

lenalidomide by the European Medicines Agency (EMA). Here, we present the clinical course and the results of cytogenetic follow-up including fluorescence *in situ* hybridization (FISH) on selected CD34<sup>+</sup> progenitor cells of 3 patients with isolated deletion del(5)(q13q33) who were treated with lenalidomide. Two of the patients initially presented with erythrocyte transfusion-dependent MDS from diagnosis (UPN1, UPN2). One patient (UPN3) had refractory anemia with excess blasts >10% (RAEB 2), and was in complete hematologic remission after allogeneic hematopoietic stem cell transplantation (HSCT) but was threatened with an imminent relapse, as predicted by an increase of endogenous CD34<sup>+</sup> cells that still had the del(5q) leukemic abnormality.

Briefly, the individual details and results were the following: *UPN1*: Patient UPN1 achieved transfusion independence, and hemoglobin returned to within the normal range two months after the start of lenalidomide therapy (Figure 1A). The 5q- progenitor cells were eradicated. Also, parallel histology did not detect any more features of MDS in the biopsy specimen. Therefore, lenalidomide was stopped. The patient is still in complete hematologic remission. *UPN2*: Like UPN1, patient UPN2 responded to lenalidomide with an increase in hemoglobin level (Figure 1B) and did not need further transfusions. In addition, the bone marrow blasts decreased to 2%. Cytogenetic investigation seven months after the start of lenalidomide therapy revealed that the 5q- cells had been eradicated from the CD34<sup>+</sup> subset. However, an unrelated clone (46,XY,del(18)(q21)) had emerged. The deletion 18q was also confirmed by FISH in the del(5q) negative CD34<sup>+</sup> cells. *UPN3*: Because of the allogeneic setting, patient UPN3 was a special case of lenalidomide administration. The aim was to eradicate the minimal residual disease (MRD) after an abrupt drop in the percentage of donor cell chimerism in the CD34<sup>+</sup> cell subset following allogeneic hematopoietic stem cell transplantation (HSCT) (Figure 1C). A dramatic decrease of donor cell chimerism in the CD34<sup>+</sup> subset foreshadows hematologic relapse after allogeneic HSCT.<sup>4,5</sup> In patient UPN3 lenalidomide transiently suppressed the del(5q) stem cells, delaying relapse for about 18 months (Figure 1C). Subsequent hematologic relapse occurred, with bone marrow blasts reappearing. Additionally, karyotype analysis revealed clonal evolution among the aberrant recipient cells, with a new unbalanced translocation der(6)t(1;6) in addition to the deletion del(5)(q13q33). The patient successfully underwent a second HSCT.

Taken together, our results confirm that lenalidomide is effective in MDS patients with single del(5q), moreover in UPN1 and UPN2 the del(5q) CD34<sup>+</sup> stem cells were eradicated. This has not been previously reported, and possibly suggests that short-term lenalidomide could cure a subset of del(5q) MDS patients. However, long-term follow-up will be needed to exclude the persistence and repopulation of disseminated 5q- stem cells. Given that lenalidomide suppresses the del(5q) clone, a prerequisite for response is that normal residual stem cells are present.<sup>1</sup> The best responder (*UPN1*) fitted into this category, with only 48% of CD34<sup>+</sup> stem cells harboring the deletion 5q before the start of therapy.

Despite the eradication of the del(5q) stem cells, patient *UPN2* developed an unrelated aberrant clone; however, there have been no signs of any disease progression so far.

This is in contrast to patient *UPN3*, whose del(5q) CD34<sup>+</sup> cells were not eradicated. This patient had a longer history of MDS with excess blasts and was a different

case because of the allogeneic setting. Lenalidomide exerts immunomodulatory effects, which might have caused the donor T cells to only transiently control the del(5q) CD34<sup>+</sup> cells. The documented clonal progression is attributable to selection pressure favoring a more proliferative subclone unresponsive to lenalidomide.

Long-term lenalidomide therapy might support clonal selection. Hence, close monitoring of bone marrow cytology and cytogenetics, possibly including selected CD34<sup>+</sup> cells, is imperative in these patients.

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*Acknowledgments: we thank Barbara Brocard, Ulrike Fitze, Jeannette Mundt, Anne Richter, Manuela Neumann, Heidrun Zengler, and Cornelia Grosse for their technical assistance and Michael Kramer for his assistance in preparing the figures.*

*Key words: myelodysplastic syndrome, deletion 5q, CD34<sup>+</sup>, lenalidomide.*

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*Citation: Mohr B, Oelschlaegel U, Thiede C, Stewart MM, Ehninger G, Platzbecker U. The response to lenalidomide of myelodysplastic syndrome patients with deletion del(5q) can be sequentially monitored in CD34<sup>+</sup> progenitor cells. Haematologica 2009; 94:430-431. doi:10.3324/haematol.13865*

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## Myelofibrotic transformation in essential thrombocythemia

