# The Role of JAK2 Mutations in RARS and Other MDS

*Eva Hellström-Lindberg[1] and Mario Cazzola[2]*

*[1]Karolinska Institutet, Stockholm, Sweden; [2]University of Pavia and Fondazione IRCCS Policlinico San Matteo, Pavia, Italy*

**Acquired sideroblastic anemia with unilineage dysplasia (WHO RARS) is a clonal stem cell disorder characterized by erythroid dysplasia, mitochondrial accumulation of mitochondrial ferritin, defective erythroid maturation and anemia. A fraction of these patients also show elevated platelet counts; since 2001 this has been defined as RARS with marked thrombocytosis (RARS-T). It has recently been described that around half of RARS-T patients, along with a small subset of other MDS and mixed myelodysplastic/myeloproliferative disorders, carry the *JAK2* mutation, and that *MPL* mutations are found in single patients. Clinically, RARS-T patients show features of both RARS, essential thrombocythmia (ET) and to some extent also myelofibrosis. However, the degree of anemia and overall survival is more similar to RARS than myeloproliferative disorders. The occurrence of JAK2 mutations and features of ET in RARS is too frequent to be the result of chance only, and it is possible that this link may provide a key to an increased understanding of the genetic abnormalities causing ring sideroblast formation.**

## Myelodysplastic Syndromes

Myelodysplastic syndromes (MDS) are characterized by cytopenia due to ineffective hemopoiesis and an increased risk for leukemic evolution. Erythroid failure resulting in anemia is the most common feature in MDS and may range from mild, with only slightly decreased hemoglobin levels and increased red cell volume, to severe, with a complete inability to produce red blood cells. Defective erythropoiesis in MDS could be divided into a hypoproliferative/suppressed and a hyperproliferative/ineffective form.[1] Hypoproliferative erythropoiesis, characterized by a decreased relative number of erythroid progenitors in the bone marrow, is typically seen in advanced MDS, in hypoplastic MDS, in some cases with 5q- syndrome, and in MDS with severe marrow fibrosis. Ineffective hyperproliferative erythropoiesis is characterized by an increased percentage of marrow erythroblasts, of which many undergo intramedullary apoptosis before they mature into erythrocytes. This type of erythropoiesis is typically observed in refractory anemia with ringed sideroblasts (RARS), but is also common in a subset of refractory anemia (RA) and sometimes in RA with excess blasts (RAEB) with a moderate increase of marrow blasts.

The WHO classification from 2001[2] has recently been revised, and the updated classifications of MDS and mixed MDS/MPD are available as working documents. A new subgroup; refractory cytopenia with unilineage dysplasia was suggested, encompassing three entities; RA, refractory neutropenia and refractory thrombocytopenia. The 5q- syndrome subgroup has been renamed "MDS associated with isolated del(5q)," but still requires < 5% marrow blasts. The classification of "mixed myelodysplastic/myeloproliferative neoplasms (mixed MDS/MPN) was also updated. Within this group remains the provisional entity RA with ringed sideroblasts and marked thrombocytosis (RARS-T), defined by < 5% marrow blasts, ≥ 15% ringed sideroblasts and a persistent platelet count of $> 600 \times 10^9$/L. In the 2008 proposal, the cut-off value for thrombocytosis was decreased to $450 \times 10^9$/L, which may be problematic as the majority of published articles on the subject used the higher cut-off level. The revised WHO classification of MDS and mixed MDS/MPN are outlined in **Tables 1** and **2**.

## Refractory Anemia with Ringed Sideroblasts

The sideroblastic anemias constitute a heterogeneous group of inherited and acquired disorders characterized by anemia of varying severity and the presence of ringed sideroblasts in the bone marrow.[3] Ringed 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.[4] The presence of ringed sideroblasts in the bone marrow (15% or more of erythroblasts) is a marker of the myelodysplastic syndromes subgroup RARS, refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS), and RARS-T.[2]

The division of the previous French-American-British (FAB) RARS subgroup into the WHO subgroups RARS and RCMD-RS has significant clinical relevance. RARS patients do not show pancytopenia and have an overall good prognosis, with a 5-year survival well above 50% and a very low risk for transformation to AML.[5] By con-

