David S. Ritchie
Hematology Immunology Translational Research Laboratory, Department of Hematology and Medical Oncology, Peter MacCallum Cancer Centre, University of Melbourne, Melbourne, Australia

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Correspondence: Paul Neeson, Cancer Immunology Program, Peter MacCallum Cancer Centre, East Melbourne, Victoria 3002, Australia; e-mail: paul.neeson@petermac.org.

References

1. Carter CL, Feyler S, Smalle N, et al. Effect of combined dexamethasone/lenalidomide therapy on NK cell–receptor levels in myeloma patients. *Blood.* 2011; 118(24):6465-6466.
2. Hsu AK, Quach H, Tai T, et al. The immunostimulatory effect of lenalidomide on NK-cell function is profoundly inhibited by concurrent dexamethasone therapy. *Blood.* 2011;117(5):1605-1613.
3. El-Sherbiny YM, Meade JL, Holmes TD, et al. The requirement for DNAM-1, NKG2D, and NKp46 in the natural killer cell-mediated killing of myeloma cells. *Cancer Res.* 2007;67(18):8444-8449.
4. Jinushi M, Vanneman M, Munshi NC, et al. MHC class I chain-related protein A antibodies and shedding are associated with the progression of multiple myeloma. *Proc Natl Acad Sci U S A.* 2008;105(4):1285-1290.
5. Mitsiades CS. How "immunomodulatory" are IMIDs? *Blood.* 2011;117(5):1440-1441.

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 or/and mutation of *JAK* genes.[1] Rearrangement of the *JAK2* gene, which presumably alters *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* 46/1 (GGCC) haplotype (represented in Figure 1A) predisposing to the *JAK2*V617F mutation.[5,6] The *JAK2*V617F mutation facilitates the acquisition of homozygous status for the *JAK2*V617F by mitotic homologous recombination (HR) occurring between the *JAK2WT* and *JAK2*V617F alleles, resulting in chromosome 9p uniparental disomy (9pUPD).[7,8] Here we report 2 cases where high *JAK2* mRNA expression was associated with a novel early step in MPN development, HR preceding *JAK2* mutation.

Patients Na1061 and Na1253 presented with a high hematocrit, slightly elevated leukocyte counts, normal (Na1061) or elevated (Na1253) platelet counts, aquagenic pruritus, absence of splenomegaly, and presence of *JAK2*V617F (20.7% for Na1061, 30.0% for Na1253), and were diagnosed with PV (see supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Sequencing and allele-specific qPCR analysis in granulocyte DNA of marker rs12343867 (C/T) in intron 14 of *JAK2*, characteristic of the 46/1 haplotype, revealed rs12343867 ratios sharply different from *JAK2*V617F ratios: 80% C-alleles for Na1061 and 100% T-alleles for Na1253 (Figure 1B-C). For both patients, $CD3^+$ lymphocytes were unambiguously heterozygous for rs12343867 (Figure 1C). This indicated granulocyte acquisition of homozygosity for rs12343867 but not for the V617F mutation. In other words, the acquisition of homozygosity for rs12343867 must have preceded *JAK2* mutation in these patients. This was confirmed by further analysis of *JAK2* in granulocytes and $CD3^+$ lymphocytes (Figure 1C), and of chromosome 9p using SNP arrays (Figure 1D). These studies showed that the DNA regions recombined involved *JAK2* exons 6-25 for Na1061, and the complete 46/1 haplotype for Na1253. Moreover, SNP array studies revealed the presence of 1 subclone for Na1253 (28.24 Mb) or 2 subclones for Na1061 (5.7 and 24.54 Mb) with partial 9pUPD (supplemental Figures 2-3 and Figure 1E). Sequencing of the complete *JAK2* cDNA excluded any mutation other than V617F.

These 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 myelopoiesis, the V617F mutation facilitating additional HR involving the *JAK2*V617F-mutated allele, leading to 9pUPD and *JAK2*V617F homozygosity (Figure 1E-F). The new model allows that a nonidentified somatic genetic event may facilitate *JAK2* recombination and subsequent genetic alterations eventually leading to PV phenotype (Figure 1F).