The exact incidence of myelofibrotic transformation in essential thrombocythemia (ET) is still a critical issue<sup>1,2</sup> and is certainly influenced by risk status and diagnostic criteria. In a recently published study by **Passamonti** and co-workers in this journal,<sup>3</sup> a cohort of 605 patients with ET is described recruited during a lengthy period (1975 to 2008) from one center. Unfortunately, as explicitly stated, diagnosis of ET was performed using criteria at the time of the first observation, implicating that a large fraction, probably the majority of patients, were diagnosed by following the cited criteria of the Polycythemia Vera Study Group (PVSG),<sup>4</sup> contrasting the more recently entered

fraction to whom the WHO classification was applied.<sup>5</sup>

Consequently, the diagnostic guidelines are inconsistent regarding a strict differentiation of ET from early stages of primary myelofibrosis (PMF) with associated thrombocytosis. It has been demonstrated by different groups that significant differences exist between the PVSG versus the WHO classification schemes for ET: depending on risk factors and selection of patients only about 20-40% of ET cases diagnosed according to the PVSG<sup>4</sup> are validated by the WHO criteria.<sup>6,8</sup> The diagnosis of post-ET myelofibrosis was made by the authors using the criteria of the International Working Group on Myelofibrosis (IWG-MRT).<sup>9</sup>

However, these criteria do explicitly include as first major requirement the documentation of a previous diagnosis of WHO-defined essential thrombocythemia. This postulate can not be fulfilled by the obviously predominant PVSG-diagnosed series of patients incorporated into this investigation. Moreover, following the IWG-MRT criteria, bone marrow fibrosis grade 2-3 consistent with the cited European Consensus Grading System<sup>10</sup> is the second major requirement. This important feature implicates the evaluation of sequential bone marrow biopsy specimens - has this been performed in all patients under study? Concerning the frequency of post-ET myelofibrosis, the transformation rate was 2.8% at a median follow-up of 9.1 years (or a 10-year risk of 3.9% and a 15-year risk of 6%).<sup>3</sup> This relatively low incidence may possibly be created by including an unknown number of WHO-diagnosed ET cases into this study. When strictly following the diagnostic criteria of the PVSG<sup>4</sup> applied in the UK-PT1 Study for high risk ET,<sup>11</sup> in our retrospective evaluation of 539 patients under standard therapy, after three years overall incidence of myelofibrosis proved to be significantly different depending on diagnostic criteria (Table 1).

This difference was generated by discrimination of the total cohort according to the WHO classification<sup>5</sup> into (true) ET and PMF with accompanying thrombocytosis mimicking this condition. On the other hand, when maintaining the PVSG guidelines,<sup>4</sup> a comparable rate (2.8% vs. 2.6%)<sup>11</sup> of progression into myelofibrosis was revealed during an observation time of about three years. Regarding leukemic transformation, although no systemic study has yet been published, similar discrepancies associated with applied classification schemes may be encountered. In conclusion, a thorough re-evaluation of this series of 605 patients with distinction into those diagnosed as ET cases according to PVSG criteria<sup>4</sup> versus those in whom diagnosis was based on the WHO classi-

fication seems to be warranted, including a repeatedly performed calculation of progression rates into myelofibrosis and leukemia. As criteria for myelofibrotic transformation, the corresponding guidelines of the UK-PT1 Study<sup>11</sup> should be used for the presumably prevalent ET group diagnosed after PVSG, and for the WHO classified series the IWG-MRT parameters can be applied. As has been reviewed for myelofibrosis in chronic myeloproliferative neoplasms,<sup>12</sup> a significant difference between both groups of patients can be predicted consistent with the diagnostic criteria applied.

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Key words: essential thrombocythemia, myelofibrotic transformation, Polycythemia Vera Study Group, WHO classification, diagnostic criteria

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Citation: Thiele J, Kvasnicka HM. Myelofibrotic transformation in essential thrombocythemia. *Haematologica* 2009; 94:431-432. doi:10.3324/haematol.2008.001446

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**Table 1.** Risk of myelofibrotic transformation (post-essential thrombocythemia myelofibrosis) within 36 months in 539 high-risk patients with essential thrombocythemia strictly diagnosed according to the UK-PT1 Study criteria<sup>11</sup> receiving standard therapy regimens.

Diagnosis	PVSG criteria <sup>4</sup>		WHO classification <sup>5</sup>	
	ET	ET	ET	PMF (prefibrotic-early)
N. of patients (%)	539(100)	111 (20.6)	428 (79.4)	
Relative increase in bone marrow fibers at least per one grade (%) <sup>a</sup>	10.8	0	13.6	
Relative incidence of myelofibrosis (%) <sup>b</sup>	2.8	0	3.5 <sup>c</sup>	

<sup>a</sup>Bone marrow fiber quantity according to the European Consensus Grading System.<sup>10</sup> <sup>b</sup>Post-ET myelofibrosis diagnosed according to the British guidelines (PVSG criteria)<sup>11</sup> or the International Working Group on Myelofibrosis (WHO classification).<sup>9</sup> <sup>c</sup>p=0.002 (two-sided exact significance) for ET versus prefibrotic-early fibrotic stages of PMF. ET: essential thrombocythemia; PMF: primary myelofibrosis.

