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### Authors
Shannon, Kevin
Van Etten, Richard A



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label the therapy as primitive would not do justice to the resounding success in the number of lives saved and the dedicated professionals who pioneered cures for these children. The fact is that if the 75% cure rate seen today was established with only modest insight into the underlying biology of ALL, then we should expect nothing short of 100% cure as well as successful preventive strategies in the decades to come.

William L. Carroll* and
Elizabeth A. Raetz

Stephen D. Hassenfeld Children's Center for Cancer and Blood Diseases
New York University Cancer Institute
517 East 34th Street
New York, New York 10016
*E-mail: william.carroll@nyumc.org

**Selected reading**

Cario, G., Stanulla, M., Fine, B.M., Teuffel, O., Neuhoff, N.V., Schrauder, A., Flohr, T., Schafer, B.W., Bartram, C.R., Welte, K., et al. (2005). Blood *105*, 821–826.

Den Boer, M.L., Harms, D.O., Piters, R., Kazemier, K.M., Gobel, U., Korholz, D., Graubner, U., Haas, R.J., Jorch, N., Spaar, H.J., et al. (2003). J. Clin. Oncol. *21*, 3262–3268.

Gaynon, P.S., Desai, A.A., Bostrom, B.C., Hutchinson, R.J., Lange, B.J., Nachman, J.B., Reaman, G.H., Sarther, H.N., Steinherz, P.G., Trigg, M.E., et al. (1997). Cancer *80*, 1717–1726.

Holleman, A., Cheok, M.H., den Boer, M.L., Yang, W., Veerman, A.J.P., Kazemier, K.M., Cheng, C., Pui, C.-H., Relling, M.V., Janka-Schaub, G.E., et al. (2004). N. Engl. J. Med. *351*, 533–542.

Lugthart, S., Cheok, M.H., den Boer, M.L., Yang, W., Holleman, A., Cheng, C., Pui, C.-H., Relling, M.V., Janka-Schaub, G.E., Pieters, R., and Evans, W.E. (2005). Cancer Cell, this issue.

Moos, P.J., Raetz, E.A., Carlson, M.A., Szabo, A., Smith, F.E., Willman, C., Wei, Q., Hunger, S.P., and Carroll, W.L. (2002). Clin. Cancer Res. *8*, 3118–3130.

Nachman, J.B., Sather, H.N., Sensel, M.G., Trigg, M.E., Cherlow, J.M., Lukens, J.N., Wolff, L., Uckun, F.M., and Gaynon, P.S. (1998). N. Engl. J. Med. *338*, 1663–1671.

Pui, C.-H., Relling, M.V., and Downing, J.R. (2004). N. Engl. J. Med. *350*, 1535–1548.

Smith, M., Arthur, D., Camitta, B., Carroll, A.J., Crist, W., Gaynon, P., Heerema, N., Korn, E.L., Link, M., Murphy, S., et al. (1996). J. Clin. Oncol. *14*, 18–24.

Szczepanski, T., Orfao, A., van der Velden, V.H., San Miguel, J.F., and van Dongen, J.J. (2001). Lancet Oncol. *7*, 409–417.

Yeoh, E.J., Ross, M.E., Shurtleff, S.A., Williams, W.K., Patel, D., Mahfouz, R., Behm, F.G., Raimondi, S.C., Relling, M.V., Patel, A., et al. (2002). Cancer Cell *1*, 133–143.