**Table 1. Proposal for updated WHO classification of myelodysplastic syndromes 2008**

| Disease | Blood findings | Bone marrow findings |
|---|---|---|
| Refractory cytopenias with unilineage dysplasia (RCUD):<br>Refractory anemia (RA)<br>Refractory neutropenia (RN)<br>Refractory thrombocytopenia (RT) | Unicytopenia or bicytopenia*<br>No or rare blasts (<1%)† | Unilineage dysplasia; ≥10% of the cells of the affected lineage are dysplastic<br>< 5% blasts<br>< 15% of the erythroid precursors are ringed sideroblasts |
| Refractory anemia with ringed sideroblasts (RARS) | Anemia<br>No blasts | Erythroid dysplasia only<br>≥ 15% of erythroid precursors are ringed sideroblasts<br>< 5% blasts |
| Refractory cytopenia with multilineage dysplasia (RCMD) | Cytopenia(s)<br>No or rare blasts (<1%)†<br>No Auer rods<br>$< 1 \times 10^9$/L monocytes | Dysplasia in ≥ 10% of cells in two or more myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes)<br>< 5% blasts<br>No Auer rods<br>± 15% ringed sideroblasts |
| Refractory anemia with excess blasts-1 (RAEB-1) | Cytopenia(s)<br>< 5% blasts†<br>No Auer rods<br>$< 1 \times 10^9$/L monocytes | Unilineage or multilineage dysplasia<br>5-9% blasts†<br>No Auer rods |
| Refractory anemia with excess blasts-2 (RAEB-2) | Cytopenia(s)<br>5-19% blasts<br>Auer rods ± ‡<br>$< 1 \times 10^9$/L monocytes | Unilineage or multilineage dysplasia<br>10-19% blasts<br>Auer rods ± |
| Myelodysplastic syndrome – unclassified (MDS-U) | Cytopenias<br>≤ 1% blasts† | Unequivocal dysplasia in less than 10% of cells in one or more myeloid cell lines<br>< 5% blasts† |
| MDS associated with isolated del(5q) | Anemia<br>Usually normal or increased platelet count<br>No or rare blasts (< 1%) | Normal to increased megakaryocytes with hypolobated nuclei<br>< 5% blasts<br>Isolated del(5q) cytogenetic abnormality<br>No Auer rods |

*Bicytopenia may occasionally be observed. Cases with pancytopenia should be classified as MDS-U

† If the marrow myeloblast percentage is < 5% but there are 2% to 4% myeloblasts in the blood, the diagnostic classification is RAEB-1. If the marrow myeloblast percentage is < 5% and there are 1% myeloblasts in the blood, the case should be classified as MDS-U.

‡ Cases with Auer rods and < 5% myeloblasts in the blood and < 10% in the marrow should be classified as RAEB-2.

trast, patients with RCMD-RS have a 5-year survival of 37%, a cumulative risk for AML transformation of 9%, and a substantial risk for developing more advanced MDS.[6] The two subgroups also differ in their response to treatment with erythropoietin (EPO) plus granulocyte colony-stimulating factor (G-CSF), as described below.[7] The different clinical profiles of RARS and RCMD-RS indicate that at least partly different biological mechanisms are active in these disorders.

## Biology of Acquired Sideroblastic Anemia

Ringed sideroblast formation may be caused by exogenous factors, such as lead intoxication and treatment with isoniazid, both of which inhibit δ-aminolevulinic acid (ALA) dehydratase activity, block hemoglobin formation and cause ringed sideroblast formation.[3]

A number of hereditary conditions are associated with ringed sideroblast formation. The most common of these inherited forms is X-linked sideroblastic anemia (OMIM 301300), which is caused by mutations in the erythroid-specific ALA synthase gene (*ALAS2*).[3] Defective ALAS2 enzyme activity in bone marrow erythroid cells leads to insufficient protoporphyrin IX synthesis, mitochondrial iron overload, and intramedullary death of red cell precursors. Most but not all XLSA patients are, to a variable extent, responsive to pyridoxine, which is metabolized to pyridoxal phosphate.