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### Myelofibrotic transformation in essential thrombocythemia. Author reply

We thank Juergen Thiele and Hans Kvasnicka for commenting on our recently published paper including 605 patients with essential thrombocythemia (ET).<sup>1</sup> We provided evidence that progression to myelofibrosis (post-ET MF) has a prevalence of 2.8% (10-year risk of 3.9%), and that progression to acute leukemia (AL) has a prevalence of 2.3% (10-year risk of 2.6%). The first question of Thiele and Kvasnicka concerns the evolution of ET in post-ET MF and AL. They asked that the prevalence of post-ET MF and AL in patients diagnosed according to the PVSG criteria<sup>2</sup> and with the WHO criteria,<sup>3</sup> be evaluated separately. The analysis suggested would be strongly biased by the fact that PVSG-classified patients have longer follow-up than WHO-classified patients. In the paper, we mentioned that the longer the follow-up, the higher the risk of transformation into myelofibrosis or leukemia. We regret that their request could not be satisfied, but a direct comparison of these two cohorts with different follow-up may give a misleading message. The second question from Thiele and Kvasnicka concerns the diagnostic differentiation between ET and prefibrotic/early fibrotic phase of primary myelofibrosis (PMF), an entity recognized on the basis of bone marrow features by the WHO classification of 2001.<sup>4</sup> However, the recent WHO classification requires the combination of histological picture, clonal markers and clinical parameters to diagnose PMF at prefibrotic or fibrotic phase.<sup>5</sup> In our series we excluded cases of PMF (excluded combination of leukoerythroblastosis, anemia, elevated LDH, spleen enlargement). Concerning the discussion on sequential bone marrow evaluations, we perform bone marrow biopsy at diagnosis in all the patients and during follow-up when we suspect clinical progression of the disease. We are glad to know that the prevalence of myelofibrosis reported by Thiele and Kvasnicka ranges between 2.8% and 3.5%. We find that this is a reassuringly low prevalence for patients with ET.

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Citation: Passamonti F, Rumi E, Boveri E, and Lazzarino M. Myelofibrotic transformation in essential thrombocythemia. Author reply. *Haematologica* 2009; 94: 433-433. doi:10.3324/haematol.2008.002071

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### A JAK2-V617F activating mutation in addition to KIT and FLT3 mutations is associated with clinical outcome in patients with t(8;21)(q22;q22) acute myeloid leukemia

A JAK2-V617F mutation was found in 3 of 45 (6.7%) patients with t(8;21) acute myeloid leukemia (AML), whereas only one of 137 (0.7%) patients with *de novo* AML other than t(8;21) had the same mutation ( $p=0.047$ ). We examined the clinical significance of *KIT*, *FLT3* and *JAK2* mutations as a collective group. There was a significant difference in the cumulative incidence of relapse: 77% in the 21 patients with the mutations and 26% in 19 lacking mutations respectively ( $p=0.0083$ ). Our study highlights the importance of *JAK2* mutations in addition to *KIT* and *FLT3* mutations as a prognostic factor in t(8;21) AML patients.

RUNX1(AML1)-RUNX1T1(MTG8) generated by t(8;21)(q22;q22) contributes to leukemic transformation, but additional events are required for full leukemogenesis.<sup>1,2</sup> Mutations in the receptor tyrosine kinases (RTK) including the *KIT* and *FLT3* genes are the genetic events that appear to cause acute myeloid leukemia (AML) harboring t(8;21) and are associated with unfavorable prognosis.<sup>3,4</sup> The activating missense mutation in the pseudokinase domain of the JAK2 cytoplasmic tyrosine kinase has been identified in a significant proportion of patients with myeloproliferative disorders.<sup>5</sup> Although the same somatic mutation has been found in a small number of AML patients, a relatively high incidence of JAK2-V617F mutation is often seen in *de novo* and therapy-related t(8;21) AML patients.<sup>6-10</sup> Nevertheless, whether JAK2-V617F mutation is associated with other biological parameters including clinical prognosis in patients with t(8;21) AML remains to be fully determined.

To examine its biological and prognostic impact, we studied the JAK2 mutation in 45 patients with *de novo* t(8;21) AML. Approval for this study was obtained from the Institutional Review Board of Kumamoto University School of Medicine. The results of *KIT*, *FLT3*, *N-RAS*, *K-RAS* and *PDGFR $\alpha$*  mutations in 37 of the 45 patients have been reported previously.<sup>3</sup> Of the 45 patients, activating mutations in *KIT* and internal tandem duplications in *FLT3* were observed in 18 (40%) and 3 (6.7%) respectively. Mutations of JAK2-V617F were identified by allele specific RT-PCR and direct sequencing.<sup>11</sup> We detected the het-