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Correspondence

ABL Kinase Domain Pseudoexon Insertion Is Not Uncommon in BCR-ABL Transcripts

To the Editor-in-Chief:

We read with interest an article in the March issue of *The Journal of Molecular Diagnostics*, in which Laudadio and colleagues[1] reported detection of a 35-base insertion in the *ABL* kinase domain of *BCR-ABL* transcripts from three chronic myelogenous leukemia patients. We wish to direct your readers to an Association for Molecular Pathology meeting abstract published in *JMD* 4 years ago in which we described our discovery of the same mutation in the course of validating our *ABL* kinase domain DNA sequencing assay.[2]

After discovering and characterizing this mutation in June of 2004, one of us (N.B.Q.) communicated our observations to Susan Branford (the Institute of Medical and Veterinary Science, University of Adelaide, Adelaide, Australia), who had helped us months earlier with *ABL* sequencing primer design for our assay. Susan Branford indicated that their group had also seen *ABL* gene insertion mutations in some chronic myelogenous leukemia patients, but that they had not characterized those mutations (personal communication).

Although we were only able to indicate our main findings in the abstract (ie, discovery of a novel frame-shift insertion mutation in the *ABL* kinase domain of *BCR-ABL* transcripts, mapping the source of the insert to intron 8 of the *ABL* gene, proposal of aberrant splicing as its genesis, discovery of the same insertion in *BCR-ABL* transcripts from other chronic myelogenous leukemia patients, and lack of apparent correlation between insertion mutation transcript abundance and clinical symptoms), the companion poster (available on request), described in considerable detail our characterization of this novel mutation.

In addition to the shared observations that were made both in our abstract[2] and in the recent article by Laudadio and colleagues,[1] our poster made two key additional experimental observations that hinted strongly at the possible ubiquity of this alternate splicing event. We reported in the poster that RNA extracted from eight of eight chronic myelogenous leukemia patients undergoing Gleevec therapy (but not K562 or HL60 RNA) contained at least some detectable transcripts carrying the 35-base insertion (ranging in relative abundance from $<5\%$ up to $\sim 80\%$ of all *BCR-ABL* transcripts) and the same 35-base insert-carrying transcripts were also detected in low abundance among the normal (nonfusion) *ABL* gene transcript pool, as assessed by *ABL* gene amplification without prior enrichment amplification for *BCR-ABL* transcripts. We proposed in the poster that, since splice variants are known for many genes, one could expect other *BCR-ABL* splicing anomalies would be found as more sequencing analysis is performed (as reported recently by Volpe et al[3]). We observed also that, based on the literature,[4] the 35-base insert qualified as a legitimate pseudoexon but that the complex factors regulating selection among possible alternate splice points were not well understood.

The recent article by Laudadio and colleagues[1] provides valuable independent confirmation in three additional chronic myelogenous leukemia patients of the presence of the 35-base pseudoexon in the *ABL* kinase domain that we reported in 2004. It is our present contention that this aberrant splicing event is quite commonly detected in the *BCR-ABL* transcript pool (and perhaps also in the corresponding normal *ABL* gene transcript pool) in chronic myelogenous leukemia patients and that its abundance is dynamic in individual patients and probably variable across many chronic myelogenous leukemia patients.

It would be very interesting to know whether or not the abundance of these alternately spliced transcripts will be correlated in the future with therapy response or prognosis. To this end, we have designed a TaqMan probe and two pairs of real-time polymerase chain reaction primers with which to quantify the relative abundance of these alternate transcripts in chronic myelogenous leukemia patients and healthy donors.

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Authors' Reply:

In response to the informative correspondence of Neil Quigley and colleagues regarding our description of an insertion mutation in the BCR-ABL kinase domain of patients with imatinib-treated chronic myelogenous leukemia,[1] we readily agree that this mutation is dynamic and not uncommon. But what exactly is the prevalence of BCR-ABL deletion/insertion/splice variants as compared to their better-studied point mutation counterparts? Although the prevalence of the specific 35-bp intron 8 insertion variant that we reported has, to our knowledge, never been documented in the peer-reviewed literature, our group recently found a 15% prevalence of other deletion/insertion/splice variants among an unselected cohort of 95 imatinib-treated chronic myelogenous leukemia patients.[2] To similarly address this prevalence issue (of the 35-bp intron 8 insertion), Quigley and colleagues[3] reported in their abstract that "close inspection of sequence electropherograms from other BCR-ABL-positive patients revealed that a very small proportion (<5%) of their BCR-ABL mRNAs also carried the same insertion." Given the well-known analytical sensitivity limit of direct DNA sequencing for detecting low-level variants (~20%), we wonder how these much lower-abundance (<5%) variants could have been distinguished from background signals. We will therefore eagerly await the publication of results from Quigley and colleagues allele-specific TaqMan assay, which will, of course, be a much better assay for both detecting and quantitating low-level variants. Perhaps more important, however, from a practical perspective, will be a direct functional characterization of the kinase, drug resistance, and transforming activities of the intron 8 insertion variant—which we predict will be absent because of the loss of the entire *ABL* last exon region. We thank Quigley and colleagues for their comments and for continuing this important discussion on BCR-ABL kinase domain mutations in chronic myelogenous leukemia patients being treated with tyrosine kinase inhibitors.

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