To the editor:

The V617F *JAK2* mutation is uncommon in cancers and in myeloid malignancies other than the classic myeloproliferative disorders

Four groups recently reported the existence of an activating mutation of *JAK2* in many patients with one of the classic myeloproliferative disorders (MPDs).[1-4] Mutant Janus kinase 2 (JAK2) has increased kinase activity, renders BaF3 cells cytokine independent, and produces erythrocytosis in vivo. The V617F mutation is present in most patients with polycythemia vera, and a substantial proportion of patients with idiopathic myelofibrosis (IMF) or essential thrombocythemia (ET). It remains unclear whether the V617F *JAK2* mutation contributes to other hematologic malignancies or to nonhematologic tumors.

Sequence analysis was used to determine the *JAK2* status of 618 cell lines derived from hematologic (132 lines) and nonhematologic (486 lines representing more than 30 different tumor types) malignancies (Table 1). The V617F mutation was not detected in most of the cell lines but was present in 2 acute myeloid leukemia (AML) lines (HEL and HEL-92.1.7). Microsatellite analysis demonstrated that these were clonally related. We therefore analyzed 211 primary hematologic malignancies (including 90 AML samples) using allele-specific polymerase chain reaction (PCR)[1] to detect the V617F allele. The mutation was present in 5 (5.6%) of the AML samples (Table 1). None of the 5 patients had the FMS-like tyrosine kinase 3–internal tandem duplication (FLT3-ITD) mutation, or the t(8;21), inv(16), or t(15;17) rearrangements. All 5 were male ($P = .06$, Fisher exact test) and significantly older than their unaffected counterparts (median ages at diagnosis were 68 years for V617F-positive patients and 52 years for V617F-negative patients; $P = .05$, Wilcoxon rank-sum test). None of these V617F-positive patients had a prior history of an overt MPD. Although a prior study did not detect *JAK2* mutations in AML patients,[5] *JAK2* rearrangements occur infrequently in hematologic malignancies. The t(9;12), t(8;9), and t(9;22) translocations present in ALL and atypical chronic myeloid leukemia (CML) each disrupt *JAK2,* generating the translocation Ets leukemia (TEL)/JAK2, pericentriolar material 1 (PCM1)/JAK2, and breakpoint cluster region (BCR)/JAK2 proteins, respectively.[6-8] The kinase activity of the TEL/JAK2 fusion protein is required for its leukemogenic properties,[9] consistent with the concept that constitutive JAK2 activity resulting from the V617F mutation contributes to the leukemic phenotype.

The V617F mutation was not present in patients with CMML or CLL, or in the majority of patients with *BCR/ABL*-positive CML or MDS. We did detect this mutation in single instances of CML and MDS (Table 1). The V617F-positive CML patient had a 12-year history of *BCR/ABL*-negative thrombocytosis prior to presenting with CML. His subsequent clinical course suggested the coexistence of imatinib-sensitive CML and imatinib-resistant ET.[10] Similarly, the V617F-positive MDS patient had trephine features of myelodysplasia with fibrosis, consistent with an MDS/MPD overlap syndrome.

The molecular basis of the V617F-negative MPDs remains obscure. We therefore screened samples from 24 MPD patients without the V617F allele for alternative *JAK2* mutations and for mutations in other components of the JAK/signal transducer and activator of transcription (STAT) pathway. All 128 coding exons of *JAK1, JAK2, JAK3, TYK2, STAT5A,* and *STAT5B* were sequenced in the peripheral blood granulocyte DNA from 19 ET and 5 IMF patients who lack the V617F allele. No mutations were detected in these individuals (data not shown).

Collectively, these data suggest that the JAK2 V617F mutation does not occur in nonhematologic cancers, that this mutation is uncommon in myeloid malignancies other than the classic *BCR/ABL*-negative MPDs, and that the V617F-negative MPDs are likely to reflect mutations in other molecules that modulate the JAK/STAT pathway, or mutations in different signaling pathways.

Table 1. JAK2 V617F mutations in human cancer cell lines and primary hematopoietic cells

	Total	V617F
Hematologic		
Cell lines	132	2
AML	25	2
CML	10	0
ALL	37	0
Lymphoma	42	0
Myeloma	14	0
Other*	4	0
Primary cells	211	7
AML	90	5
MDS	20	1
CML	91	1
CMML	5	0
CLL	5	0
Nonhematologic		
Cell lines	486	0
Bladder	15	0
Bone	14	0
Brain	15	0
Breast	27	0
Colorectal	41	0
Glioma	25	0
Kidney	10	0
Liver	10	0
Lung	121	0
Neuroblastoma	15	0
Esophagus	12	0
Ovary	17	0
Pancreas	13	0
Skin	36	0
Stomach	21	0
Uterus	10	0
Other†	84	0

ALL indicates acute lymphoblastic leukemia; CMML, chronic myelomonocytic leukemia; MDS, myelodysplasia; and CLL, chronic lymphocytic leukemia.

