD. Genome-wide single-nucleotide polymorphism array-based karyotyping in myelodysplastic syndrome and chronic myelomonocytic leukemia and its impact on treatment outcomes following decitabine treatment. Ann Hematol 2013;92: 459-469.

[18] Jiang Y, Dunbar A, Gondek LP, Mohan S, Rataul M, O’Keefe C, Sekeres M, Saunthararajah Y, Maciejewski JP. Aberrant DNA methylation is a dominant mechanism in MDS progression to AML. Blood 2009;113: 1315-1325.

[19] 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. DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes. J Clin Oncol 2010;28: 605-613.

[20] Jankowska AM, Szpurka H, Tiu RV, Makishima H, Afable M, Huh J, O’Keefe CL, Ganetzky R, McDevitt MA, Maciejewski JP. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. Blood 2009;113: 6403-6410.

[21] Langemeijer SM, Kuiper BP, Berends M, Knops R, Aslanyan MG, Massop M, Stevens-Linders E, van Hoogen P, van Kessel AG, Raymakers RA, Kamping EJ, Verhoef GE, Verburgh E, Hagemeijer A, Vandenbergh P, de Witte T, van der Reijden BA, Jansen JH. Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet 2009;41: 838-842.

[22] Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, An J, Lamperti ED, Koh KP, Ganetzky R, Liu XS, Aravind L, Agarwal S, Maciejewski JP, Rao A. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature 2010;468: 839-843.

[23] Ponnaluri VK, Maciejewski JP, Mukherji M. A mechanistic overview of TET-mediated 5-methylcytosine oxidation. Biochem Biophys Res Commun 2013;436: 115-120.
[25] Ko M, An J, Bandukwala HS, Chavez L, Aijó T, Pastor WA, Segal MF, Li H, Koh KP, Lähdesmäki H, Hogan PG, Aravind L, Rao A. Modulation of TET2 expression and 5-methylcytosine oxidation by the CXCR4 domain protein IDAX. Nature 2013:497: 122-126.

[26] Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Loby D, Lobry C, Figueroa ME, Vasanthakumar A, Patel J, Zhao X, Perna F, Pandey S, Madzo J, Song C, Dai Q, He C, Ibrahim S, Beran M, Zavadil J, Nimer SD, Melnick A, Godley LA, Afantitis I, Levine RL. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell 2011;20: 11-24.

[27] Itzykson R, Kosmidis O, Cluzeau T, Mansat-De Mas V, Dreyfus F, Beyne-Raury O, Quesnel B, Vey N, Gelsi-Boyer V, Raynaud S, Preudhomme C, Adès L, Fenaux P, Fontenay M; Groupe Francophone des Myelodysplasies (GFM). Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia 2011;25: 1147-1152.

[28] Traina F, Jankowska A, Visconte V, Sugimoto Y, Szpurka H, Makishima H, Jereczek J, O'Keefe CL, Sekeres MA, Advani AS, Saunthararajah Y, Kalaycio M, Copelan E, Ollala Saad S, Maciejewski JP, Tiu RV. Impact of Molecular Mutations on Treatment Response to Hypomethylating Agents in MDS. Blood (ASH Annual Meeting Abstracts) 2011;118: 461.

[29] Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, Kandoth C, Fayton JE, Baty J, Welch J, Harris CC, Lichti CF, Townsend RR, Fulton RS, Dooling DJ, Klbodt DC, Schmidt H, Zhang Q, Osborne JR, Lin L, O'Laughlin M, McMichael JF, Delehaunty KD, McGrath SD, Fulton LA, Magrini VJ, Vickery TL, Hundal J, Cook LL, Conyers JJ, Swift GW, Reed JP, Aldredge PA, Wylie T, Walker J, Kalicki J, Watson MA, Heath S, Shannon WD. Varghese N, Nagarajan R, Westervelt P, Tomasson MH, Link DC, Graubert TA, DiPersio JF, Mardis ER, Wilson RK. DNMT3A mutations in acute myeloid leukemia. N Engl J Med 2010;363: 2424-2433.

