To the editor:

t(X;14)(p22;q32)/t(Y;14)(p11;q32) *CRLF2-IGH* translocations from human B-lineage ALLs involve CpG-type breaks at *CRLF2*, but *CRLF2/P2RY8* intrachromosomal deletions do not

Rearrangements involving the type I cytokine receptor subunit *CRLF2* occur in 5% to 7% of all adult and pediatric B-cell precursor acute lymphoblastic leukemias (B-ALL) and in 60% of B-ALL in children with Down syndrome.[1-4] *CRLF2* rearrangement places full-length *CRLF2* under alternate transcriptional control and can result from either an intrachromosomal *CRLF2-P2RY8* deletion or a *CRLF2-IGH* translocation. All of the reported *CRLF2-P2RY8* deletions involve V(D)J-type breaks at highly localized heptamer sequences upstream of *CRLF2* (Figure 1A) and within intron 1 of *P2RY8*.[2-4] In contrast, the breakpoints upstream of *CRLF2* in *CRLF2-IGH* translocations distribute over a 24 717-bp region in a patchy manner (Figure 1A). This patchy clustering differs from the random distribution of breakpoints over multikilobase domains that we described for translocations including TEL-AML1 and BCR-ABL that occur within multipotent progenitors before V(D)J recombination.[5] Among *CRLF2* breakpoints, there is a moderately prominent 311-bp cluster from positions 1 307 403 to 1 307 713, which contains 6 of 19 described breakpoints (Figure 1A).[4] Comparing this *CRLF2* cluster to the remaining 24 406 bp, there is a 36-fold "enrichment" of breakpoints in the region.

We previously reported that some translocations occurring during B-cell ontogeny preferentially localize to the dinucleotide CpG, with 30% or more occurring directly at a CpG and 70% or more within 8 bases of a CpG.[5,6] Of 19 total *CRLF2* breakpoints, 7 are directly at CpGs ($P < .001$) and breakpoints are much closer to CpGs than expected by random chance ($P < .001$). Thirteen breakpoints are within 8 bp of a CpG, far more than if breakpoints were randomly distributed throughout the region (Figure 1B). In contrast, breakpoints are not significantly clustered around ▼CAC or ▼CACA ($P > .1$ in all tests, Figure 1C; ▼ indicates the target site for breakage by the V(D)J recombinase), the essential sequence motif that defines V(D)J recognition signal sequences (RSSs). Methods have been described previously.[5]

Similar to *BCL2-IGH* translocations described previously, at least 18 of the 19 *IGH* breaks in *CRLF2-IGH* translocations are compatible with standard V(D)J recombination, in that the *IGH* junction is within 30 bp 5′ of an RSS. In addition, 18 of 19 *CRLF2-IGH* junctions contain nontemplated nucleotide additions consistent with the activity of terminal deoxynucleotide transferase (TdT). Thus, the *CRLF2* region from *CRLF2-IGH* translocations shares all the key features of the 4 regions previously described as having CpG-type breaks: (1) preferential localization to the dinucleotide sequence CpG, with breakpoints on either side (ie, 5′ or 3′) of CpG, (2) significantly weaker or no preferential localization to any other dinucleotide motif or ▼CAC, (3) propensity to cluster into zones of 20 to 600 bp, and (4) evidence of occurrence at the pro-B/pre-B stage, based on the involvement of *IGH* breaks from V(D)J recombination and the presence of TdT additions.

With this letter, the list of pathologic chromosomal rearrangements involving a CpG-type mechanism now consists of the *BCL2-IGH* translocation of follicular lymphomas, the *BCL1-IGH*
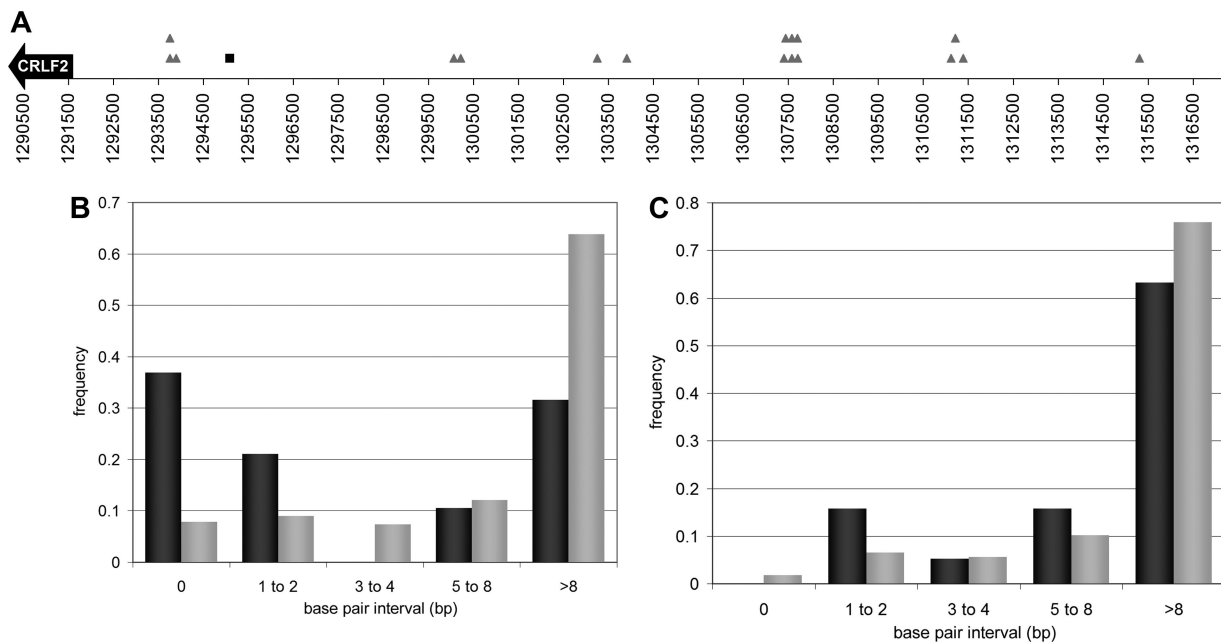
Figure 1. Distribution of breakpoints on CRLF2. (A) Each △ denotes an individual CRLF2 breakpoint sequenced from a B-ALL with a CRLF2-IGH translocation. The ■ denotes the position of the CRLF2 breakpoints sequenced from B-ALL with CRLF2-P2RY8 intrachromosomal deletions. The arrow labeled "CRLF2" indicates the position and transcriptional direction of the CRLF2 gene. Numbering is per the March 2006 (hg18) build from the UCSC Genome Browser (http://genome.ucsc.edu/). (B) Overall frequency of CRLF2 breakpoints at various distance intervals from CpG. (C) Overall frequency of CRLF2 breakpoints at various distance intervals from CAC. Proportions of CRLF2 breakpoints at distances of 0 bp, 1-2 bp, 3-4 bp, 5-8 bp, and > 8 bp from CpG (B) or CAC (C) are graphed for the CRLF2 region. The distribution for actual leukemia breakpoints is shown in black, and that for a random distribution between the farthest breakpoints is shown in gray. If the black and gray bars parallel one another, then the patient breakpoints appear random in their distribution relative to the specified motif. However, when they follow opposite trends (ie, the gray bars rise with increasing distance from the specified motif while the black bars fall), then the breakage process appears to concentrate around the motif.

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.

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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.