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## REVIEW

# Significance of *JAK2* and *TET2* mutations in myelodysplastic syndromes

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## SUMMARY

The pathogenesis of myelodysplastic syndromes involves a pattern of genetic, epigenetic, and immune-mediated mechanisms but little is known about what causes the specific disease features and promotes disease progression in the individual patient. The identification of *JAK2* and *MPL* mutations, and more recently *TET2*, *CBL* and *ASXL-1* mutations in these disorders provide a basis for increased understanding of disease biology and mechanisms behind progression. Such mutations are more commonly found in patients with a significant amount of marrow ring sideroblasts, and in patients belonging to the category of mixed myelodysplastic/myeloproliferative neoplasms, entities which are in focus for this review.

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## Introduction

Myelodysplastic syndromes (MDS) are characterized by cytopenia caused by ineffective hematopoiesis and an increased risk for leukemic evolution. While certain patients remain stable for many years, the majority experience progressive disease with worsened cytopenia with or without increasing blast percentage over time. MDS pathogenesis involves a pattern of genetic, epigenetic, and immune-mediated mechanisms and with a few exceptions, little is known about what causes the specific disease features and promotes disease progression in the individual patient. The genetic background of most MDS subtypes is complex, and balanced chromosomal translocations and fusion proteins, such as in chronic myeloid leukemia (CML) and some types of acute myeloid leukemia (AML) are rare. The only MDS subtype defined by a chromosomal aberration is the 5q– syndrome, with an interstitial deletion encompassing >40 genes on 5q31.<sup>1</sup> Haplo insufficiency of the *RPS14* gene within the commonly deleted region has elegantly been associated with the erythroid deficiency of 5q– syndrome, but it does not explain all the feature of disease, neither the growth advantage of the clonal cells.<sup>2</sup> Hence, there are no specific molecular targets described for MDS, and targeted therapy in its strict sense is yet to be developed. Morphological classification is still state-of-the-art in the diagnostic process but molecular characterization is becoming increasingly important for diagnosis and therapeutic decision-making, also in MDS. Recently, new mutational discoveries have shed light over some of the MDS subtypes, in particular the overlap syndromes between the myelodysplastic and myeloproliferative neoplasms, which is the focus for this review.

## Implications of the WHO 2008 classifications of MDS and mixed MDS/MPN

### Myelodysplastic syndromes

The recently published WHO 2008 classification<sup>3</sup> replaced the pivotal first WHO edition from 2001.<sup>4</sup> No major changes were introduced in the classification of myelodysplastic syndromes, but minor alterations were done in order to clarify the definitions of certain subgroups. The previous MDS subgroup refractory anemia (RA) and a proportion of the previously unclassifiable patients were replaced by a new entity, refractory cytopenia with uni-lineage dysplasia, encompassing three subentities; refractory anemia, refractory neutropenia (RN) and refractory thrombocytopenia (RT). The 5q– syndrome subgroup was renamed to “MDS associated with isolated del(5q)” and is still defined by <5% marrow blasts. In the category “MDS unclassifiable” remain cases with severe marrow fibrosis making an estimation of blast percentage difficult, and cases without diagnostic morphology but with characteristic chromosomal aberrations, such as monosomy 7. The WHO 2001 classification was first to distinguish MDS with uni-lineage dysplasia from MDS with multilineage dysplasia and large cohort studies have since then confirmed the prognostic relevance of doing so.<sup>5</sup> MDS patients with uni-lineage dysplasia have a significantly better prognosis than those with multilineage dysplasia, in spite of similar percentages of marrow blasts. This has led to a need for revised prognostic scoring systems since the IPSS prognostic scoring system does not take these variables into account.<sup>6</sup>

### Mixed myelodysplastic/myeloproliferative neoplasms

The classification of mixed myelodysplastic/myeloproliferative neoplasms (mixed MDS/MPN) was also updated in 2008. This

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category encompasses chronic myelomonocytic leukemia (CMML), which is divided into CMML I with 5–9% marrow blasts, and CMML II with 10–19% blasts. An absolute monocyte count of  $>10^9/l$  is a prerequisite for a diagnosis of CMML, however, the clinical observation that some CMML patients have more dysplastic features and others more proliferative features is not reflected in the current classification and it is doubtful whether this distinction has clinical relevance. The two CMML subtypes differ significantly with respect to overall survival and risk for leukemic transformation.<sup>7</sup> Within the mixed MDS/MPN category remain also the provisional entity refractory anemia with ringed sideroblasts and marked thrombocytosis (RARS-T), described in detail below.