[30] Jankowska AM, Makishima H, Tiu RV, Szpurka H, Huang Y, Traina F, Visconte V, Sugimoto Y, Prince C, O'Keefe C, Hsi ED, List A, Sekeres MA, Rao A, McDevitt MA, Maciejewski JP. Mutational spectrum analysis of chronic myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX, EZH2, and DNMT3A. Blood 2011;118: 3932-3941.

[31] Thol F, Winschel C, Lüdeking A, Yun H, Friesen I, Damm F, Wagner K, Krauter J, Heuser M, Ganser A. Rare occurrence of DNMT3A mutations in myelodysplastic syndromes. Haematologica 2011;96: 1870-1873.

[32] Challen GA, Sun D, Jeong M, Luo M, Jelinek J, Berg JS, Bock C, Vasanthakumar A, Gu H, Xi Y, Liang S, Lu Y, Darlington GJ, Meissner A, Issa JP, Godley LA, Li W, Goodell MA. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet 2012;44: 23-31.

[33] Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan M, Fulton R, Schmidt H, Kaliciki-Keizer J, O'Laughlin M, Kandoth C, Baty J, Westervelt P, DiPersio JF, Mardis ER, Wilson RK, Ley TJ, Graubert TA. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. Leukemia 2011;25: 1153-1158.

[34] Cazzola M. IDH1 and IDH2 mutations in myeloid neoplasms--novel paradigms and clinical implications. Haematologica 2010;95: 1623-1627.

[35] Dinardo CD, Propert KJ, Loren AW, Paitetta E, Sun Z, Levine RL, Staley KS, Yen K, Patel JP, Agresta S, Abdel-Wahab O, Perl AE, Litzow MR, Rowe JM, Lazarus HM, Fernandez HF, Margolis DJ, Tallman MS, Luger SM, Carroll M. Serum 2-hydroxyglutarate levels predict isocitrate dehydrogenase mutations and clinical outcome in acute myeloid leukemia. Blood 2013;121: 4917-4924.

[36] Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li Y, Bhagwat N, Vasanthakumar A, Fernandez HF, Tallman MS, Sun Z, Wolniak K, Peeters JK, Liu W, Choe SE, Fantin VR, Paitetta E, Löwenberg B, Licht JD, Godley LA, Delwel R, Valk PJ, Thompson CB, Levine RL, Melnick A. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell 2010;18: 553-567.

[37] Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, Kantarjian H, Raza A, Levine RL, Neuberg D, Ebert BL. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 2011;364: 2496-2506.

[38] Shih AH, Abdel-Wahab O, Patel JP, Levine RL. The role of mutations in epigenetic regulators in myeloid malignancies. Nat Rev Cancer 2012;12: 599-612.

[39] Davids MS, Steensma DP. The molecular pathogenesis of myelodysplastic syndromes. Cancer Biol Ther 2010;10: 309-319.

[40] Faustino NA, Cooper TA. Pre-mRNA splicing and human disease. Genes Dev 2003;17: 419-437.

[41] 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, Kawaihara R, Yamaguchi T, Otsu M, Obara N, Sakata-Yanagimoto M, Ishiyama K, Mori H, Nolte F, Hofmann WK, Miyawaki S, Sugano S, Haferlach C, Koeffler HP, Shih LY, Haferlach T, Chiba S, Nakauchi H, Miyano S, Ogawa S. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 2011;478: 64-69.

[42] Papaemmanuil E, Cazzola M, Boulwood J, Malcovati L, Vyas P, Bowen D, Pellagatti A, Wainscoat JS, Hellstrom-Lindberg E, Gambacorti-Passerini C, Godfrey AL, Rapado I, Cvejic A, Rance R, Mcgee C, Ellis P, Mudie LJ, Stephens PJ, McLaren S, Massie CE, Tarpey PS, Varela I, Nik-Zainal S, Davies HR, Shlien A, Jones D, Raine K, Hinton J, Butler AP, Teague JW, Baxter EJ, Score J, Galli A, Delia Porta MG, Travaglini E, Groves M, Tauro S, Munshi NC, Anderson KC, El-Naggar A, Fischer A, Mustonen V, Warren AJ, Cross NC, Green AR, Futreal PA, Stratton MR, Campbell PJ; Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium. Somatic
SF3B1 mutation in myelodysplasia with ring sideroblasts. Nature Engl J Med 2011;365: 1384-1395.