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# JAKing up hematopoietic proliferation

**Mutations that deregulate proliferation and survival pathways have emerged as a common molecular theme in the pathogenesis of myeloproliferative disorders (MPDs). Three studies now report an amino acid substitution in the JAK2 kinase in most patients with polycythemia vera as well as in some cases of essential thrombocythemia and chronic idiopathic myelofibrosis. Functional analysis demonstrates that this mutation confers erythropoietin-independent growth in vitro, deregulates signaling pathways downstream of JAK2, and causes polycythemia in mice. These results open new avenues for diagnosing and classifying patients with these disorders, and identify a new molecular target for drug discovery.**

Myeloproliferative disorders (MPDs) are clonal malignancies characterized by overproduction of one or more hematopoietic lineages with relatively normal differentiation (Van Etten and Shannon, 2004). The World Health Organization (WHO) classifies chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (CIMF), and the related disorders chronic eosinophilic leukemia (CEL) and idiopathic hypereosinophilic syndrome (HES) as distinct MPDs. Atypical CML, chronic myelomonocytic leukemia (CMML), and juvenile myelomonocytic leukemia (JMML) comprise a related group of "overlap" disorders in which myeloproliferation is prominent, but the bone marrow also shows aberrant maturation (myelodysplasia). Laboratory and clinical observations such as de novo chromosomal translocations (e.g., t[9;22] in CML and t[5;12] in some cases of CMML), an increased risk of JMML in children with neurofibromatosis and Noonan syndrome, and the unexpected responses of some patients with HES to imatinab mesylate provided clues that facilitated identifying molecular lesions that play a central role in the pathogenesis of MPDs and "overlap" diseases (Figure 1). Aberrant activation of kinase signaling cascades and hyperactive Ras have emerged as common biochemical themes in these disorders, and studies in animal models strongly imply that many of the mutations found in human patients can initiate MPD-like diseases in vivo (Van Etten and Shannon, 2004).

A paper by Levine et al. (2005) in this issue of *Cancer Cell*, and data published in the *Lancet* and *Nature* (Baxter et al., 2005; James et al., 2005), report *JAK2* point mutations in most patients with PV and in a substantial proportion of ET and CIMF. These results are satisfying, as they follow logically from the known role of the JAK2 kinase in hematopoietic proliferation and are consistent with previous studies of PV patient samples. The four mammalian Janus (JAK) kinases are recruited by ligand binding to cytokine receptors, where they are activated by *trans*-phosphorylation and, in turn, phosphorylate critical tyrosine residues on the receptor that can then serve as docking sites for members of the STAT (signal transducer and activation of transcription) family and for other signaling molecules (O'Shea et al., 2002). Specific cytokine receptors recruit and activate distinct pairs of JAK and STAT proteins. JAK2 is the primary tyrosine kinase activated by erythropoietin (EPO), and is essential for definitive erythropoiesis (Parganas et al., 1998). Many of the effects of JAK2 are mediated through the recruitment of STAT5 to phospotyrosyl residues on the EPO, interleukin 3 (IL-3), and granulocyte-macrophage colony stimulating factor (GM-CSF) receptors. Interestingly,