#### WHO classification and anemia

Erythroid failure resulting in anemia is the most common feature of MDS and may range from mild, with only slightly decreased hemoglobin levels and increased red cell volume to severe, with a complete loss of red blood cell production. Severe transfusion-dependent anemia is associated with both a shorter overall survival and an increased risk of leukemic transformation.<sup>8</sup> The poorer survival has several potential explanations, such as increasing iron overload, a negative effect of repeated transfusions, and persistent severe anemia leading to poor organ oxygenation. The increased transformation rate is less likely explained by such factors but may instead indicate that severe erythroid failure reflects a disease biology with a higher risk for leukemic evolution. Based on these findings, Malcovati et al.<sup>9</sup> have developed a new risk score, the WPSS system based on WHO 2001 subgroup, presence or absence of transfusion need and cytogenetic subgroup. WPSS has yet to be validated in prospective clinical and molecular studies, but is likely to improve future management of MDS.

Defective erythropoiesis in MDS can be divided into a hypo-proliferative/suppressed and a hyper-proliferative/ineffective form.<sup>10</sup> Hypo-proliferative erythropoiesis, characterized by a decreased relative number of erythroid progenitors in the bone marrow is usually seen in advanced MDS, hypoplastic MDS, in the 5q– syndrome, and in MDS with severe marrow fibrosis. Ineffective hyper-proliferative erythropoiesis is characterized by an increased percentage of marrow erythroblasts, of which many undergo intramedullary apoptosis. This type of erythropoiesis is typically observed in refractory anemia with ringed sideroblasts (RARS), but is also common in a subset of RA and sometimes in RAEB with a moderate increase of marrow blasts.

#### Refractory anemia with ringed sideroblasts

Ring sideroblasts are erythroblasts with iron-loaded mitochondria visualized by Prussian blue staining as a perinuclear ring of blue granules and with most of the iron deposited in the form of mitochondrial ferritin.<sup>11</sup> The sideroblastic anemias constitute a heterogeneous group of inherited and acquired disorders characterized by anemia of varying severity and the presence of ring sideroblasts in the bone marrow (Table 1).<sup>12,13</sup> The majority of these patients have clonal myeloid disorders and are classified either as MDS or MDS/MPN. The presence of 15% or more ringsideroblasts of the total number of erythroblasts in the bone marrow is a marker of the MDS subgroups RARS, refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS), and RARS-T.<sup>3</sup>

The division of the previous FAB RARS subgroup into RARS and RCMD-RS has significant clinical relevance. RARS patients present with anemia only, and have a five-year survival well above 50% and a very low risk, if any, for transformation to AML.<sup>14</sup> By contrast, patients with RCMD-RS have a five-year survival of 37%, a cumulative risk for AML transformation of 9%, and a substantial risk for developing more advanced MDS.<sup>14</sup> The two subgroups also differ

in their response to treatment with erythropoietin plus Granulocyte-CSF (G-CSF).<sup>15</sup> RARS patients show a response rate of 71% while the corresponding figure for patients with RCMD-RS is 30% (expanded unpublished data from the Nordic MDS database). The different clinical profiles of RARS and RCMD-RS indicate that at least partly different biological mechanisms are active in these disorders. Whether the molecular mechanism underlying sideroblast formation is the same in these different conditions remains to be investigated.

#### RARS with marked thrombocytosis (RARS-T)

Although myelodysplastic syndromes and myeloproliferative disorders appear to have different pathophysiological mechanisms, the existence of conditions with overlapping features is well established. In 2002, Schmitt-Graeff et al.<sup>16</sup> published a pivotal study of 38 patients showing both thrombocytosis in peripheral blood and ringed sideroblasts in the bone marrow, a condition that was at that time defined as “essential thrombocythemia with ringed sideroblasts (ET-RS)”. Findings of this study provided evidence that ET/RS includes a wide spectrum of conditions ranging from myelodysplastic syndromes in the strict sense to myeloproliferative disorders (essential thrombocythemia, prefibrotic primary myelofibrosis). The 2001 WHO classification introduced the mixed myelodysplastic/myeloproliferative neoplasms (mixed MDS/MPN) entity, which encompass the previous MDS group chronic myelomonocytic anemia (CMML), juvenile myelomonocytic leukemia, atypical chronic myelogenous leukemia, unclassifiable mixed MDS/MPN, and RARS-T. RARS-T was then defined as a provisional entity encompassing patients with “clinical and morphological features of myelodysplastic syndrome subtype RARS, but who also have marked thrombocytosis associated with abnormal megakaryocytes similar to those observed in *BCR-ABL1* negative MPN, such as ET or early stage primary myelofibrosis (PMF)”.<sup>4</sup> Fig. 1 depicts a representative bone marrow from a patient with RARS-T showing numerous ringsideroblasts and abnormal and abundant megakaryocytes. The 2001 classification defined a cut-off value for thrombocytosis of  $600 \times 10^9/l$ , and the majority of hitherto published clinical studies on the subject have used this definition. In the WHO 2008 classification, however, the cut-off value for thrombocytosis was decreased from 600 to  $450 \times 10^9/l$ , to make it in line with the threshold for essential thrombocytopenia.<sup>17</sup> Whether this was the right choice or not depends on how we perceive the provisional entity of RARS-T; as a myeloproliferative disorder, which with time develop myelodysplastic features, or a form of MDS which acquires additional molecular abnormalities resulting in abnormal megakaryocytopoiesis. In this context it should be mentioned that moderate thrombocytosis ( $400\text{--}500 \times 10^9/l$ ) is quite common in RARS.