Another interesting inherited condition is the X-linked sideroblastic anemia associated with cerebellar ataxia (XLSA/A; OMIM 301310), characterized by neurological manifestations early in infancy with impaired gross motor and cognitive development. This X-linked sideroblastic anemia differs clinically from the classic XLSA (OMIM 301300), which does not have neurological manifestations, is associated with iron overload, and is generally at least partially responsive to pyridoxine. XLSA/A is caused by

**Table 2. Proposal for updated WHO classification of myelodysplastic/myeloproliferative neoplasms.**

| Disease | Blood findings | Bone marrow findings |
|---|---|---|
| Chronic myelomonocytic leukemia (CMML) | Peripheral blood monocytosis > $1 \times 10^9$/L<br>No BCR/ABL-1 fusion gene<br>< 20% blasts | Dysplasia in one or more myeloid lineage*<br>< 20% blasts. Blasts include myeloblasts, monoblasts and promonocytes.<br>No rearrangement of *PDGFRA* or *PDGFRB* |
| Atypical chronic myeloid leukemia, BCR-ABL1 negative (aCML) | Leukocytosis, Neutrophilia<br>Neutrophilic dysplasia<br>Neutrophil precursors ≥ 10% of leukocytes<br>Blasts < 20%<br>No BCR-ABL1 fusion gene<br>No rearrangement of *PDGFRA* or *PDGFRB*<br>Minimal basophilia<br>Monocytes < 10% of leukocytes | Neutrophil dysplasia with or without other dysplastic lineages<br>< 20% blasts |
| Juvenile myelomonocytic leukemia (JMML) | Peripheral blood monocytosis > $1 \times 10^9$/L<br>< 20% blasts<br>Usually WBC > $10 \times 10^9$/L<br>< 20% blasts | < 20% blasts. Blasts include myeloblasts, monoblasts, and promonocytes. |
| Myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN) | Mixed MDS and MPN features<br>No prior diagnosis of MDS or MPN<br>No history of recent growth factor or cytotoxic therapy to explain MDS or MPN features<br>No BCR-ABL1 fusion gene or rearrangements of *PDGFRA or PDGFRB* | Mixed MDS and MPN features<br>< 20% blasts |
| † Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) (provisional entity) | Persistent thrombocytosis > $450 \times 10^9$/L<br>Anemia<br>*BCR-ABL1* negative<br>Cases with t(3;3)(q21;q26), inv(3)(q21q26), and isolated del (5q) are excluded | Morphologic features of RARS;<br>≥ 15% of erythroid precursors are ringed sideroblasts<br>Abnormal megakaryocytes similar to those observed in *BCR-ABL1*–negative MPN |

* If myelodysplasia is minimal or absent, CMML can still be diagnosed if the other requirements are met and there is an acquired clonal cytogenetic or molecular genetic abnormality present in the hematopoietic cells, or the monocytosis has persisted for at least 3 months and all other causes of monocytosis have been excluded.

† Provisional entity. In WHO classification from 2002 the cut-off for platelet count was $600 \times 10^9$/L. In the WHO 2008 classification the cut-off value is $450 \times 10^9$/L, to be in line with the classification of essential thrombocythemia. Around 50% of cases with RARS-T carry the JAK2 mutation, but this is not a diagnostic criterion.

missense mutations in the human *ABCB7* gene, which encodes a membrane-associated protein belonging to the superfamily of ATP-binding cassette (ABC) transporters.[8] The ABCB7 protein functions to enable transport of iron from the mitochondria to the cytoplasm. It has been reported that *ABCB7* is essential for hematopoiesis[9] and that RNA silencing of the gene in HeLa cells causes an iron-deficient phenotype with mitochondrial iron overload.[10] Moreover, in a recent study we showed that *ABCB7* expression is also markedly reduced in acquired RARS and RCMD-RS, and that its expression levels are inversely related to the percentage of ringed sideroblasts in these conditions.[11] However, no gene mutations were observed in this cohort of patients, indicating that the *ABCB7* gene may be suppressed via other factors and pathways.

A defective mitochondrial enzyme function of RARS bone marrow cells was suggested decades ago, and there is now 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.[12] In addition, the occurrence of mitochondrial DNA mutations has been reported in MDS; however, these are of uncertain significance.[13,14] 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.[15] 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 Fas-associated protein with death domain (FADD)-mediated erythroid apoptosis.[16] Recently it was also shown that cultured erythroblasts in RARS accumulate mitochondrial ferritin during early differentiation, long before morphological signs of erythroid differentiation are visible.[17]

In spite of the isolated anemia and erythroid dysplasia observed in RARS, apoptosis seems to be initiated at the stem cell level.[18] Moreover, stem cells have also been shown to be clonal, as assessed by HUMARA analysis, 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.[19] Thus, the genetic defects present in RARS must both give rise to a proliferative advantage leading to expansion of the clone, but also to ineffective erythropoiesis and abnormal erythroid iron metabolism, leading to accumulation of the metal in the mitochondria of immature red cells. It is likely that this defect would encompass gene(s) involved in erythroid differentiation, mitochondrial function, or cellular iron metabolism.[20]

