To the editor:

Homologous recombination of wild-type JAK2, a novel early step in the development of myeloproliferative neoplasm

Transformation of hematopoietic cells depends on the acquisition of genetic events leading to cytokine independence, typically associated with acquisition of an autocrine cytokine loop or/and increased expression of/and mutation of JAK genes.1 Rearrangement of the JAK2 gene, which presumably alternates JAK2 transcription, is reported in hematopoietic cells.2 Murine models of myeloproliferative neoplasms (MPN) demonstrated that the polycythemia vera (PV) phenotype requires the combination of high expression and activation of Jak2.3 Indeed, expression of both wild-type (WT) and mutant JAK2 transcripts can be high in PV.4 PV is characterized by a high frequency of the JAK2 V617F allele, resulting in chromosome 9p uniparental disomy (9pUPD).5 Here we report 2 cases where high JAK2 mRNA expression was associated with a novel early step in MPN development, HR preceding JAK2 homozygosity (Figure 1E-F). The new model allows that a non-identified somatic genetic event may facilitate JAK2 recombination and subsequent genetic alterations eventually leading to PV phenotype (Figure 1F).

The first cases of HR of JAK2WT led us to propose a new model for MPN: the 46/1 haplotype may predispose carriers to diverse alteration of JAK2 including early HR of wild-type JAK2, associated or not with mutation in JAK2 or other genes important for myeloproliferation, the V617F mutation facilitating additional HR involving the JAK2V617F-mutated allele, leading to 9pUPD and JAK2V617F homozygosity (Figure 1E-F). This model is consistent with the acquisition of homozgyosity for the V617F mutation solely in HR of JAK2WT cells. Finally, finding recurrent JAK2 recombination associated with high mRNA expression suggests that residual JAK2V617F disease may be best assessed in cDNA.

Mathias Vilaine
Inserm U892, Institut de Recherche Thérapeutique, Université de Nantes, Nantes, France

Damla Olcaydu
Center for Molecular Medicine (CeMM) of the Austrian Academy of Sciences, Vienna, Austria

Ashot Harutyunyan
Center for Molecular Medicine (CeMM) of the Austrian Academy of Sciences, Vienna, Austria

Jonathan Bergeman
Inserm U892, Institut de Recherche Thérapeutique, Université de Nantes, Nantes, France

Mourad Tiab
Centre Hospitalier Départemental de Vendée, La Roche sur Yon, France

References
Figure 1. Analysis of the JAK2 gene in patients Na1061 and Na1253 and proposition of a new pathogenic model for polycythemia vera. (A) Representation of the 46/1 haplotype. The 46/1 haplotype is an approximately 280 Kb-long region of chromosome 9p that includes the entire JAK2, INSL6, and INSL4 genes. (B) Schematic representation of the JAK2 gene. JAK2 exons are represented by black boxes. JAK2 SNP rs10429491 (in exon 6), rs7034539 (in intron 18) and rs2230724 (in exon 19) positions are indicated with black bars. (C) Analysis by direct sequencing of JAK2 SNPs and JAK2V617F allelic ratios in gDNA of granulocytes and CD3/H11001 lymphocytes (used as a control, healthy cells) of PV patients Na1061 and Na1253 (see primers in supplemental Table 3 and supplemental Figure 1). Black arrows indicate the different SNPs and JAK2V617F. Both patients were heterozygous for SNPs in CD3/H11001 lymphocytes yet had SNP rs12343867 C-allele ratios in granulocyte gDNA (80% and 100%) compatible with homologous recombination of JAK2. (D) Detailed view of the JAK2 region. Results of the distortion of SNP allelic differences showed HR of part of JAK2 (exons 6-25) for Na1061 and of the whole 46/1 haplotype for Na1253. Regions of pre-JAK2V617F homologous recombination, not readily visible unless one looks for them, are indicated by double black arrows. (E) Karyoview of chromosomal aberrations. Bars depict the physical position and size of the aberration (purple, homologous recombination; blue, uniparental disomy events). Black arrows indicate the chronology of events, as deduced from the rs12343867 and V617F allelic ratios. For both patients, the distortion of SNP allelic differences because of homologous recombination was higher at the telomeric end than in the centromeric region of chromosome 9p indicating 2 distinct partial 9pUPDs for Na1061 and 1 partial 9pUPD for Na1253. For both patients, SNPs distal to JAK2V617F mutation revealed pre-JAK2 homologous recombination (in purple). (F) Main and new pathogenic models for polycythemia vera and other MPN. The current model states that MPN patients carry or acquire a predisposition to MPN and mutation in the JAK2 gene; the JAK2GGCC haplotype is one such genetic predisposition. In other patients, another genetic abnormality, congenital or acquired, presumably in a myeloid progenitor, is responsible for clonality, growth advantage and eventually, acquisition of JAK2 mutation - V617F being the most frequent - and MPN phenotype. Because high JAK2V617F loads are usually acquired via 9pUPD and most frequent in PV, acquisition of the PV phenotype is assumed to result from 9pUPD facilitated by JAK2V617F. Both JAK2 mutation and 9pUPD may occur more than once, leading to the development of one or several JAK2V617F-homozygous subclone(s). Disease phenotype and evolution, and occurrence of 9pUPD, may vary depending on parallel genetic events (eg, TET2 mutations) and the type of JAK2 mutation (eg, high mutant loads and 9pUPD are rare in patients with JAK2 exon 12 mutations). The new model adds an early step to the conventional model, stating that subsets of patients carrying the JAK2GGCC haplotype may be predisposed to homologous recombination (HR) of JAK2 associated with growth advantage, followed or not by mutation in the JAK2 gene on the recombined allele and high JAK2mRNA expression. Early JAK2HR is compatible with all of the later steps leading to MPN according to the conventional pathogenic model: JAK2 mutation, 9pUPD, acquisition of parallel events in other genes than JAK2. The new model allows that a non-identified genetic event may facilitate JAK2 recombination and subsequent genetic alterations eventually leading to PV phenotype.
Jean-François Ramée
Clinique Catholique de Sienne, Nantes, France
Jian-Min Chen
Inserm U613 and EFS-Bretagne, Brest, France
Robert Kralovics
Center for Molecular Medicine (CeMM) of the Austrian Academy of Sciences, Vienna, Austria
Sylvie Hermouet
Inserm U892, Institut de Recherche Thérapeutique, Université de Nantes, Laboratoire d’Hématologie, Centre Hospitalier Universitaire, Nantes, France
The online version of this article contains a data supplement.