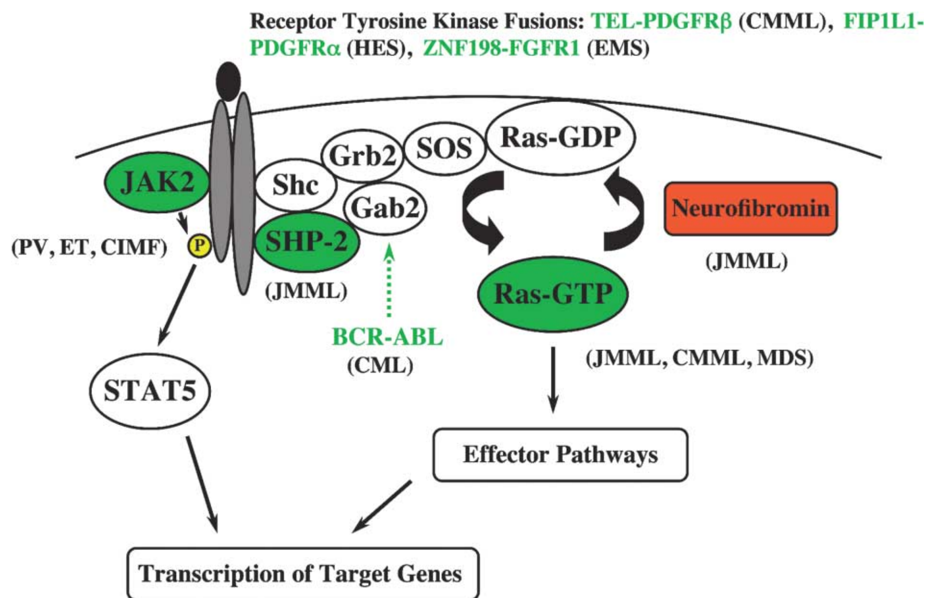


**Figure 1.** Molecular lesions identified in MPD

Overview of mutant proteins detected in specific subtypes of MPD and "overlap" diseases with putative connections to cellular signaling networks. Proteins that are altered by dominantly acting mutations are shown in green, while the known instance of tumor suppressor gene inactivation (loss of *NF1*) is shown in red. JAK2 phosphorylates tyrosine residues on activated growth factor receptors that serve as docking sites for STAT5 and other effectors. See text and Van Etten and Shannon (2004) for further discussion and additional information.

compound *Stat5a*/*b* mutant mice demonstrate fetal anemia and impaired erythroid progenitor colony growth, which is associated with failure to induce the antiapoptotic family member Bcl-$X_L$ in response to EPO (Socolovsky et al., 1999). By contrast, bone marrow cells from PV patients form EPO-independent endogenous erythroid colonies (EEC) in methylceullose, and Bcl-$X_L$ protein levels are increased in PV erythroblasts (Silva et al., 1998). While the molecular basis of EEC was unknown until now, it was thought likely that the relevant mutation(s) were downstream of the EPO receptor, which is not mutated in PV and has normal expression and affinity for EPO. The new reports confirm this speculation, and identify a single amino acid substitution in the JH2 pseudokinase domain of JAK2—V617F—as a prevalent molecular lesion in PV, ET, and CIMF.

*JAK2* mutations were detected in PV, ET, and CIMF specimens through two general experimental strategies. James and coworkers (James et al., 2005) identified *JAK2* as a candidate PV gene on the basis of experiments in which they found that a JAK2 kinase inhibitor and a short interfering RNA molecule impaired EEC formation from PV bone marrows. The other two groups uncovered *JAK2* mutations by pursuing systematic "kinome" sequencing efforts of MPD specimens (Baxter et al., 2005; Levine et al., 2005). Analysis of over 300 primary PV specimens revealed the V617F substitution in 73%–97% of cases (Baxter et al., 2005; James et al., 2005; Levine et al., 2005). The true incidence may approach the 97% frequency reported by Baxter et al. (2005), as these investigators used a sensitive allele-specific PCR-based strategy to detect alleles that were present in a minority of nucleated bone marrow cells. The mutation was present in differentiated myeloid and erythroid cells and in progenitor colonies grown from PV patient samples, but was not identified in T lymphocytes (Baxter et al., 2005; James et al., 2005; Levine et al., 2005). Interestingly, the mutation was also identified in buccal swab DNA from a small proportion of patients, which infers that the inciting genetic lesion is occasionally present in the germline (Levine et al., 2005). The V617F substitution was also detected in a significant percentage of ET and CIMF specimens, thereby implicating the same molecular lesion in all three types of MPD. Another striking result was the finding of biallelic mutations in ~30% of the PV specimens. Analysis of polymorphisms showed this to be due to a mitotic recombination event that ablated the remaining normal allele. These new data, which are remarkably consistent with a previous study that demonstrated loss of constitutional heterozygosity in PV specimens within a segment of chromosome band 9p that contains the *JAK2* locus (Kralovics et al., 2002), emphasize that this genetic mechanism is not invariably associated with tumor suppressor gene inactivation, but can also be a marker of proto-oncogene activation.