## *JAK2* and *MPL* Mutations in Myelodysplastic Syndromes

Philadelphia chromosome–negative chronic myeloproliferative disorders (polycythemia vera, ET, primary myelofibrosis) are characterized by various combinations of erythrocytosis, leukocytosis and thrombocytosis, i.e., the opposite of the cytopenia found in MDS. These conditions are typical clonal disorders of hematopoietic stem cells. The occurrence of the unique V617F mutation of *JAK2* exon 14,[21-23] of several mutations of *JAK2* exon 12[24-25] and of *MPL* mutations[26-27] in a multipotent stem cell generates a myeloid clone that expands to replace hematopoietic cells without the mutation.

Although MDS and MPD appear to have entirely different pathophysiological mechanisms, the existence of conditions with overlapping features is well established. In 2002, Schmitt-Graeff and coworkers[28] published an interesting study on 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 MDS in the strict sense to MPD (ET, prefibrotic primary myelofibrosis).

Szpurka and coworkers[29] studied 57 patients with MDS/MPD and found that 11 of them carried *JAK2* (V617F). In particular, this mutation was detected in 6 of 9 patients with RARS-T, and the authors suggested that RARS-T constitutes another *JAK2* mutation–associated form of MPD. Several other studies on mutation analysis of *JAK2* and *MPL* in MDS/MPD have been published in the last 2 years,[30-38] and their findings are summarized in **Table 3**. Schmitt-Graeff and coworkers[37] have recently reported findings from a study evaluating *JAK2* (V617F) status in 23

**Table 3. JAK2 and MPL mutations in myelodysplastic syndromes and mixed myelodysplastic/myeloproliferative neoplasms.**

| Authors | Proportion of cases that were found to be positive for *JAK2* (V617F) | *MPL* mutations |
|---|---|---|
| Szpurka et al[29] | 6/9 RARS-T<br>3/26 MDS/MPD, U<br>2/22 CMML | Not studied |
| Remacha et al[30] | 6/9 RARS-T | Not studied |
| Wang et al[31] | 6/12 RARS-T with plt count $\geq 600 \times 10^9$/L<br>0/19 RARS-T with plt count $< 600 \times 10^9$/L<br>0/11 MDS/MPD, U | Not studied |
| Boissinot et al[32] | 5/16 RARS-T<br>(5/8 RARS-T with ET features) | Not studied |
| Ceesay et al[33] | 4/6 RARS-T | Not studied |
| Renneville et al[34] | 5/7 RARS-T<br>2/15 CMML | Not studied |
| Gattermann et al[35] | 9/10 RARS-T | Not studied |
| Schnittger et al[36] | — | *MPL* (W515) mutation in a case with features of both ET and RARS-T |
| Schmitt-Graeff et al[37] | 11/23 RARS-T | *MPL* (W515) mutation in 1 *JAK2* (V617F)-negative patient with RARS-T |
| Raya et al[38] | 14/23 (61%) RARS-T with plt count $\geq 600 \times 10^9$/L<br>3/24 RARS-T with plt count 400-$600 \times 10^9$/L<br>(all had plt count $> 500 \times 10^9$/L | Not studied |
| Ingram et al[39] | 6/97 MDS with del(5q) | Not studied |

Abbreviations: CMML, chronic myelomonocytic leukemia; ET, essential thrombocythemia; MDS/MPD, U, myelodysplastic/myeloproliferative disease, unclassifiable; RARS-T, refractory anemia with ringed sideroblasts associated with marked thrombocytosis