#### Biology of acquired sideroblastic anemia

Ringsideroblast formation may be caused by exogenous factors, such as lead intoxication and treatment with isoniazid, which inhibits delta-aminolevulinic acid (ALA) dehydratase activity and blocks hemoglobin formation (Table 1). Several hereditary conditions are also associated with ring sideroblast formation, the most common of these being X-linked sideroblastic anemia (XLSA), which is caused by mutations in the erythroid-specific ALA synthase gene (*ALAS2*).<sup>13</sup> Recently, a new mutation associated with autosomal recessive congenital sideroblastic anemia (CSA) was characterized.<sup>18</sup> This gene encodes for an erythroid-specific mitochondrial carrier family protein, *SLC25A38*, important for the biosynthesis of heme in eukaryotes. Silencing of *SLC25A38* leads to an anemic phenotype in zebrafish. *SLC25A38* mutation is a quite common cause of CSA and seems to account for 17% of cases.

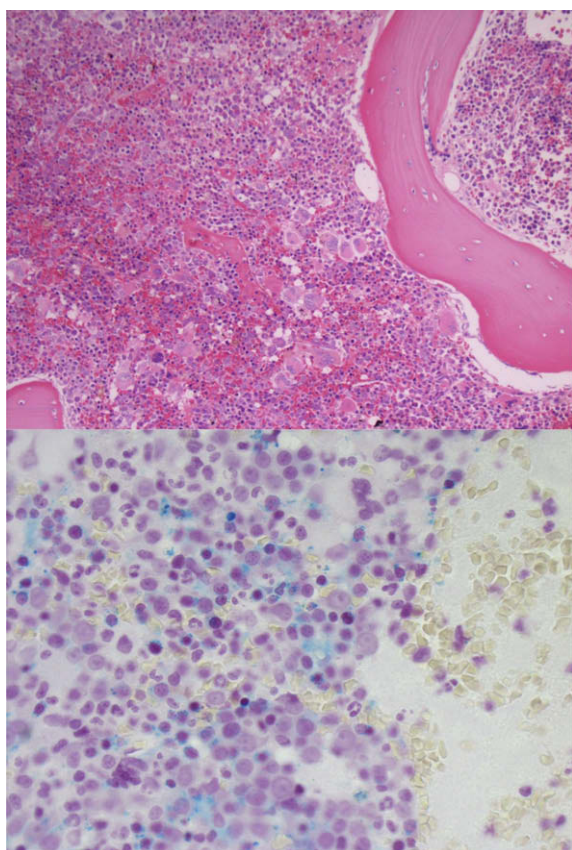
**Table 1**  
Hereditary and acquired conditions with bone marrow ringsideroblasts.

Group	Disorder	Gene	Gene expression with relevance for hereditary SA
Hereditary <sup>a</sup>	X-linked SA	ALAS2	
Sideroblastic anemias	GLRXS-related SA	GLRXS	
Nonsyndromic forms	SLC25A38-related SA	SLC25A38	
Others	Other	Unknown	
Hereditary <sup>a</sup>	SA/ataxia	ABCB7	
Sideroblastic anemias	Pearson' syndrome	Mt DNA deletions	
Syndromic forms	Kearns-Sayre syndrome	Mt DNA deletions	
	Mitochondrial myopathy SA	PUS1	
	Thiamine-responsive megaloblastic anemia	SLC19A2	
Acquired sideroblastic anemia as part of MDS	RARS	TET2 mutations frequent	ALAS2 expression increased <sup>b</sup>
	RCMD-RS	JAK2 mutations rare	ABCB7 expression decreased
		"sideroblast" mutation unknown	
Acquired sideroblastic anemia as part of Mixed MDS-MPN	RARS-T	TET2 mutations frequent	ALAS2 expression increased
		JAK2 mutations 50%	ABCB7 expression decrease
		MPL mutations occur	
		"sideroblast" mutation unknown	
Acquired sideroblastic anemia caused by exogenous factors or deficiency syndromes	Lead poisoning	ALAS2 inhibition	ALAS2 expression increased
	Isoniazid treatment	ALAS2 inhibition	ABCB7 expression decreased for difference RARS/RARS-T <sup>c</sup>
	Copper deficiency	Unknown	
	B12 deficiency	Unknown	

<sup>a</sup> List of hereditary sideroblastic anemias adapted from Camaschella C. *Semin Hematol* 2009.<sup>13</sup>

<sup>b</sup> ALAS2 expression increased in CD34+ selected bone marrow progenitors, however only slightly increased in intermediate erythroblasts.

