translocation of mantle cell lymphomas, the *MALT1-IGH* translocation of MALT lymphomas,[6] the *E2A-PBX1* translocation of B-ALL, and the *CRLF2-IGH* translocation of B-ALL. Despite the differences in stage of arrested differentiation between the 5 diseases, all 5 translocations appear to occur within pro-B/pre-B cells.

Albert G. Tsai
Norris Comprehensive Cancer Center and Departments of Pathology, Biochemistry & Molecular Biology, Molecular Microbiology & Immunology, and Biological Sciences (Section of Molecular and Computational Biology), University of Southern California Keck School of Medicine, Los Angeles, CA

Akinori Yoda
Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

David M. Weinstock
Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

Michael R. Lieber
Norris Comprehensive Cancer Center and Departments of Pathology, Biochemistry & Molecular Biology, Molecular Microbiology & Immunology, and Biological Sciences (Section of Molecular and Computational Biology), University of Southern California Keck School of Medicine, Los Angeles, CA

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Correspondence: David M. Weinstock, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney St, Dana 510B, Boston, MA 02115; e-mail: dweinstock@partners.org.

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To the editor:

Detection of the *JAK2*V617F mutation with the Ipsogen MutaScreen kit: absence of *JAK2*V617F does not mean absence of myeloproliferative neoplasm

We have analyzed with great interest the recent correspondence by Warshawsky et al describing 2 cases of myeloproliferative neoplasms (MPNs) where the Ipsogen MutaScreen kit failed to identify *JAK2*V617F mutations when another variant was present.[1] These variants were, however, detected with a "home-brew" melting curve analysis method.[2] The authors therefore considered that the real-time quantitative polymerase chain reaction–based MutaScreen kit is inferior to other technologies and generates a potential clinical risk. We do not support these views and would like to discuss these cases within the appropriate context.

Patient 1, with polycythemia vera (PV), was homozygous for 2 mutations in codon 617 (G1849T and C1851T) and 1 in codon 618. The MutaScreen assay reported "no signal," and the test was not interpretable.[1] This cannot be considered as a false-negative result. Patient 2, with essential thrombocythemia (ET), was heterozygous for 2 mutations in codons 617 and 618, and the MutaScreen assay reported "wild-type."[1] This is a real false-negative result.

The MutaScreen kit has been designed to detect the *JAK2*V617F G1849T mutation with high specificity, and the probe used cannot bind to *JAK2*V617F in the presence of 1 or more mismatches. The assay performance has been systematically assessed,[3,4] and high concordance with "home-brew" methods and reliable detection of *JAK2*V617F were shown.

It is well known that single nucleotide polymorphism detection methods using primer annealing can miss rare variants or mutations. Conversely, these can be detected by melting curve analysis. However this method has moderate to poor analytical sensitivity (5%-10% depending on the instrument used)[2] and the likelihood of not detecting a clinically relevant mutation is high. Biologists and clinicians therefore face a classical trade-off: guaranteed identification of patients with low (1%-10%) *JAK2*V617F allele burden (up to 20% of ET and 5% of PV patients; C. Marzac, unpublished data, May 31, 2010) versus the ability to detect rare variants reported in approximately 0.2% of all *JAK2*V617F carriers.[5]

The absence of *JAK2*V617F does not mean absence of MPN. Such a conclusion would constitute inappropriate use of the MPN World Health Organization guidelines,[6] which state that the presence of *JAK2*V617F or a similar mutation is only one of 2 possible main criteria for PV, and one of the major criteria for ET and primary myelofibrosis. They also indicate that if *JAK2*V617F is not detected in the presence of clinical manifestations consistent with MPN, additional tests are required to propose a final diagnosis. The MutaScreen instructions for use[7] state that the absence of *JAK2*V617F does not exclude the presence of other mutations. Therefore, a missed *JAK2*V617F mutation does not create a clinical risk for patients provided they are diagnosed and managed according to the most recent standards.

In conclusion, we believe that the Mutascreen assay has the appropriate design and performance characteristics to allow accurate detection of *JAK2*V617F. Every molecular laboratory should select and evaluate available assays based on performance and potential limitations, their objective when using the products, the regulatory framework, and current practice guidelines.

Hélène Peyro-Saint-Paul
Medical Affairs Department, Ipsogen SA, Luminy Biotech Entreprises, Marseille, France

Fabienne Hermitte
Research and Development Department, Ipsogen SA, Luminy Biotech Entreprises, Marseille, France

Contribution: H.P.-S.-P. and F.H. contributed equally to writing of the article.