patients with RARS-T by using allele-specific PCR. The mutation was detected in 11 of 23 patients, and in 6 patients with RARS-T the allelic ratio of *JAK2* (V617F) was above 50%, indicating the presence of cells homozygous for the mutation. Interestingly, in 2 of these latter patients a transition from JAK2-V617F heterozygosity to homozygosity was documented, and this was accompanied by rising platelet counts in sequential samples. The *MPL* (W515L) mutation was detected in 1 *JAK2* (V617F)-negative patient. This study clearly indicates that RARS-T has several features of MPD in addition to overproduction of platelets, including striking megakaryocytic proliferation, leukocytosis, abnormalities of chromosomes 8 and 20, vascular events, and marrow fibrosis. A recent preliminary report of the hitherto largest series of 47 patients showed the JAK2 mutation in 61% of patients with a platelet count higher than $600 \times 10^9$/L, as compared with 12.5% in patients with a platelet count between 400 and $600 \times 10^9$/L.[38] All patients who were JAK2 positive had a platelet count higher than $500 \times 10^9$/L. Patients who were JAK2 positive had significantly higher hemoglobin levels and white blood cell counts, but no difference in survival or marrow fibrosis was observed ($P = .38$). Finally, an additional study investigated *JAK2* status in 97 cases with MDS and del(5q).[39] A mutation was found in 6 patients, all with an isolated del(5q). These patients displayed higher platelet counts, but otherwise no major differences compared with patients without the mutation.

The mechanisms of anemia in a *JAK2* (V617F)–positive RARS-T are unclear, considering that this mutation is normally associated with erythrocytosis. In order to properly answer this question, it should be first considered that the combination of ringed sideroblasts and *JAK2* (V617F) mutation is very unlikely in terms of probability, considering the low prevalence of both RARS and ET in the general population. This means that this combination cannot be simply coincidental. Thus, there must be a pathophysiological mechanism that predisposes RARS patients to acquire *JAK2* (V617F), or alternatively patients with *JAK2* (V617F)–positive myeloproliferative disorder to develop mitochondrial iron loading and ineffective erythropoiesis. Further studies are required to elucidate which of the two mechanisms is more likely.

### Diagnosis of RARS-T

Published studies on the subject are in agreement that patients with MDS and a *JAK2* mutation have significantly higher platelet counts than other MDS, and some, but not all, indicate that white blood cell counts and bone marrow cellularity also are elevated. **Figure 1** (see Color Figures, page 491) shows a representative bone marrow from a patient with RARS-T. It should be remembered that slightly elevated platelet counts (350 to $500 \times 10^9$/L) are common also in WHO-RARS. There is at present some confusion as to the best cut-off platelet count level for a diagnosis of RARS-T as compared to RARS. The 2001 WHO classification uses $600 \times 10^9$/L, while the 2008 classification suggests $450 \times 10^9$/L, to be in line with the criteria for ET (**Tables 1** and **2**). Most reports cite above have used a cut-off of $600 \times 10^9$/L, and a few have used $500 \times 10^9$/L, while a cut-off level of $450 \times 10^9$/L has not been evaluated. One study[31] failed to demonstrate any JAK2 mutations in RARS patients with a platelet count of 400 to $600 \times 10^9$/L and the recent report by Raya et al strongly supports the present cut-off of $600 \times 10^9$/L[38] (**Table 3**). We conclude that diagnostic criteria for RARS-T need to be better defined, as this disorder currently appears to represent a condition that is borderline to RARS, ET and primary myelofibrosis. As a practical recommendation, we suggest that a Perls staining on bone marrow aspirate should be performed in patients with myeloid neoplasm and thrombocytosis whether carrying mutations of *JAK2* or *MPL* or not. In analogy, analysis of JAK2 mutational status should be performed in RARS patients with an elevated platelet count.

### Treatment of the Anemia in MDS

The first-line treatment for the anemia of low- and intermediate-1 risk MDS is erythropoietic stimulating agents: erythropoietin or darbepoetin, with or without the addition of G-CSF, leading to an erythroid response rate of 40% to 50%. The median response duration in patients achieving normalized hemoglobin levels sis around 2 years, with 30 months in patients with a low IPSS score.[40] The combined information of pretreatment transfusion need (≤ 2 units/month) and serum erythropoietin level (≤ 500 U/L) predicts for a response to treatment, and while patients with low transfusion need and S-EPO levels show a response rate of 74%, the corresponding figure for those with a high transfusion need and S-EPO level is 7%.[41] Two retrospective studies comparing patients treated with EPO with or without the addition of G-CSF with untreated patients from the IPSS registry have demonstrated that treatment is safe and leads to no increased risk for leukemic transformation.[40,42] Moreover, the French study showed by univariate comparison that the survival of treated patients was better than that of the untreated cohort. We have recently performed an epidemiological study comparing treated and untreated but otherwise matched cohorts of patients with low- and intermediate-risk MDS enrolled from 1990 through 1999.[43] This study, which had information of and could control for all major risk factors, showed a very clear survival advantage for the EPO plus G-CSF–treated cohort (relative risk 0.61, 95% confidence interval 0.44-0.83), and also decreased risk of non-leukemic death (HR = 0.66, 95% CI 0.44-0.99, $P = .042$). There was no association with the risk of AML evolution (HR = 0.89, 95% CI 0.52-1.52, $P = .66$). A subgroup analysis revealed that EPO plus G-CSF treatment was associated with enhanced survival only in