Levine et al. (2005) observed EPO-independent survival and hypersensitivity in the Ba/F3-EPOR cells engineered to express V617F JAK2. These investigators also coexpressed wild-type JAK2 with the mutant protein in 293T cells, but did not find that this interferes with autophosphorylation of V617F JAK2. They also identified the PV-associated mutation in HEL cells, and showed that this was associated with elevated levels of STAT5 and ERK phosphorylation, and with sensitivity to a small molecule inhibitor. James et al. (2005) performed an extensive functional analysis of V617F JAK2 in cell lines and in murine bone marrow. Importantly, they demonstrated that V617F JAK2, but not the wild-type protein, could activate STAT5-dependent transcription. Other studies showed that V617F JAK2 induces EPO hypersensitivity and aberrant activation of STAT5, Akt, and ERK in cell lines. Interestingly, coexpressing wild-type JAK2 with the mutant protein restored EPO dependence in Ba/F3 cells. This finding, which is consistent with loss of the wild-type *JAK2* allele in ~30% of human PV specimens, infers a dominant interfering activity of the normal protein and a selective growth advantage for cells that have deleted it. Based on the data of Levine et al. in 293T cells (Levine et al., 2005), it is possible that wild-type JAK2 does not directly downregulate the mutant protein, but instead competes for binding sites on cytokine receptors. Since JAK2 molecules that are recruited to activated homo- or heterodimeric receptors *trans*-phosphorylate each other, it is not difficult to envision how retaining normal protein expression could impede the ability of V617F JAK2 to constitutively activate downstream effectors. Further work will be required to resolve this question. In a final series of experiments that directly establish the relevance of the mutation to the PV phenotype, James and coworkers (James et al., 2005) found that recipient mice that were transplanted with cells engineered to express V617F JAK2 developed erythrocytosis.

Together, these three reports firmly establish somatic *JAK2* mutations as a prevalent genetic lesion in PV that plays an integral role in the disease phenotype. Remarkably, every patient sample contained the same amino acid substitution. Based on the predicted JAK2 structure, the V-to-P substitution at codon 617 disrupts an autoinhibitory interaction between the JH2 and kinase domains of the protein. This pathogenic mechanism is reminiscent of somatic *PTPN11* mutations in JMML, which encode amino acid substitutions that constitutively activate the SHP-2 phosphatase. Other parallels with PV include the consistent GM-CSF hypersensitivity seen in JMML, the finding of germline mutations in some patients, the ability of wild-type and mutant SHP-2 to activate multiple downstream effector pathways by binding to phosphotyrosyl residues on growth factor receptors, the limited spectrum of *PTPN11* mutations found in leukemias, and occasional JMML samples that demonstrate both a somatic *PTPN11* mutations and loss of the normal allele (Loh et al., 2004; Tartaglia et al., 2003).

These three studies also raise provocative new questions regarding the role of *JAK2* mutations in MPD. Perhaps the most interesting of these is how the same mutation is associated with three diseases that have distinct clinical features. EECs are observed in approximately half of ETs, and it seems likely that these cases will be associated with the V617F JAK2 substitution, whereas the myelofibrosis patients could be PV patients presenting in the so-called "spent" phase of the disease. While it is possible that the diverse clinical spectrum reflects transformation of distinct progenitors, recent data in mouse models supports the idea that proliferative mutations such as *JunB* deficiency and oncogenic *Kras* expression do not enhance self-renewal and must occur in the hematopoietic stem cell (HSC) compartment to induce overt disease (Braun et al., 2004; Passegue et al., 2004). Although T lymphocytes are not involved in the clonal outgrowth of V617F JAK2-expressing cells in PV, they are also predominantly negative for the Ph chromosome in CML, and hence this does not preclude the possibility that the initiating mutation occurs in HSCs with subsequent selection against their progeny during lymphocyte differentiation. Generating conditional knockin strains of mice harboring the V617F JAK2 mutation may help to resolve this issue. Alternatively, somatic *JAK2* mutations may initiate PV (and perhaps ET), but represent cooperating events in CIMF. A similar model has been proposed to explain the existence of *KRAS2* and *NRAS* mutations in both MPD and acute myeloid leukemia (Braun et al., 2004). The underlying cause of the MPD in those patients lacking the JAK2 V617F mutation awaits further investigation, but the work of Levine et al. (2005) suggest that the offending lesion is not in another tyrosine kinase. Finally, these exciting studies open new avenues for diagnosing and classifying patients with MPD, and for developing targeted therapeutics to inhibit the mutant V617F JAK2 kinase.