Acknowledgments: The authors thank Dr Ariane Plet (Nantes, France), Dr Eric Lippert (Bordeaux, France), and Dr Richard Redon (Nantes, France) for reading the manuscript.

This study was performed thanks to grants from the Association pour la Recherche contre le Cancer (ARC) and the Comités Morbihan and Ille-et-Vilaine of the Ligue Nationale contre le Cancer to S.H. and the MPN Research Foundation to R.K. M.V. is recipient of a scholarship from the French Ministry of Research (2009-2012) and benefited from a scholarship for short term scientific missions (November 2010) from MPN & MPN-EuroNet (COST Action BM0902). M.V., J.B., and S.H. are members of MPN & MPN-EuroNet.

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

Contribution: S.H. designed the research, analyzed data, and wrote the paper; R.K. designed the research and analyzed data; M.V. performed research, analyzed data, and wrote the paper; D.O., A.H., and J.B. performed research and analyzed data; M.T. and J.-F.R. contributed patient samples and clinical data; and J.-M.C. contributed with scientific and technical advice and helped write the paper.

Correspondence: Sylvie Hermouet, Inserm U892, Institut de Recherche Thérapeutique, Université de Nantes, 8 quai Moncousu, 44007 Nantes cedex 1, France; e-mail: sylvie.hermouet@univ-nantes.fr.

References

To the editor:

Differential expression of MMP-2 and MMP-9 activity in megakaryocytes and platelets

In the May 31, 2011 online issue of Blood, Cecchetti et al presented a transcriptome analysis of matrix metalloproteinase (MMP) expression in megakaryocytes and platelets.1 Their results demonstrate that megakaryocytes and platelets differentially express mRNAs and proteins for MMPs. The authors found that platelets constitutively expressed MMP-2 protein and released it on thrombin activation, although platelets lacked mRNA for MMP-2. On the other hand, they did not detect pro–MMP-9 protein in both inactivated and thrombin-stimulated platelet lysates, even though MMP-9 mRNA was present; moreover, the authors also detected MMP activity in megakaryocyte releasates but not in their lysates.

The data are incongruent with previous quantitative studies and the peculiar subcellular localization in platelets of both zymogen and activated forms of MMP-9 (also named Gelatinase B).2-5 Cecchetti et al suggested several hypotheses for this unclear discrepancy/variance: (1) MMP-9 protein is secreted during pro-platelet formation in lieu of being retained in mature platelets, (2) the lack of MMP-9 proenzyme is due to more efficient platelet preparations (clearing CD45+ leukocytes and thus limiting the MMP-9 contamination).

Although no MMP zymogram was displayed by the authors, we believe that there could be a simpler explanation for the incongruence with the literature data, evidencing a neglected methodologic aspect. Cecchetti et al performed all cell lysates with a buffer containing 1mM of EDTA and Na3VO4, chemicals with well-known inhibitory effects on MMP activity. In fact, the Ca/Zn-dependent gelatinases are sensitive to and partially inhibited by the EDTA chelation activity,6,7 whereas orthovanadate (classic phosphotyrosine phosphatase inhibitor) has also been characterized as an MMP inhibitor (eg, for gelatinase B8 and collagenase9).

To reveal the possible partial inhibitory effect of EDTA and Na3VO4 on platelet gelatinases, in Figure 1 we show all gelatinolytic MMP forms present in whole cord blood10 and in purified mature platelets according to Cecchetti et al’s procedure.1 Whole cord blood contained both MMP-2 proenzyme (at 72 kDa) and pro–MMP-9 forms (monomer at 92 kDa, and complexed forms at 130 and 225 kDa; lane standard); proforms are activable by 1mM APMA (lane 2). The EDTA/Na3VO4 treatment is able to partially inhibit MMP-9 in platelet lysates; in particular, both the pro-enzyme and APMA-activated MMP-9 forms showed a significant reduction of gelatinolytic activity (lane 3). Our observations may at least in part explain both the incongruent “absence” of MMP-9 activity in platelet lysates (similar to collagenase inhibition, like for MMP-3), as well as the presence of MMP-9 activity in only megakaryocyte releasates or untreated culture media (but not in their lysates with EDTA/Na3VO4).

To avoid misinterpretation and possible technical pitfalls (possibly because of the neglected peculiar MMP inhibition by EDTA/Na3VO4), we believe that a more careful MMP analysis in
Homologous recombination of wild-type JAK2, a novel early step in the development of myeloproliferative neoplasm

Mathias Vilaine, Damla Olcaydu, Ashot Harutyunyan, Jonathan Bergeman, Mourad Tiab, Jean-François Ramée, Jian-Min Chen, Robert Kralovics and Sylvie Hermouet