patients receiving more than 2 units per month at start of observation ($HR_{<2\ U/month} = 0.44$, 95% CI 0.29-0.66, $P < .001$, $HR_{=2\ units/month} = 1.04$, 95% CI 0.57-1.89, $P = .91$). This group of patients corresponds well with the good prognosis group, according to the predictive model.[41]

### Treatment of RARS

Immunosuppressive treatment and thalidomide, which may be efficacious in subsets of RA, have proven relatively inefficacious in RARS.[42] Lenalidomide has been shown to abrogate transfusion need in around 25% of patients with EPO-refractory RARS, with a median duration of response of 43 weeks, and is presently evaluated in combination with EPO and other drugs in clinical trials.[43] Interestingly, while several reports have shown that the response rate to EPO in RARS is lower than the response in RA or early RAEB, the effect of the combination of EPO and G-CSF is better in RARS than in other subsets of MDS, with an overall and complete erythroid response rate of 50% and 38%, respectively.[39] RARS and RCMD-RS also showed significant differences in response to treatment with EPO+G-CSF.[43] The survival of patients with WHO RARS was 121+ months, with a response rate of 71% and a response duration of 28 months (8-116+ months). Interestingly, RCMD-RS patients showed a lower response rate, 30%, but a similar response duration (25 months, range 27-95) and a shorter survival, 31 months (14-43 months). No patients with WHO RARS developed AML as compared to 10% in the RCMD-RS cohort. Hence, treatment with EPO+G-CSF in patients with MDS and ringed sideroblasts is safe and leads to prolonged responses. It is, however, clear that patients with RARS show a higher response rate than those with RCMD-RS, 71% versus 30%, confirming previous results from a smaller study.[7] These response rates indicate that while the biology of RARS is relatively homogeneous, that of RCMD-RS shows a larger variation.

### Outcome and Management of RARS-T

The current literature does not provide good information on the outcome of this patient group, partly because of the recent identification of the syndrome and the still unclear diagnostic criteria. Compared to patients with ET, patients with RARS-T have a worse outcome, more similar to that of patients with RARS in general. A recent study reported that 11 of 23 patients with RARS-T had *JAK2* mutations, and that no AML evolution and a better survival were observed in this group compared to those without JAK2 mutation.[37] By contrast, a recent relatively large report[37] failed to show any difference in survival in the JAK2 cohort, despite significantly more myeloproliferative features. Other reports have observed AML transformation and progressive disease also in *JAK2*-positive RARS-T. Some studies have indicated a lower degree of anemia, while others see no difference. In our EPO+G-CSF–treated cohort we identified 4 patients with pretreatment platelet counts > $450 \times 10^9$/L (452-585 $\times$ $10^9$/L, unpublished observations). This small group did not differ from the RARS/RCMD-RS cohort regarding response rate and duration. There is no published evidence that the thrombocytosis in RARS-T is associated with an elevated risk for thrombotic complications, and there is no reason to believe that treatment of very high platelet counts in RARS-T should differ from that of ET. Thus, the available evidence is insufficient for recommending any specific treatment. Whether 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

More than 50 years have elapsed since Sven Erik Bjorkman described 4 patients with RA and accumulation of "iron granules" in the cytoplasm. These observations eventually led to the inclusion of acquired sideroblastic anemia in the classification of MDS. Although there has been progress as regard to the biology and biochemical alterations of this disorder, the mechanisms underlying accumulation of aberrant ferritin in the mitochondria and associated erythroid apoptosis and anemia still remain to be discovered. The occurrence of JAK2 mutations and thrombocytosis in RARS is too frequent to be the result of chance only, and it is possible that this link may provide a key to an increased understanding of the genetic abnormalities causing ringed sideroblast formation.

#### Disclosures

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Off-label drug use: None disclosed.

#### Correspondence

Eva Hellström-Lindberg, MD, PhD, Dept. of Med., Div. of Hem, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, S-14186, Sweden; Phone: 46 (8) 858582506; Fax: 46 (8) 87748725; e-mail: eva.hellstrom-lindberg@ki.se.