In the context of inherited gene mutations, meiotic HR can increase expression of the gene involved.[9] In the case of *JAK2*, mitotic HR could result in a configuration that amplifies *JAK2* expression and subsequently cell growth after activation of Jak2 by cytokine receptors. This is of importance because MPN progenitors produce Jak2-activating cytokines.[10] For both patients, cDNA quantitative analysis revealed high *JAK2* mRNA levels with $> 96\%$ *JAK2*V617F (see supplemental Table 2), implying an mRNA expression almost 100-fold higher for recombined alleles in *V617F/V617F* cells than for alleles in *WT/WT* cells. Finally, finding recurrent *JAK2* recombination associated with high mRNA expression suggests that residual *JAK2*V617F 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

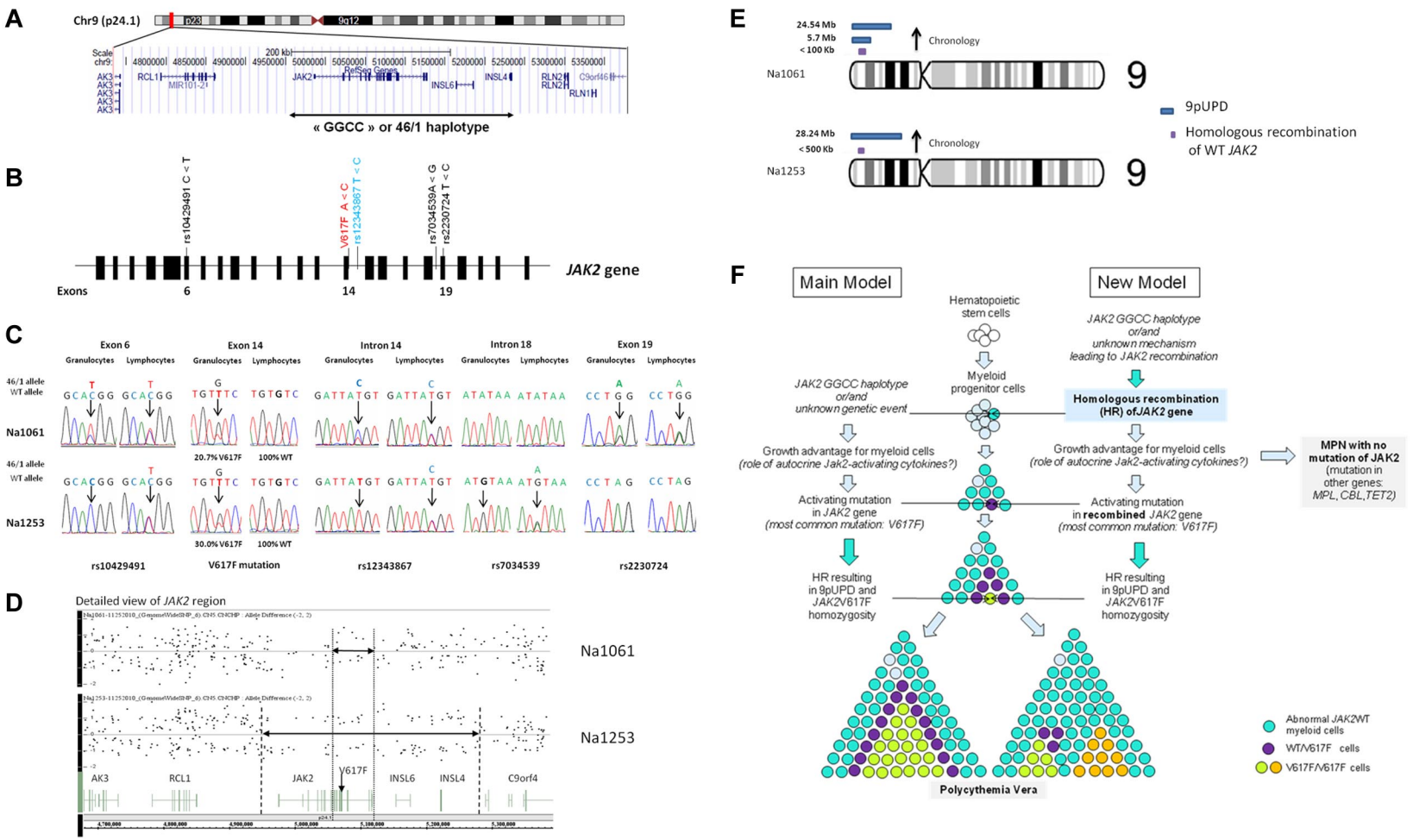

Figure 1. Analysis of the *JAK2* gene in patients Na1061 and Na1253 and proposition of a new pathogenic model for polycythemia vera. (A) Representation of the 46/1 haplotype. The 46/1 haplotype is an approximately 280 Kb-long region of chromosome 9p that includes the entire *JAK2*, *INSL6* and *INSL4* 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 *JAK2*V617F allelic ratios in gDNA of granulocytes and $CD3^+$ 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 *JAK2*V617F. Both patients were heterozygous for SNPs in $CD3^+$ 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-*JAK2*V617F 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, SNP allelic distortion 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 *JAK2* GGCC 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 *JAK2*V617F loads are usually acquired via 9pUPD and most frequent in PV, acquisition of the PV phenotype is assumed to result from 9pUPD facilitated by *JAK2*V617F. Both *JAK2* mutation and 9pUPD may occur more than once, leading to the development of one or several *JAK2*V617F-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 *JAK2* GGCC 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 *JAK2* mRNA expression. Early *JAK2* HR 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 genes other 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 Catherine 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

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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