Kevin Shannon[1,*] and
Richard A. Van Etten[2,*]

[1]Department of Pediatrics and Comprehensive Cancer Center
University of California
San Francisco, California 94143
[2]Molecular Oncology Research Institute
Tufts-New England Medical Center
Boston, Massachusetts 02111
*E-mail: kevins@itsa.ucsf.edu or rvanetten@tufts-nemc.org

**Selected reading**

Baxter, E.J., Scott, L.M., Campbell, P.J., East, C., Fourouclas, N., Swanton, S., Vassiliou, G.S., Bench, A.J., Boyd, E.M., Curtin, N., et al. (2005). Lancet *365*, 1054–1061.

Braun, B.S., Tuveson, D.A., Kong, N., Le, D.T., Kogan, S.C., Rozmus, J., Le Beau, M.M., Jacks, T.E., and Shannon, K.M. (2004). Proc. Natl. Acad. Sci. USA *101*, 597–602.

James, C., Ugo, V., Le Couedic, J.P., Staerk, J., Delhommeau, F., Lacout, C., Garcon, L., Raslova, H., Berger, R., Bennaceur-Griscelli, A., et al. (2005). Nature. Published online March 27, 2005. 10.1038/nature03546.

Kralovics, R., Guan, Y., and Prchal, J.T. (2002). Exp. Hematol. *30*, 229–236.

Levine, R., Wadleigh, M., Cools, J., Ebert, B., Wering, G., Huntly, B., Boggon, T., Wlodarska, I., Clark, J., Moore, S., et al. (2005). Cancer Cell, this issue. Published online March 24, 2005. 10.1016/j.ccr.2005.03.023.

Loh, M.L., Vattikuti, S., Schubbert, S., Reynolds, M.G., Carlson, E., Lieuw, K.H., Cheng, J.W., Lee, C.M., Stokoe, D., Bonifas, J.M., et al. (2004). Blood *103*, 2325–2331.

O'Shea, J.J., Gadina, M., and Schreiber, R.D. (2002). Cell *109* (*Suppl*), S121–S131.

Parganas, E., Wang, D., Stravopodis, D., Topham, D.J., Marine, J.C., Teglund, S., Vanin, E.F., Bodner, S., Colamonici, O.R., van Deursen, J.M., et al. (1998). Cell *93*, 385–395.

Passegue, E., Wagner, E.F., and Weissman, I.L. (2004). Cell *119*, 431–443.

Silva, M., Richard, C., Benito, A., Sanz, C., Olalla, I., and Fernandez-Luna, J.L. (1998). N. Engl. J. Med. *338*, 564–571.

Socolovsky, M., Fallon, A.E., Wang, S., Brugnara, C., and Lodish, H.F. (1999). Cell *98*, 181–191.

Tartaglia, M., Niemeyer, C.M., Fragale, A., Song, X., Buechner, J., Jung, A., Hahlen, K., Hasle, H., Licht, J.D., and Gelb, B.D. (2003). Nat. Genet. *34*, 148–150.

Van Etten, R.A., and Shannon, K.M. (2004). Cancer Cell *6*, 547–552.

DOI: 10.1016/j.ccr.2005.04.002