<sup>c</sup> For differences in gene expression profiling between RARS and RARS-T, see Malcovati et al.<sup>33</sup>



**Fig. 1.** Bone marrow biopsy sections from a patient with RARS-T. Upper panel: Hematoxylin-Eosin staining showing abundant and abnormal megakaryocytes. Lower panel: Perls staining showing numerous ring sideroblasts. Photograph a courtesy of Birgitta Sander, Karolinska Institutet.

Some of the inherited sideroblastic anemias are part of a syndrome phenotype and associated with other symptoms and abnormalities. One example is the X-linked sideroblastic anemia

associated with ataxia (XLSA/A), which is caused by missense mutations in the human *ABCB7* gene, encoding a membrane-associated protein belonging to the superfamily of ATP-binding cassette (ABC) transporters.<sup>19</sup> The *ABCB7* protein enables transport of iron from the mitochondria to the cytoplasm. It has been reported that *ABCB7* is essential for hematopoiesis,<sup>20</sup> and that RNA silencing of the gene in HeLa cells causes an iron-deficient phenotype with mitochondrial iron overload.<sup>21</sup> In a recent study we showed that *ABCB7* expression is markedly reduced in acquired RARS and RCMD-RS, and that its expression level is inversely related to the percentage of ring sideroblasts in these conditions.<sup>22</sup> Neither mutations nor hypermethylation of this gene have been observed in patients with acquired sideroblastic anemia, indicating that the *ABCB7* gene may be suppressed through other mechanisms and pathways.<sup>23,24</sup>

Gene expression studies of RARS CD34+ progenitors show a similar pattern to that observed in IFN- $\gamma$ -exposed normal progenitors. They also show altered expressions of heme biosynthesis and mitochondrial genes.<sup>25</sup> In these studies, *ALAS2* is one of the most significantly overexpressed genes (14 $\times$  compared to control). The CD34 compartment of MDS bone marrow is, however, heterogeneous and expression pattern may therefore reflect a different mix of progenitors depending on factors such as degree of erythroid hypo versus hyperplasia. To specifically address the expression pattern in differentiating erythroblasts we performed gene expression profiling on cultured RARS and normal intermediate erythroid progenitors and showed that *ALAS2* expression was close to normal in RARS erythroblasts, while *ABCB7* down-regulation was even more pronounced than in the CD34+ compartment.<sup>24</sup>

There is accumulating evidence that mitochondria play a central role in the pathophysiology of ineffective erythropoiesis in MDS. Electron microscopy has demonstrated pronounced ultra-structural mitochondrial changes not only in RARS but also in other types of MDS.<sup>26</sup> In addition, mitochondrial DNA mutations, however of uncertain significance have been reported in MDS.<sup>27,28</sup> Studies of an *in vitro* model of erythroid differentiation showed that early erythroid progenitor cells from low-risk MDS spontaneously release excessive amounts of cytochrome *c* from mitochondria, resulting in activation of caspase-9, and subsequent

cell death.<sup>29</sup> Inhibition of caspase-9 activity abrogated the enhanced sensitivity to Fas-ligation, hence, the increased sensitivity of MDS progenitor cells to death receptor stimulation seems to be due to a constitutive activation of the mitochondrial axis of the apoptotic signaling pathway in these cells. Other investigators have shown involvement of the Fas-caspase-8 pathway and FADD-mediated erythroid apoptosis.<sup>30</sup> It has also been shown that cultured erythroblasts in RARS accumulate mitochondrial ferritin already during early differentiation.<sup>31</sup>

In spite of the isolated anemia and erythroid dysplasia observed in RARS, apoptosis is initiated at the stem cell or common myeloid progenitor level.<sup>32</sup> Moreover, Marrow CD34+ cells are clonal, as assessed by HUMARA analysis, also in pure sideroblastic anemia (WHO-RARS), further confirming that this disorder is a clonal stem cell disorder and that the initial pathogenetic event occurs in multipotent stem cells.<sup>33</sup> Thus, the genetic defects present in RARS must explain the proliferative advantage leading to expansion of the clone, as well as the ineffective erythropoiesis and abnormal erythroid iron metabolism leading to accumulation of the metal in the mitochondria of immature red cells. These features could be explained by several mutations, which is likely, or by a genetic alteration with differential effects on the hemopoietic stem cell and differentiating erythroid cells.

#### JAK2 and MPL mutations in MDS and mixed MDS/MPN

Mutations of *JAK2*, located at chromosome 9p, and *MPL*, located at 1p, are implicated in the pathogenesis of Philadelphia-negative chronic myeloproliferative disorders. The occurrence of *JAK2* and of *MPL* mutations in the multipotent stem cells of these disorders generates a myeloid clone that expands to replace hematopoietic cells without the mutation.<sup>34–36</sup> The discovery of conditions with overlapping features between myelodysplastic syndromes and myeloproliferative disorders resulted in a new field of study and a number of investigators have explored the mutational status of these genes also in patients with MDS and MDS/MPN. Szpurka et al.<sup>37</sup> reported on 57 patients with mixed MDS/MPN and found that 11 of them, and in particular RARS-T patients carried *JAK2* (V617F). This initial study were followed by a number of subse-