1. Hornakova T, Springuel L, Devreux J, et al. Oncogenic JAK1 and JAK2-activating mutations resistant to ATP-competitive inhibitors. *Haematologica.* 2011;96(6):845-853.
2. Van Roosbroeck K, Cox L, Tousseyn T, et al. JAK2 rearrangements, including the novel SEC31A-JAK2 fusion, are recurrent in classical Hodgkin lymphoma. *Blood.* 2011;117(15):4056-4064.
3. Tiedt R, Hao-Shen H, Sobas MA, et al. Ratio of mutant JAK2-V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. *Blood.* 2008; 111(8):3931-3940.
4. Lippert E, Boissinot M, Kralovics R, et al. The JAK2-V617F mutation is frequently present at diagnosis in patients with essential thrombocythemia and polycythemia vera. *Blood.* 2006;108(6):1865-1867.
5. Olcaydu D, Harutyunyan A, Jäger R, et al. A common *JAK2* haplotype confers susceptibility to myeloproliferative neoplasms. *Nat Genet.* 2009;41(4):450-454.
6. James C, Ugo V, Le Couédic JP, et al. A unique clonal *JAK2* mutation leading to constitutive signalling causes polycythaemia vera. *Nature.* 2005;434(7037): 1144-1148.
7. Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. *Exp Hematol.* 2002; 30(3):229-236.
8. Klampfl T, Harutyunyan A, Berg T, et al. Genome integrity of myeloproliferative neoplasms in chronic phase and during disease progression. *Blood.* 2011; 118(1):167-76.
9. Lupski JR, Stankiewicz P. Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. *PLoS Genet.* 2005;1(6):e49.
10. Boissinot M, Cleyrat C, Vilaine M, Jacques Y, Corre I, Hermouet S. Anti-inflammatory cytokines hepatocyte growth factor and interleukin-11 are over-expressed in Polycythemia vera and contribute to the growth of clonal erythroblasts independently of JAK2V617F. *Oncogene.* 2011;30(8):990-1001.

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 hypothesis 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 Na_3VO_4, 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 B[8] and collagenase[9]).

To reveal the possible partial inhibitory effect of EDTA and Na_3VO_4 on platelet gelatinases, in Figure 1 we show all gelatinolytic MMP forms present in whole cord blood[10] 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/Na_3VO_4 treatment is able to partially inhibit MMP-9 in platelets lysates; in particular, both the proenzyme 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/Na_3VO_4).

To avoid misinterpretation and possible technical pitfalls (possibly because of the neglected peculiar MMP inhibition by EDTA/Na_3VO_4), we believe that a more careful MMP analysis in