To the editor:

Independently acquired biallelic JAK2 mutations are present in a minority of patients with essential thrombocythaemia

It has recently been reported that the majority of patients with essential thrombocythaemia (ET) bear a JAK2 V617F mutation on both parental alleles.1 However, a prior report using different methodology indicated that biallelic JAK2 mutations are infrequent (<5% of MPN patients).2 We have undertaken a systematic investigation to clarify this discrepancy and elucidate the true prevalence of biallelic JAK2 mutations in ET.

Initial studies focused on reproducing the methodology and results of both previous reports.1,2 First, granulocyte genomic DNA (gDNA) from V617F-positive ET patients was analyzed by allele-specific polymerase chain reaction (AS-PCR),3 which amplifies only the V617F-mutant allele and generates an amplicon for sequencing which includes an informative intron 14 SNP (Figure 1A). Using this approach, biallelic JAK2 mutations were detected in 2 of 30 patients (eg, Figure 1B patient 1), in keeping with Olcaydu and colleagues, who used similar methodology.2 In the report of Lambert and colleagues,3 the frequency of biallelic JAK2 mutations was determined by PCR amplification of granulocyte cDNA and genotyping by restriction enzyme digestion. Given the difficulty of ensuring complete enzymatic digestion, bacterial cloning was also performed. We therefore used granulocyte cDNA to generate a similar amplicon (encompassing the V617F mutation and an informative exon 19 SNP, Figure 1A) for bacterial cloning and genotyping. In contrast to the AS-PCR/sequencing assay, this methodology suggested the presence of biallelic JAK2 mutations in all 4 patients studied (Figure 1C), consistent with Lambert and colleagues.1 Thus, our data reproduced the findings of both previous reports and demonstrated that the 2 methodologies give conflicting results.

We pursued several experimental strategies to explain these discrepant results. First, an 8-kb gDNA amplicon encompassing exons 14 to 19 was studied by bacterial cloning. This approach gave results concordant with AS-PCR/sequencing (Figure 1C), excluding insensitivity of the AS-PCR/sequencing assay or genotyping of a proximal versus distant SNP as explanations for our results. Next, a 2-kb cDNA amplicon encompassing exons 6 to 19 was similarly studied. This approach also gave results concordant with AS-PCR/sequencing (Figure 1C), excluding the use of cDNA compared with gDNA as an explanation.

Finally, we investigated template-switching as a source of discrepancy. This is an assay-specific phenomenon, reflecting differences in template, primers, and/or PCR conditions, which may occur when mixed templates (eg, heterozygous SNP and/or mutant/wild-type alleles) are amplified5,6 (Figure 1D). As the likelihood of template-switching increases in the later stages of the PCR reaction,5,6 we studied granulocyte cDNA from 10 of our 30 ET patients and compared results obtained using 25 and 35 PCR cycles, with PCR products analyzed quantitatively by bacterial cloning and sequencing (Figure 1E). Using 35 cycles, apparent biallelic mutations were present in all 10 patients. In marked contrast, using 25 cycles, biallelic mutations were found only in patient 1, who also had biallelic mutations detected by AS-PCR/sequencing.

These data indicate that apparent biallelic JAK2 mutations in ET patients frequently reflect assay-specific generation of chimeric DNA molecules during PCR amplification. Our study suggests that...
the true prevalence of biallelic JAK2 mutations in ET is approximately 5% to 10%.

Acknowledgments: We thank Prof Nick Cross for helpful comments on the manuscript and the Addenbrooke’s Hematology Disorders Sample Bank for processing and managing patient samples. Work in the authors’ laboratories is supported by the United Kingdom Medical Research Council, Leukaemia and Lymphoma Research, the Kay Kendall Leukaemia Fund, the National Institute for Health Research Cambridge Biomedical Research Center, and the Leukaemia & Lymphoma Society of America.

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

References


To the editor:

Poor outcome after reintroduction of imatinib in patients with chronic myeloid leukemia who interrupt therapy on account of pregnancy without having achieved an optimal response

Since the introduction of imatinib as the first-line therapy in chronic phase chronic myeloid leukemia (CML), the prognosis for patients has dramatically improved. As female patients who wish to conceive are currently advised to discontinue imatinib during conception and pregnancy due to the recognized teratogenic effects of the drug, these patients are often without ideal therapy for a prolonged period of time. Responses to imatinib can be regained after prolonged cessation of therapy on account of side effects or pregnancy; however, the ideal degree of response that patients wishing to conceive should achieve before stopping therapy is not clear, nor is it clear whether a lengthy discontinuation of the treatment is safe in all the cases. Here we report the effect of discontinuing imatinib on the clinical response in 7 patients.

The median age was 32 years (range, 25-34 years). All patients received 400 mg of imatinib daily as initial therapy for CML in chronic phase. In 4 patients the imatinib was discontinued as soon as the pregnancy was confirmed, and 3 patients stopped imatinib to conceive. Before discontinuation, imatinib was received for a median time of 19 months (range, 7-42 months, Table 1), and all patients were still receiving 400 mg. The median period of drug interruption was 9 months (range, 6-23 months). All patients had lost their cytogenetic response, and 4 lost the complete hematologic response (CHR) before the imatinib was reintroduced, but only patient no. 3 required therapy (leukapheresis) during imatinib discontinuation.

Before imatinib discontinuation, patients 2, 3, 4, and 6 were suboptimal responders (Table 1). None of these 4 patients obtained an appropriate response when imatinib was reintroduced after the delivery. Patients 2, 4, and 6 (Table 1) failed to achieve complete cytogenetic response (CCyR) or subsequently restarting imatinib therapy. Patient 3 achieved CCyR but failed to achieve major molecular response (MMR) after 26 months of therapy. The 3 patients (nos. 1, 5, and 7) who had obtained an optimal response at the time of stopping imatinib regained at least MMR after restarting therapy (Table 1). None of the 7 patients had developed kinase domain mutations.

Our results demonstrate that an adequate response after restarting imatinib after discontinuation in pregnancy is seen only in patients who had an optimal response (MMR) before stopping the drug. Suboptimal responders to initial treatment with imatinib either demonstrated the same response after discontinuation or, more of concern, deterioration of the response eventually meeting criteria for imatinib failure. Indeed, it is not clear whether the poor results after reintroduction of imatinib occurred because the patients had not yet achieved an appropriate response before conception or because they were poor-risk patients. It is possible that the use of nilotinib or dasatinib in suboptimal responders to obtain MMR before therapy discontinuation may reduce the risk of treatment failure after the reintroduction of therapy. Our findings emphasize the
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