quent reports on *JAK2* and *MPL* mutations in MDS and mixed MDS/MPN, as listed in Table 2.<sup>33,37–48</sup> The overall conclusion is that *JAK2* mutations occur in around 50% of patients with RARS-T, but is exceedingly rare in RARS with a normal platelet count. Moreover, *JAK2* mutations are also observed in a proportion of patients with CMML and unclassified mixed MDS/MPN, but are rare in MDS in general. Two larger studies have recently shed more light over the clinical implications of such mutations. Raya et al.<sup>46</sup> described 76 RARS patients, of which 61% of patients with platelet counts  $>600 \times 10^9/l$  had *JAK2* mutations. Mutations were also found in a smaller proportion of patients with platelet counts  $500–600 \times 10^9/l$ , but were not found in 23 patients with a platelet count  $<500 \times 10^9/l$ . *JAK2* (V617F) positive patients had significantly higher hemoglobin levels and white blood cell counts, but did not differ with regard to survival or marrow fibrosis compared with the *JAK2* negative patients ( $p = 0.38$ ). Recently, Malcovati et al.<sup>33</sup> described 187 patients with  $\geq 15\%$  marrow ringsideroblasts and/or a platelet count  $>450 \times 10^9/l$  as part of a myeloid neoplasm. Nineteen patients fulfilled the criteria for RARS-T according to the WHO 2008 classification. *JAK2V617F* mutations were found in 53% of RARS-T patients, and in 2/5 patients with MDS/MPN-unclassified. Two of these patients had in addition also a *MPL* mutation, and one patient had a *JAK2* exon 12 mutation only. This study indicated that anemia was significantly less severe, however not abrogated in MDS patients with *JAK2* or *MPL* mutations, and that WBC counts were higher in this cohort. An additional study investigated *JAK2* status in 97 cases with MDS and del(5q).<sup>40</sup> A mutation was found in six cases, all with isolated del(5q). These patients had higher platelet counts, but did otherwise not differ from patients without the mutation. A recent case report described a patient with *JAK2* mutation and 5q- aberration detected in genetically discordant clones, indicating an underlying genetic course for both these events<sup>49</sup>.

#### JAK2 mutations in MDS and MDS/MPN with significant marrow fibrosis

Chronic myeloid malignancies with marked fibrosis other than classical primary myelofibrosis constitute a diagnostic challenge. Morphological estimation of blast percentage and dysplasia is usu-

**Table 2**  
*JAK2* and *MPL* mutations in myelodysplastic syndromes and mixed myelodysplastic/myeloproliferative neoplasms.

Authors	Proportion of cases positive for <i>JAK2</i> (V617F)	<i>MPL</i> mutations
Szpurka et al. (2006) <sup>37</sup>	6/9 RARS-T 3/26 MDS/MPN, U 2/22 CMML	Not studied
Remacha et al. (2006) <sup>41</sup>	6/9 RARS-T	Not studied
Wang et al. (2006) <sup>43</sup>	6/12 RARS-T with PLT count $\geq 600 \times 10^9/l$ 0/19 RARS-T with PLT count $<600 \times 10^9/l$ 0/11 MDS/MPN, U	Not studied
Boissinot et al. (2006) <sup>38</sup>	5/16 RARS-T	Not studied
Ceesay et al. (2006) <sup>39</sup>	4/6 RARS-T	Not studied
Renneville et al. (2006) <sup>42</sup>	5/7 RARS-T 2/15 CMML	Not studied
Gattermann et al. (2007) <sup>44</sup>	9/10 RARS-T	Not studied
Schnittger et al. (2008) <sup>48</sup>		<i>MPL</i> (W515) mutation in a case with features of both ET and RARS-T
Schmitt-Graeff et al. (2008) <sup>47</sup>	11/23 RARS-T	<i>MPL</i> (W515) mutation in one <i>JAK2</i> (V617F)-negative patient with RARS-T
Raya et al. (2008) <sup>46</sup>	14/23 (61%) RARS-T with PLT count $\geq 600 \times 10^9/l$ 3/24 RARS-T with PLT count $400–600 \times 10^9/l$ (all had plt count $>500 \times 10^9/l$ )	Not studied
Olsen et al. (2008) <sup>45</sup>	0/14 MDS-F, 18/19 MPN/MPN-AML with fibrosis, 0/2 CMML	Not studied
Malcovati et al. (2009) <sup>33</sup>	11/19 RARS-T with PLT count $\geq 450 \times 10^9/l$ ; 10 <i>JAK2V617F</i> , 1 exon 12	2 pts with <i>JAK2V617F</i> had also <i>MPL</i> mutation
Ingram et al. (2006) <sup>40</sup>	6/97 MDS with del(5q)	Not studied

CMML = chronic myelomonocytic leukemia.

MDS/MPN, U = myelodysplastic/myeloproliferative neoplasm, unclassifiable.