[43] Visconte V, Makishima H, Jankowska A, Szpurka H, Traina F, Jerez A, O'Keefe C, Rogers HJ, Sekeres MA, Maciejewski JP, Tiu RV. SF3B1, a splicing factor is frequently mutated in refractory anemia with ring sideroblasts. Leukemia 2012;26: 542-545.

[44] Visconte V, Tabarroki A, Rogers HJ, Hasrouni E, Traina F, Makishima H, Hamilton BK, Liu Y, O’Keefe C, Lichtin A, Horwitz L, Sekeres MA, Hsieh FH, Tiu RV. (SF3B1) mutations are infrequently found in non-Myelodysplastic bone marrow failure syndromes and mast cell diseases but, if present, are associated with the ring sideroblast phenotype. Haematologica 2013, “in press”.

[45] Visconte V, Rogers HJ, Singh J, Barnard J, Bupathi M, Traina F, McMahon J, Makishima H, Szpurka H, Jankowska A, Jerez A, Sekeres MA, Saunthararajah Y, Advani AS, Copelan E, Koski H, Isono K, Padgett RA, Osman S, Koide K, O’Keefe C, Maciejewski JP, Tiu RV. SF3B1 haploinsufficiency leads to formation of ring sideroblasts in myelodysplastic syndromes. Blood 2012;120: 3173-3186.

[46] Cazzola M, Rossi M, Malcovati L. Biologic and clinical significance of somatic mutations of SF3B1 in myeloid and lymphoid neoplasms. Blood 2012;120:260-269.

[47] Patnaik MM, Lasho TL, Hodnefield JM, Knudson RA, Kettinger RP, Garcia-Manero G, Steensma DP, Pardanani A, Hanson CA, Tefferi A. SF3B1 mutations are prevalent in myelodysplastic syndromes with ring sideroblasts but do not hold independent prognostic value. Blood 2012;119: 569-572.

[48] Lagisetti C, Pourpak A, Goronga T, Jiang Q, Cui X, Hyle J, Lahti JM, Morris SW, Webb TR. Synthetic mRNA splicing modifier compounds with in vivo antitumor activity. J Med Chem 2009;52: 6979-6990.

[49] Bonnal S, Vigevan L, Valcarchel J. The spliceosome as a target of novel antitumor drugs. Nat Rev Drug Discov 2012;11: 847-859.

[50] Visconte V, Makishima H, Maciejewski JP, Tiu RV. Emerging roles of the spliceosomal machinery in myelodysplastic syndromes and other hematological disorders. Leukemia 2012;26: 2447-2454.

[51] Wan Y, Wu CJ. SF3B1 mutations in chronic lymphocytic leukemia. Blood 2013;121: 4627-4634.

[52] Visconte V, Mahfouz R, Tabarroki A, Rogers HJ, Bodo J, Hasrouni E, Sharhag-Moshtaghin R, Aviash N, Bupathi M, Hamilton BK, Liu Y, Makishima H, Jankowska A, Barnard J, Traina F, Saunthararajah Y, Copelan EA, Heuer A, Lindner D, Maciejewski JP, Sekeres MA, and Tiu RV. Biological Rationale for the Favorable Clinical Outcomes of Patients Carrying SF3B1 Mutations in Myelodysplastic Syndromes with Ring Sideroblasts. Blood (ASH Annual Meeting Abstracts) 2012;120: 922.

[53] Graubert TA, Shen D, Ding L, Okeyo-Owuor T, Lunn CL, Shao J, Krysiak K, Harris CC, Koboldt DC, Larson DE, McLellan MD, Dooling DJ, Abbott RM, Fulton RS, Schmidt H, Kalicki-Veizer J, O’Laughlin M, Grillot M, Baty J, Heath S, Frater JL, Nasim T, Link DC, Tomasson MH, Westervelt P, DiPersio JF, Mardis ER, Ley TJ, Wilson RK, Walter MJ. Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. Nat Genet 2012;44: 53-57.

[54] Makishima H, Visconte V, Sakaguchi H, Jankowska AM, Abu Kar S, Jerez A, Przychodzen B, Bupathi M, Guinta K, Afable MG, Sekeres MA, Padgett RA, Tiu RV, Maciejewski JP. Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. Blood 2012;119: 3203-3210.