*Chronic B-cell leukemia (3), refractory anemia (1).

†Adrenal (6), bile duct (3), cervix (7), cornea (1), head/neck (7), intestine (2), muscle (1), neuroblastoma (8), placenta (3), pleura (5), primitive neuroectodermal tumor (5), prostate (1), respiratory tract (3), retina (1), rhabdomyosarcoma (6), synovium (1), testis (4), thyroid (8), tongue (6), ureter (4), and vulva (2).

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To the editor:

Replacement of unfractionated heparin by low-molecular-weight heparin for postorthopedic surgery antithrombotic prophylaxis lowers the overall risk of symptomatic thrombosis because of a lower frequency of heparin-induced thrombocytopenia

Heparin-induced thrombocytopenia (HIT) is a prothrombotic immune-mediated adverse drug reaction that usually begins 5 or more days after starting heparin.[1] One randomized clinical trial reported a significant difference in HIT between unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) in postoperative orthopedic patients.[2] To corroborate that LMWH is safer than UFH in this patient population, we performed a "before-after" prospective cohort study in which 231 consecutive patients over a 9-month period received UFH (Liquemin [Roche, Basel, Switzerland], 5000 IU 3 times/day subcutaneously) following hip or knee replacement. During the subsequent 12-month period, 271 patients received LMWH (enoxaparin [Clexane; Sanofi-Aventis, Frankfurt, Germany], 40 mg/d subcutaneously). Both anticoagulants were started the night before surgery. The primary study end point was HIT, defined a priori as a positive test for HIT antibodies (platelet activation assay[3]) plus a 30% or greater fall in the platelet count between postoperative days 4 to 16, and/or symptomatic, objectively documented thrombosis (venous or arterial). We predefined thrombotic events occurring between days 5 to 16 as potentially HIT related. Routine testing for HIT antibodies was also performed in most patients at end of study (day 16). Stored sera were also tested posthoc for anti-PF4/polyanion antibodies (GTI, Brookfield, WI).[4] Clinical events were adjudicated by 2 investigators by consensus. The study was approved by the ethics committee of the University of Greifswald. The patients we report overlap somewhat with those included in a previous publication[5] reflecting the participating surgeons' perspective; however, that report included an additional 130 nonstudy patients, used a different definition of HIT (> 50% platelet count fall), and did not report systematic serologic investigations.

We found a higher frequency of HIT in patients who received UFH (5.2%; 95% confidence interval [CI], 2.7%-8.9%), compared with LMWH (0%; 95% CI, 0.0%-1.4%); $P < .001$ (Table 1). In addition to the 6 patients who had thrombosis associated with HIT, 5 patients had thrombosis without HIT (3 in the UFH group, 2 in the LMWH group). Thus, total thrombosis (both HIT and non-HIT) was significantly more frequent in UFH-treated patients (Table 1). Two other findings deserve comment. First, among the 431 patients undergoing serologic testing who did not develop HIT manifesting as a platelet count fall, there was a strong association between symptomatic thrombosis after day 4 and a positive platelet activation test for HIT antibodies: 3 (50.0%) of 6

Table 1. Frequency of HIT, thrombosis (HIT, non-HIT associated, total), and HIT antibodies in before-after prospective cohort study comparing UFH and LMWH after orthopedic surgery

	UFH, n = 231 (%)	LMWH, n = 271 (%)	P
Patients with HIT*	12 (5.2)	0 (0)	< .001
All symptomatic thrombotic events, HIT and non-HIT associated	9 (3.9)	2 (0.7)	.028
All symptomatic thrombotic events before or on day 4†	1 (0.4)	1 (0.4)	.910
All symptomatic non-HIT-associated thrombotic events after day 4‡	2 (0.9)	1 (0.4)	.597
All symptomatic HIT-associated thrombotic events after day 4 (HIT)§	6 (2.6)	0 (0)	.009
HIT antibody status at day 16/platelet activation assay	25/202 (12.4)	13/238 (5.5)	.010
HIT antibody status at day 16/immunoassay	46/196 (23.5)	19/228 (8.3)	< .001

*The 12 patients who met the a priori definition for HIT included 6 with platelet count fall (> 30%) alone, 3 with platelet fall (> 30%) plus symptomatic thrombosis, and 3 with symptomatic thrombosis alone; all HIT-associated symptomatic thrombi occurred after day 4. (Six of the 12 HIT patients had a platelet count fall more than 50%, 2 with thrombosis.)

†One UFH-treated patient developed pulmonary embolism (day 1), and one LMWH-treated patient developed cardiac shock (day 2).

‡Two UFH-treated patients developed pulmonary embolism (both day 10), and 1 LMWH-treated patient developed acute coronary syndrome (day 7).

§Four UFH-treated patients developed deep vein thromboses (DVTs; days 8, 8, 10, and 16), and 2 developed pulmonary embolism (days 7 and 15).