RARS-T = refractory anemia with ring sideroblasts associated with marked thrombocytosis.

**Table 3**

TET2 mutations in myelodysplastic syndromes and mixed myelodysplastic/myeloproliferative neoplasms.

Authors	Proportion of cases positive for TET2 mutations	Association of TET2 mutation with prognosis of MDS
Delhommeau et al. (2009) <sup>56</sup>	15/81 MDS 2/9 CMML 24/198 MPN 21/181 JAK2 V617F+ MPN	Not studied
Langemeyer et al. (2009) <sup>60</sup>	1/6 MPL(W515)+ MPN 27/102 MDS 4 homozygous 1 hemizygous 22 heterozygous	Mutation detected in IPSS Low (41%) INT-1 (27%) INT-2 (13%) High (14%) Not studied
Tefferi et al. (2009) <sup>62</sup>	3/15 CMML 1/16 MDS 3/7 AML evolving from MDS or MDS/MPN	Not studied
Jankowska et al. (2009) <sup>57</sup>	21/68 pts 3/14 MDS (all with RS) 11/30 MDS/MPN 7/14 MDS/MPN evolving to AML	Trend towards higher transformation rate. No association with survival
Mohamedali et al. (2009) <sup>61</sup>	5/9 MDS with 4q UPD	Not studied
Kosmider et al. (2009) <sup>58</sup>	22/96 MDS	Mutation associated with better overall, leukemia-free and event-free survival
Kosmider et al. (2009) <sup>59</sup>	44/88 CMML 18/43 pts studied at diagnosis	Poorer survival in CMML-1
Flach et al. (2009) <sup>63</sup>	5/19 RARS-T, 3 of which also had JAK2 mutation	Not studied
Abdel-Wahab et al. (2009) <sup>55</sup>	3/15 CMML, 1/16 MDS	Not studied

ally difficult due to insufficient quality of the bone marrow smears, which makes it difficult to verify a diagnosis of MDS. Severe fibrosis may also hamper the possibility to achieve a high-quality cytogenetic analysis. The observed incidence of marrow fibrosis in MDS varies due to difference in staining methods and interpretation, as well as differences between the analyzed cohorts, but is perceived to be around 10–15%.<sup>50</sup> Several publications report on a poorer survival and response to therapy in patients with significant marrow fibrosis.<sup>50–52</sup> The latter large epidemiological study by Della Porta et al.<sup>52</sup> convincingly showed that moderate to severe marrow fibrosis in MDS also is independently associated with a poorer overall survival and should be considered in the decision-making process before curative therapeutic regimens.

Recently, Olsen et al.<sup>45</sup> reported on a cohort of 45 patients with hematological malignancies including MPN, MDS, and acute leukemia and with a significant degree of marrow fibrosis. Patients underwent a thorough clinical and morphological re-evaluation with regard to consensus diagnosis and, separately, an analysis for JAK2 mutational status. Interestingly, while patients who were reclassified as having myeloproliferative neoplasms or MDS/MPN had a very high frequency of mutations, none of the fourteen patients classified and MDS with severe fibrosis (MDS-F) carried the mutation. It is possible therefore that JAK2 analysis could be used as a differential diagnostic tool in patients with MDS or MDS/MPN like features and marrow fibrosis.

### Biological implications of JAK2 and MPL mutations in RARS

The recent publication by Malcovati et al. has shed considerable light over the biology of JAK2 mutations in MDS.<sup>33</sup> First, the authors reported 3 patients who evolved from RARS with normal platelet counts to RARS-T. Two of these were analyzed for JAK2 mutational status and were initially negative, but developed small JAK2 mutated clones at the time of transformation. The opposite evolution, acquisition of ring sideroblasts in patients with MPN with thrombocytosis has not yet been reported. Next, X-chromosome inactivation patterns (XCIP) were analyzed in 8 female patients with RARS-T and confirmed clonal XCIP in 7 of these. The proportion of clonal granulocytes ranged from 95% to 100%. Three of these patients were JAK2 positive, but interestingly, the proportion of JAK2 positive cells was much smaller, ranging from 11% to 55%. To-

gether, these findings support the conclusion that JAK2 (and MPL) mutations most likely are secondary to other genetic events responsible for (initial) clonal preference and sideroblast formation. Hence, there is likely to be a biological mechanism that predisposes RARS patients to acquire JAK2 (V617F) or MPL mutations.

RARS and RARS-T CD34+ progenitors were also compared by gene expression profiling. While there were no difference in the expression of ALAS2 and ABCB7, there were genes of yet unknown importance, which differed between the two groups. However, no difference was observed between JAK2 wild type and mutated RARS-T.

Allelic burden of JAK2 mutations differ between different MPN subtypes and may increase with time. Schmitt-Graeff et al.<sup>47</sup> evaluated JAK2 (V617F) status in patients with RARS-T and showed that the allelic ratio was above 50% in six cases, indicating the presence of cells homozygous for the mutation. In two of these, transition from JAK2 (V617F) heterozygosity to homozygosity accompanied by rising platelet counts was documented.

