Mutations of *JAK2* and *TET2*, but not *CBL* are detectable in a high portion of patients with refractory anemia with ring sideroblasts and thrombocytosis

The WHO classification of 2008 characterized refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T) by the presence of less than 5% marrow blasts, 15% or more ring sideroblasts and a persistent platelet count over 450×10^9/L to be in line with the revised classification of essential thrombocytosis (ET). RARS-T forms a provisional entity with clinical and morphological features of both MDS and BCR-ABL negative myeloproliferative neoplasias (MPN).[1] Although the identification of the *JAK2*^{V617F} mutation was an important first step in distinguishing this entity from other hematologic diseases,[2] further genetic characterization is needed.

In order to gain further insights into the genetic markers specific for RARS-T, we performed comprehensive cytogenetic and molecular genetic investigations including *JAK2*^{V617F}, *TET2*, *CBL* and *MPL*^{W515}, markers common to MPN, as well as single-nucleotide polymorphism array analysis (SNP-A), which allows for the detection of both cryptic chromosomal changes as well as uniparental disomy (UPD). We analyzed a total of 23 RARS-T patients with platelet counts ranging from 466×10^9/L to 1500×10^9/L (median: 680×10^9/L). The median age of patients at initial diagnosis was 76.1 years (range: 46.8 to 86.8). Compared to a total cohort of 1,674 MDS patients analyzed in our laboratory, there was no difference in age (median age 71.5 years; range: 4.8-92.3 years). However, as compared to 239 ET patients, *t* test revealed that RARS-T patients were significantly older (median 62.2 years; P=0.005). A melting-curve based LightCycler analysis detected the presence of *JAK2*^{V617F} in 15 out of 19 analyzed patients (78.9%). Mutational ratios (*JAK2*^{V617F}/*JAK2*^{wt}) ranged from 0.05 to 1.4 (median 0.44) with ratios above a value of 1 considered as homozygous. The *JAK2*^{V617F} mutation was homozygous in 4/15 *JAK2*^{V617F} positive patients. Interestingly, higher platelet counts showed a tendency to higher mutational ratios. Those patients negative for *JAK2*^{V617F} were screened for *MPL*^{W515} mutations. None of these patients was found to have mutated *MPL*. Also, none of 19 analyzed patients carried mutations in exons 8 and 9 of *CBL*, which have been detected in a small number of MPN patients.[3] Conventional cytogenetics did not reveal any recurrent cytogenetic abnormalities in RARS-T patients. We found one case with loss of the Y chromosome, one patient with t(X;11)(p22;p13) and a potentially constitutional t(11;12)(q25;q14). Szpurka *et al.* reported UPD(9p) in one out of 18 RARS-T patients and UPD(1p) in 4 out of 18 RARS-T patients.[4] To gain further data, we performed SNP arrays in 10 patients but were not able to identify UPD in chromosomal loci containing the *JAK2* and *MPL* genes, 9p or 1p, respectively. Our SNP investigations did not detect additional recurrent chromosomal gains or losses nor did we observe recurring regions of UPD. However, one patient showed a deletion spanning a 1.3Mb region on the long

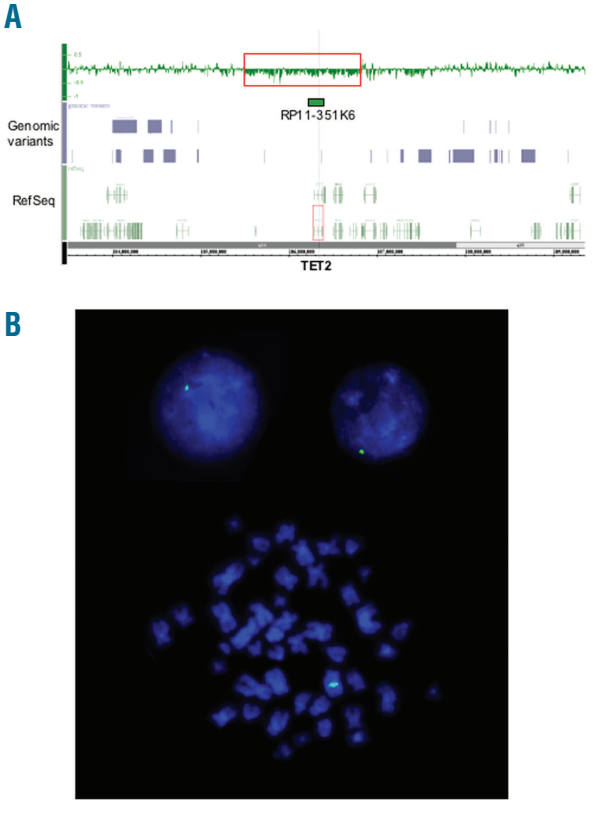

Figure 1. Deletion of *TET2*. (A) SNP-A karyogram (Affymetrix Genotyping Console V.3.0.1) of patient 4 showing the deleted region on the long arm of chromosome 4 (region 4q24) (red box). Genomic variants and RefSeq genes within this region are shown. The approximate location of probe RP11-351K6 is shown in green. (B) Fluorescence *in situ* hybridization on two interphase nuclei (top) and one metaphase (bottom) detects only one signal for probe RP11-351K6 spanning the *TET2* locus (patient 4). Blue: DAPI, Green: probe RP11-351K6 (BlueGnome, Cambridge, UK).