[55] Przychodzen B, Jerez A, Guinta K, Sekeres MA, Padgett R, Maciejewski JP, Makishima H. Patterns of missplicing due to somatic U2AF1 mutations in myeloid neoplasms. Blood 2013, “in press”.

[56] Thol F, Kade S, Schlarmann C, Löflöf P, Morgan M, Krauter J, Wlodarski MW, Kölling B, Wichmann M, Görlich K, Göhring G, Bug G, Ottmann O, Niemeyer CM, Hofmann WK, Schlegelberger B, Ganser A, Heuser M. Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood 2012;119: 3578-3584.

[57] Hamilton B, Tabarroki A, Elson P, Hasrouni E, Visconte V, Tench S, Kalaycio ME, Sekeres MA, Sobeks RM, Duong HK, Hill BT, Bolwell BJ, Copelan E, Tiu RV. Impact of allogeneic hematopoietic stem cell transplant (HSCT) on patients harboring the spliceosome complex SRSF2. J Clin Oncol 31, 2013 (suppl; abstr 7008).

[58] Makishima H, Jankowska AM, Tiu RV, Szpurka H, Sugimoto Y, Hu Z, Saunthararajah Y, Guinta K, Keddache MA, Putnam P, Sekeres MA, Moliterno AR, List AF, McDevitt MA, Maciejewski JP. Novel homo- and hemizygous mutations in EZH2 in myeloid malignancies. Leukemia 2010;24: 1799-1804.

[59] Makishima H, Jankowska AM, McDevitt MA, O’Keefe C, Dujardin S, Cazzolli H, Przychodzen B, Prince C, Nicolli J, Siddiaha H, Shailk M, Szpurka H, Hsi E, Advani A, Paquette R, Maciejewski JP, CBL, CBLB, TET2, ASXL1, and IDH1/2 mutations and additional chromosomal aberrations constitute molecular events in chronic myelogenous leukemia. Blood 2011;117: e198-206.

[60] Kulasekararaj AG, Smith AE, Mian SA, Mohamedali AM, Krishnamurthy P, Lea NC, Gáken J, Pennanenach C, Ireland R, Czepulkowski B, Pomplun S, Marsh JC, Mufli GJ. TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. Br J Haematol 2013;160: 660-672.

[61] Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galli N, Raza A, Root DE, Attar E, Ellis SR, Golub TR. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. Nature 2008;451: 335-345.

[62] Pirola A, di Celle AM, Krishnamurthy P, Attar E, McDevitt MA, Maciejewski JP, Papaemmanuil E, Spinelli R, Bafaloukos D, Cazzolli H, Przychodzen B, Attar E, McDevitt MA, Maciejewski JP. Novel homo- and hemizygous mutations in EZH2 in myeloid malignancies. Leukemia 2010;24: 1799-1804.
NC. Gambacorti-Passerini C. Recurrent SETBP1 mutations in atypical chronic myeloid leukemia. Nat Genet 2013;45: 18-24.

[63] Damm F, Itzykson R, Kosmider O, Droin N, Renneville A, Chesnais V, Gelsi-Boyer V, de Botton S, Vey N, Preudhomme C, Clavert A, Delabesse E, Park S, Birnbaum D, Fontenay M, Bernard OA, Solary E. SETBP1 mutations in 658 patients with myelodysplastic syndromes, chronic myelomonocytic leukemia and secondary acute myeloid leukemias. Leukemia 2013;27: 1401-1403.

[64] Makishima H, Yoshida K, Nguyen N, Przychodzen B, Sanada M, Okuno Y, Ng KP, Gudmundsson KO, Vishwakarma BA, Jerez A, Gomez-Segui I, Takahashi M, Shiraishi Y, Nagata Y, Guinta K, Mori H, Sekeres MA, Chiba K, Tanaka H, Muraatsu H, Sakaguchi H, Paquette RL, McDevitt MA, Kojima S, Saunthararajah Y, Miyano S, Shih LY, Du Y, Ogawa S, Maciejewski JP. Somatic SETBP1 mutations in myeloid malignancies. Nat Genet 2013, “in press”.