Aberrant STAT5 phosphorylation may be an indicator of dysregulated signal-transduction pathways in MPD.<sup>53</sup> Szpurka et al. then studied patients with RARS-T and showed that JAK2 or MPL mutations were predictive for the presence of STAT5 phosphorylation, assessed by immunohistochemistry. The authors speculate that the patients without identified mutations may harbor other molecular lesion associated with STAT5 signaling.<sup>54</sup>

### Clinical implications of JAK2 mutational status in MDS

The current literature does not provide much information as regard to the outcome of patients with RARS-T, partly because of the recent identification of the syndrome and the still unsettled diagnostic criteria. Compared to patients with essential thrombocythemia, patients with RARS-T have a worse outcome, more similar to that of patients with RARS in general. One study described 23 patients with RARS-T of which eleven had JAK2 mutations. There was no AML evolution in this group, and a better survival compared to patients without JAK2 mutation.<sup>47</sup> By contrast, a recent relatively large report failed to show any difference in survival between JAK2 positive and negative patients, in spite of significantly more myeloproliferative features in the former group.<sup>46</sup> Other reports have observed AML transformation and progressive disease also

in *JAK2* positive RARS-T. Some studies have indicated less severe anemia in mutated patients, while others see no difference.

A better understanding of the prognosis and management of RARS-T and other *JAK2* positive MDS would require more detailed investigation of patients in this category. In order to gain more information, a Perl's staining on bone marrow aspirate should be performed in patients with myeloid neoplasm and thrombocytosis either carrying or not mutations of *JAK2* or *MPL*. In analogy, analysis of *JAK2* mutational status should be performed in RARS patients with an elevated platelet count and/or with significant marrow fibrosis.

### **TET2 mutations in MDS and mixed MDS/MPN**

Recently, a number of breakthrough publications have described mutations of the *TET2* gene (*Ten–Eleven Translocation-2 gene*) in a substantial fraction of MPN and MDS after first identifying a region on 4q frequently affected by uniparental disomy (UPD) (Table 3).<sup>55–63</sup> Delhommeau et al.<sup>56</sup> described somatic mutations in the *TET2* gene in 12% of patients with myeloproliferative disorders, 19% of MDS and 22% of CMML, and showed in a series of elegant experiments that HSC harboring the mutation had a growth advantage compared to normal HSC. They also demonstrated that the *TET2* mutation preceded *JAK2* mutation in five cases with longitudinal follow-up samples. Langemeijer studied a larger cohort of 102 patients with MDS and demonstrated *TET2* mutations in 26% of these individuals, four of which had homozygous and one a hemizygous mutation.<sup>60</sup> A smaller cohort was investigated for germ-line mutations but found negative. Mutations were found in the majority of cells, indicating that it is an early event, and they also identified a number of patients with more than one *TET2* mutation. Furthermore, *TET2* mRNA expression was reduced in the neutrophils of all MDS patients including those with no mutations, suggesting the possibility of non-mutational suppression of *TET2*. In this cohort, *TET2* mutations were more common in IPSS low (41%) and INT-1 risk (27%) risk MDS than in patients with INT-2 (13%) and high-risk (14%) MDS. A favorable impact of *TET2* mutations was also supported by a study of Kosmider in a series of 96 patients demonstrating better survival and a lower incidence of AML transformation in mutated patients.<sup>58</sup> Jankowska et al.<sup>57</sup> on the other hand, demonstrated the highest frequency of mutations, 37%, in patients with mixed MDS/MPN including CMML and sAML, while the incidence in other types of MDS was only 14%, all of which had  $\geq 15\%$  ring sideroblasts in the bone marrow. This study showed a trend towards a higher transformation rate in the mutated group and male patients had a significantly higher mutation rate than female patients. Kosmider et al.<sup>59</sup> reported a series of 88 CMML patients, of which 50% had mutations (42% of those investigated at diagnosis). *TET2* mutations in patients with CMML-1 was associated with poorer survival in this study. Interestingly, although *TET2* mutations seems to precede *JAK2* mutations, mutated *TET2* was only found in 2 out of 14 patients with CMML transforming to AML, indicating the presence of even earlier genetic events. Flach et al.<sup>63</sup> reported mutations in 26% of a cohort of RARS-T patients. The cellular function of *TET2* yet remains to be clarified, but a recent paper indicate that a related gene, *TET1* mediates the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, and consequently is involved in epigenetic control of gene expression. Hence, *TET2* mutations could hypothetically enhance DNA methylation and potentially provide a growth advantage of the mutated cells.<sup>64</sup>

*TET2* is the so far most frequently mutated gene in MDS and MDS/MPN, and may help to understand the stem cell biology of at least some of its subtypes. It is likely that the mutated form in some way contributes to growth advantage of the malignant clone. Whether or not it contributes to progression or leukemic transfor-

mation is less certain and there is no evidence that it directly is involved in the typical anemia of MDS or formation of ring sideroblasts in MDS and mixed MDS/MPN.