### References

1. Hellström-Lindberg E, Malcovati L. Supportive care, growth factors, and new therapies in myelodysplastic syndromes, Blood Rev. 2008;22:75-91.
2. WHO Classification Tumours of Haemopoietic and Lymphoid Tissues. Lyon: IARC Press; 2001.
3. Cazzola M, Invernizzi R. Sideroblastic anemias. In: Young NS, Gerson SL, High KA, eds. Clinical Hematology. Philadelphia: Mosby Elsevier; 2005: 721-732.
4. Cazzola M, Invernizzi R, Bergamaschi G, et al. Mitochondrial ferritin expression in erythroid cells from patients with sideroblastic anemia. Blood. 2003;101:1996-2000.
5. Cazzola M, Barosi G, Gobbi PG, Invernizzi R, Riccardi A, Ascari E. Natural history of idiopathic refractory sideroblastic

anemia. Blood. 1988;71:305-312.
6. Germing U, Gattermann N, Strupp C, Aivado M, Aul C. Validation of the WHO proposals for a new classification of primary myelodysplastic syndromes: a retrospective analysis of 1600 patients. Leuk Res. 2000;24:983-992.
7. Howe RB, Porwit-MacDonald A, Wanat R, Tehranchi R, Hellström-Lindberg E. The WHO classification of MDS does make a difference. Blood. 2004;103:3265-3270.
8. Shimada Y, Okuno S, Kawai A, et al. Cloning and chromosomal mapping of a novel ABC transporter gene (hABC7), a candidate for X-linked sideroblastic anemia with spinocerebellar ataxia. J Hum Genet. 1998;43:115-122.
9. Pondarre C, Campagna DR, Antiochos B, Sikorski L, Mulhern H, Fleming MD. Abcb7, the gene responsible for X-linked sideroblastic anemia with ataxia, is essential for hematopoiesis. Blood. 2007;109:3567-3569.
10. Cavadini P, Biasiotto G, Poli M, et al. RNA silencing of the mitochondrial ABCB7 transporter in HeLa cells causes an iron-deficient phenotype with mitochondrial iron overload. Blood. 2007;109:3552-3559.
11. Boultwood J, Pellagatti A, Nikpour M, et al. The role of the iron transporter *ABCB7* in refractory anemia with ring sideroblasts. PLoS ONE. 2008;3:e1970.
12. Van de Loosdrecht AA, Brada SJ, Blom NR, et al. Mitochondrial disruption and limited apoptosis of erythroblasts are associated with high risk myelodysplasia. An ultrastructural analysis. Leuk Res. 2001;25:385-393.
13. Gattermann N, Wulfert M, Junge B, Germing U, Haas R, Hofhaus G. Ineffective hematopoiesis linked with a mitochondrial tRNA mutation (G3242A) in a patient with myelodysplastic syndrome. Blood. 2004;103:1499-1502.
14. Shin MG, Kajigaya S, Levin BC, Young NS. Mitochondrial DNA mutations in patients with myelodysplastic syndromes. Blood. 2003;101:3118-3125.
15. Tehranchi R, Fadeel B, Forsblom AM, et al. Granulocyte colony-stimulating factor inhibits spontaneous cytochrome c release and mitochondria-dependent apoptosis of myelodysplastic syndrome hematopoietic progenitors. Blood. 2003;101:1080-1086.
16. Claessens YE, Park S, Dubart-Kupperschmitt A, et al. Rescue of early-stage myelodysplastic syndrome-deriving erythroid precursors by the ectopic expression of a dominant-negative form of FADD. Blood. 2005;105:4035-4042.
17. Tehranchi R, Invernizzi R, Grandien A, et al. Aberrant mitochondrial iron distribution and maturation arrest characterize early erythroid precursors in low-risk myelodysplastic syndromes. Blood. 2005;106:247-253.
18. Hellstrom-Lindberg E, Schmidt-Mende J, Forsblom AM, Christensson B, Fadeel B, Zhivotovsky B. Apoptosis in refractory anaemia with ringed sideroblasts is initiated at the stem cell level and associated with increased activation of caspases. Br J Haematol. 2001;112:714-726.
19. Della Porta MG, Malcovati L, Gallì A, et al. Mitochondrial ferritin expression and clonality of hematopoiesis in patients with refractory anemia with ringed sideroblasts [abstract]. Blood. 2005;106. Abstract #3444.
20. Nikpour M, Pellagatti A, Jädersten M, et al. Gene expression profiling of day 7 erythroblasts from RARS before and after treatment with G-CSF [abstract]. Blood 2007;110. Abstract #2427.
21. James C, Ugo V, Le Couédic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005;434:1144-1148.
22. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352:1779-1790.
23. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365:1054-1061.
24. Scott LM, Tong W, Levine RL, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med. 2007;356:459-468.
25. Pietra D, Li S, Brisci A, et al. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. Blood. 2008;111:1686-1689.
26. Pikman Y, Lee BH, Mercher T, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006;3:e270.
27. Beer PA, Campbell PJ, Scott LM, et al. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. Blood. 2008 May 1. Epub ahead of print.
28. Schmitt-Graeff A, Thiele J, Zuk I, Kvasnicka HM. Essential thrombocythemia with ringed sideroblasts: a heterogeneous spectrum of diseases, but not a distinct entity. Haematologica. 2002;87:392-399.
29. Szpurka H, Tiu R, Murugesan G, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2 V617F mutation. Blood. 2006;108:2173-2181.
30. Remacha AF, Nomdedeu JF, Puget G, et al. Occurrence of the JAK2 V617F mutation in the WHO provisional entity: myelodysplastic/myeloproliferative disease, unclassifiable-refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Haematologica. 2006;91:719-720.
31. Wang SA, Hasserjian RP, Loew JM, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis harbors JAK2 mutation and shows overlapping myeloproliferative and myelodysplastic features. Leukemia. 2006;20:1641-1644.
32. Boissinot M, Garand R, Hamidou M, Hermouet S. The JAK2-V617F mutation and essential thrombocythemia features in a subset of patients with refractory anemia with ring sideroblasts (RARS). Blood. 2006;108:1781-1782.
33. Ceesay MM, Lea NC, Ingram W, et al. The JAK2 V617F mutation is rare in RARS but common in RARS-T. Leukemia 2006;20:2060-2061.
34. Renneville A, Quesnel B, Charpentier A, et al. High occurrence of JAK2 V617 mutation in refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Leukemia. 2006;20:2067-2070.
35. Gattermann N, Billiet J, Kronenwett R, et al. High frequency of the JAK2 V617F mutation in patients with thrombocytosis (platelet count >600 x $10^9$/L) and ringed sideroblasts more than 15% considered as MDS/MPD, unclassifiable. Blood. 2007;109:1334-1335.
36. Schnittger S, Bacher U, Haferlach C, et al. Detection of an MPLW515 mutation in a case with features of both essential thrombocythemia and refractory anemia with ringed sideroblasts and thrombocytosis. Leukemia. 2008;22:453-455.
37. Schmitt-Graeff AH, Teo S-S, Olschewski M, et al. JAK2-V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Haematologica. 2008;93:36-43.
38. Raya JM, Arenillas L, Domingo A, et al. Anemia with ringed sideroblasts associated with thrombocytosis: clinical and analytical features according to the presence or absence of the JAK2 V617F mutation [abstract]. Haematologica. 2008;93 (suppl 1). Abstract #0716.
39. Ingram W, Lea NC, Cervera J, et al. The JAK2 V617F mutation identifies a subgroup of MDS patients with isolated deletion 5q and a proliferative bone marrow. Leukemia.

2006;20:1319-1321.
40. Jädersten M, Montgomery SM, Dybedal I, Porwit-MacDonald A, Hellström-Lindberg E. Long-term outcome of treatment of anemia in MDS with erythropoietin and G-CSF. Blood. 2005;106:803-811.
41. Hellström-Lindberg E, Gulbrandsen N, Lindberg G, et al; Scandinavian MDS Group. A validated decision model for treating the anaemia of myelodysplastic syndromes with erythropoietin + granulocyte colony-stimulating factor: significant effects on quality of life. Br J Haematol. 2003;120:1037-1046.
42. Park S, Grabar S, Kelaidi C, et al; GFM group (Groupe Francophone des Myélodysplasies). Predictive factors of response and survival in myelodysplastic syndrome treated with erythropoietin and G-CSF: the GFM experience. Blood. 2008;111:574-582.
43. Jädersten M, Malcovati L, Porwit-McDonald A, Cazzola M, Hellström-Lindberg E. Treatment with erythropoietin and G-CSF improves survival in MDS patients with low transfusion need. J Clin Oncol. 2008;26:3607-3613.