Table 1. Molecular details of *TET2* mutations found in 5 out of 19 RARS-T patients.

N.	Exon	Nucleotide Change	Consequence	Mutation type	Age	Sex	Plt×10^9/L	*JAK2*^{V617F}	SNP-A
1	3	3485_3486ins(A)	T1114NfsX15	Frameshift	77	F	529	Negative	N
2	6	C46T C52T	R1214W, R1216X	Missense Nonsense	79	M	680	Positive	N
3	7	C66G	S1290X	Nonsense	76	M	646	Negative	N
4	11	T356G	Y1631X	Nonsense	76	F	963	Positive	deletion 4q24
5	11	A441G	R1660G	Missense	76	F	501	Positive	N

Plt: platelets; N: no change detected; Note: exon counting in this study starts with exon 3 and ends with exon 11.

arm of chromosome 4 (start: 105,497,200 bp from pter; end; 106,825,780 bp from pter) (Figure 1A). Interestingly, the deleted region contained *TET2*, a gene recently found to be altered in many subtypes of myeloid malignancies[5-9] including 2 patients with RARS-T, of whom one showed a *TET2* missense and the other a frameshift mutation.[10] To further clarify the 4q24 deletion detected by SNP arrays, we performed fluorescence *in situ* hybridization (FISH). Twenty out of 100 analyzed interphase nuclei and three metaphases showed only one signal for the probe spanning the *TET2* gene in one patient (Figure 1B). Interphase FISH with the *TET2* probe was performed in 9 additional cases not analyzed by SNP arrays due to a lack of material. No additional case showing a deletion was detected. In addition to FISH, we performed *TET2* sequencing in 19/23 RARS-T. *TET2* mutations were detected in 5 out of 19 patients (26%), of which 3 out of 5 also presented mutated *JAK2*^{V617F}, whereas the remaining 2 out of 5 showed neither *JAK2*^{V617F} nor *MPL* nor *CBL* mutations. The 5 patients showed 6 individually different *TET2* mutations. Three were nonsense and two missense mutations. One patient displayed a frameshift mutation leading to a premature stop codon (Table 1). All mutations appeared to be heterozygous. The degree of homozygosity may, however, be underestimated due to a mixture of homozygous and healthy cells in the samples. As in other disease entities analyzed so far regarding *TET2* mutations, no mutation "hotspot" could be detected in our RARS-T patients. In summary, RARS-T patients show a high frequency of both *JAK2* and *TET2* mutations. Together with the less common *MPL* mutations described by others,[11,12] RARS-T presents a wide variety of mutations that overlap with the spectrum of mutations seen in MPN and other myeloid malignancies. Therefore, a combination of molecular markers including *JAK2* and *TET2* should be investigated to provide a more precise description of RARS-T as an independent entity.

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Amyloidosis relapsing after autologous stem cell transplantation treated with bortezomib: normalization of detectable serum-free light chains and reversal of tissue damage with improved suitability for transplant

Systemic amyloidosis (AL) is a plasma cell dyscrasia in which the clone secretes free kappa (κ) or lambda(λ)-immunoglobulin light chains (FLCs).[1] These light chains do not fold into the proper tertiary conformation and form protein deposits, causing organ damage.[2] The most commonly affected organs are the heart, liver, kidney, gut, and peripheral nerves.[3] Standard treatment for patients with good performance status includes high-dose melphalan with autologous stem cell transplantation (ASCT).[1,4] Patients with organ dysfunction have increased transplant-related mortality.[5,6] Medications that treat AL without increasing the mortality of definitive treatment, currently ASCT, are sought. Bortezomib is a proteasome inhibitor that is effective in the treatment of plasma cell dyscrasias.[7] We utilized bortezomib to treat 2 patients with recurrent AL after initial ASCT. Both patients provided written informed consent according to the Helsinki Convention for their initial treatment, for ASCT, for bortezomib treatment of their relapsed disease, and for anonymous data collection.

Patient # 1, a 55-year old male, presented in April 2003 with severe congestive heart failure (CHF), renal failure, and bilateral pleural effusions. Congo red staining of myocardial biopsy indicated amyloid deposits (Figure 1). Serum λ-FLC level was elevated (Figure 2A). The patient received three monthly doses of melphalan 36 mg/m^2 after which his serum λ-FLC level was 3.38 mg/dL, his CHF resolved, with left ventricular ejection fraction increasing from 40% to 55%, and his renal failure improved (serum creatinine 1.2 mg/dL). The patient underwent ASCT with melphalan 140 mg/m^2 condition-