### **Other recently identified mutations in MDS and MDS/MPN**

A recently identified mutation in MDS/mixed MDS/MPN involves the C-cbl E3 ubiquitin ligase gene (*CBL*) located on chromosome 11q. The gene is a negative regulator of proliferative signals through activated receptor tyrosine signals and mutations may thus be associated with a growth advantage of the clonal cells. The group of Maciejewski first identified this mutation in 7 of 12 patients with myeloid malignancies and UPD 11q.<sup>65</sup> They then confirmed the mutation in 5% and 9% of patients with CMML and secondary AML, respectively. Mutations were associated with a short median survival and progression to AML.<sup>66</sup> Reindl et al.<sup>67</sup> confirmed the presence *CBL* mutations in a few percent of 116 patients with MDS and AML. They also performed functional studies showing that the mutation induced growth factor-independent proliferation by a constitutive activation of the *Flt3* pathway and suggested that *Flt3* inhibitors may be active in patients with this mutation.

Another gene, that may be more commonly mutated than *CBL* is the polycomb-associated gene *ASXL-1*, which is involved in the regulation of chromatin remodeling. Gelsi-Boyer et al.<sup>68</sup> analyzed 82 patients; 35 with MDS and 39 with CMML, and a few with AML. *ASXL-1* mutations were found in 11% of MDS/AML and in as much as 43% of the CMML cases. Another recent study<sup>69</sup> supports these findings and has identified mutations in 15% and 33% of MDS and CMML, respectively.

### **Management of RARS-T and JAK2 och TET2 positive MDS and MDS/MPN**

While MDS of the RA and RCMD subtypes may respond to immunosuppressive therapy, MDS with a significant amount of ring sideroblasts almost universally fail to respond to such treatment. For these entities, EPO  $\pm$  G-CSF should be considered the primary treatment in case of symptomatic anemia.<sup>70,71</sup> In the Nordic G-CSF-EPO treated cohort of 129 patients with MDS and <10% marrow blasts<sup>71,72</sup> we identified four patients with pre-treatment platelet counts  $>450 \times 10^9/l$  ( $452\text{--}585 \times 10^9/l$ , unpublished observations). This group and a small series of additional patients (personal observations) did not differ from RARS/RCMD-RS regarding response rate and duration, and there are no other reports suggesting that RARS-T patients, with or without *JAK2* mutations differ in their response to growth factors compared to other low-risk MDS. Hence, the suggestion based on limited data is that EPO  $\pm$  G-CSF should be attempted as treatment for symptomatic anemia according to the same guidelines that are used for MDS in general. *JAK2* mutations do not seem to affect overall outcome in RARS or CMML but there are to date not enough information to draw valid conclusions. The addition of *JAK2* mutational analysis as a study parameter in clinical trials of MDS and CMML patients may help to create patient cohorts that in the future could define the clinical impact of this mutation.<sup>73</sup>

The publications cited in this review give varying views on the impact of *TET2* mutations on survival and transformation rate of MDS and CMML. While two studies suggested a favorable outcome,<sup>58,60</sup> a third study<sup>57</sup> and a couple of preliminary reported larger studies seem to point towards an overall negative impact of survival and probability of remaining in stable phase. In this review only peer-reviewed articles have been cited. Whether *TET2* mutation in the future should be included as a diagnostic test – or as a basis for therapeutic decision-making – in MDS will have to be assessed in expanded multivariate analyses.

There is no published evidence that the thrombocytosis in RARS-T is associated with an elevated risk for thrombotic complications, and there is little reason to believe that treatment of very high platelet counts in RARS-T should differ from that of essential thrombocythemia. Clinical experience suggests that antiproliferative drugs, such as hydroxyurea may be of use also in these conditions, but no study has yet focused on this particular group of patients. If there is a role for the new *JAK2* inhibitors remains to be studied, but available data do not support that the myeloproliferative component of RARS-T contributes significantly to the morbidity of these patients.

## Conclusions

The majority of recent clinical breakthroughs for patients with hematological malignancies have started with the identification of new genetic or biological alterations and the last years' explosion of new mutational discoveries in MDS and MDS/MPN, will certainly provide possibilities to understand the biology of these disorders. In order to test the significance of the herein described early results, it is important to perform confirmatory analyses on unselected cohorts of well characterized patients with adequate follow-up for survival and leukemic transformation and with longitudinal biological sampling. More mutations are likely to be found within the next years, but the main scientific task will be to sort out the hierarchy between them, and to understand their individual functions. Also, the network connecting genetic and epigenetic alterations with abnormalities within the immune system needs to be worked out. Functional studies are still largely missing and considerable efforts in terms of cellular and animal models will be required to establish if any of the new mutations may function as targets for existing biological drugs, and to test the effects of new compounds.

## Conflict of interest statement

None to declare.

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