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Correspondence: Hélène Peyro-Saint-Paul, MD, Ipsogen SA, Luminy Entreprises, Case 923, 163 Ave de Luminy, 13288 Marseille cedex 9, France; e-mail: peyro-saint-paul@ipsogen.com.

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To the editor:

Beta 2 glycoprotein I is a substrate of thiol oxidoreductases

Beta 2 glycoprotein I (β2GPI) is an abundant plasma protein recognized as the major autoantigen in the antiphospholipid syndrome. Although the crystal structure of β2GPI has been resolved,[1,2] its normal function remains unknown. We have been intrigued by the presence of a C-terminal cysteine (Cys326), which forms a loop-back disulfide link in the fifth domain of β2GPI. In the current study we examined β2GPI's potential to participate in thiol exchange reactions with the thiol oxidoreductases thioredoxin-1 (TRX-1) and protein disulfide isomerase (PDI).

The incorporation of free thiols into β2GPI after reaction with TRX-1 or PDI was shown by labeling the products of this reaction with the selective sulfhydryl probe N^a-(3-maleimidylpropionyl) biocytin (MPB). The biotinylated proteins were visualized by Western blotting with streptavidin–horseradish peroxidase. Because β2GPI does not contain unpaired cysteines, no labeling was observed after incubation with MPB (Figure 1A). Free thiols could not be introduced into β2GPI by incubation with the reducing agent dithiothreitol (DTT) alone. However, free thiols could be introduced into β2GPI after incubation with the reduced forms of the thiol oxidoreductases TRX-1 and PDI, identifying β2GPI as a thiol oxidoreductase substrate (Figure 1A-C). An interesting effect caused by the reduction of β2GPI by TRX-1 was a marked decrease in the affinity of anti-β2GPI monoclonal and polyclonal antibodies as noted on the immunoblots.

To determine the cysteine residue(s) in the β2GPI molecule involved in thiol exchange reactions, β2GPI treated with the TRX-1/TRX-1 reductase/NADPH system and labeled with MPB was resolved on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE; Figure 1B). Gel bands were excised, digested, and analyzed by liquid chromatography–tandem mass spectrometry. Mass spectral data were searched using Mascot (Version 2.2; Matrix Science) or converted to MzXML file format using ReAdW (Version 4.0.2)[3] and submitted to the database search program X!Tandem (Release 2008.12.01).[4] The analysis revealed Cys326 to be predominantly labeled with biotin (F.H.P., S.R., M.Q., M.J.R., J.W.H.W., K.T., Y.I., J.Y.Z., R.G., J.C.Q., B.G., W.E.H., P.J.H., S.A.K., manuscript submitted). The structural features of the disulfide bond containing Cys326 and all disulfide bonds of the 2 structures of β2GPI (PDB 1C1Z and 1QUB) were determined using the disulfide bond analysis tool available at www.cancerresearch.unsw.edu.au/CRCWeb.nsf/page/Disulfide+Bond+Analysis.[5] The analysis showed that the Cys288-Cys326 disulfide is a −/+ right-handed hook (−/+RHHook) configuration in both crystal structures of the protein.[1,2] Although there is no other structural similarity with β2GPI, the active site disulfides of oxidoreductases like TRX-1 or PDI are +/− RHHooks. Of the 22 Cys residues in β2GPI, Cys326 stands out as being exposed to solvent. The solvent accessibility values for Cys326 are 117 (PDB ID 1C1Z) and 103 (PDB ID 1QUB) $Å^2$ for the 2 structures. This high solvent exposure is consistent with reduction of the Cys288-Cys326 disulfide bond by thiol oxidoreductases.

Thiol oxidoreductases are becoming increasingly recognized as important mediators of platelet function.[6] The prototype member, PDI, has been implicated in the activation of the fibrinogen receptor αIIbβ3[7] and tissue factor.[8] Several novel members of the thiol isomerase family have been recently shown to translocate to the platelet surface after platelet activation.[9] In the current issue of *Blood*, Ioannou et al have developed a sensitive and specific streptavidin-capture enzyme-linked immunosorbent assay (ELISA) to detect reduced β2GPI in plasma.[10] With the same methodology β2GPI is shown to be reduced after incubation with platelets, which can be attributed partially to the TRX-1 system (Figure 1D).

This study is the first to show the potential of β2GPI to participate in thiol exchange reactions. Our finding suggests that β2GPI may participate in redox processes in vascular biology.