To the editor:

Deletions of the derivative chromosome 9 do not account for the poor prognosis associated with Philadelphia-positive acute lymphoblastic leukemia

The Philadelphia (Ph) translocation t(9;22)(q34;q11) is found in 15% to 25% of adults and 3% to 5% of children with acute lymphoblastic leukemia (ALL)[1] and identifies patients with a particularly poor prognosis.[2] The Ph translocation is also the hallmark of chronic myeloid leukemia (CML), a biphasic disease arising in the stem cell compartment, that inevitably progresses to a terminal blastic phase.[3,4] The molecular consequence of the Ph translocation is the formation of a *BCR-ABL* fusion gene.[5] In the majority of CML patients and approximately one third of patients with ALL the translocation breakpoint in *BCR* is found within a region spanning exons 12-16 termed the major breakpoint cluster region (M-*bcr*). This results in a $p210^{BCR\text{-}ABL}$ fusion protein.[6] In about two thirds of patients with ALL and in rare cases of CML, the *BCR* breakpoint occurs further upstream between exons e2′ and e2, in a region termed the minor breakpoint cluster region (m-*bcr*) resulting in a $p190^{BCR\text{-}ABL}$ protein.

We and others have recently reported deletions adjacent to the t(9;22) translocation junction on the derivative chromosome 9.[7-11] The deletions spanned several megabases, and usually resulted in loss of sequences from both chromosome 9 and 22 sides of the junction. Deletions were detected in 10% to 15% of CML patients and were associated with rapid onset of blast crisis and a significantly shorter survival,[8,10,11] raising the possibility that similar deletions may contribute to the aggressive clinical course of Ph-positive ALL.

Here we present results from a study of 67 Ph-positive ALL patients (2 children and 65 adults). Bone marrow samples were screened for deletions of the derivative chromosome 9 by fluorescence in situ hybridization (FISH). The hybridization patterns expected in nondeleted and deleted Ph-positive metaphases with major or minor *BCR* breakpoints for the 3 probe systems used are shown in Figure 1A. Using the triple-probe system (Vysis, Downers Grove, IL, as described in Sinclair et al[8] and Huntly et al[11]), loss of a blue signal on the derivative chromosome 9, indicating deletion of 9q sequences, was observed in 1 of 67 patients (Figure 1B). Forty-eight of 49 patients analyzed using the D-FISH system[12] (QBiogene, Middlesex, United Kingdom) displayed both red and green signals on the derivative chromosome 9 indicating no overt loss of chromosome 9 or 22 material from the derivative chromosome 9 (data not shown). In the single patient in whom loss of 9q sequences had been observed using the triple-probe system, D-FISH demonstrated that the deletion involved loss of both chromosome 9 and 22 sequences (Figure 1C). The frequency of deletions in patients with Ph-positive ALL (1/67), was therefore significantly lower than the frequency of deletions in our study of CML[11] (39/253, χ^2: $P = .002$), suggesting that derivative chromosome 9 deletions are rare in Ph-positive ALL cases and do not account for the aggressive clinical course of this disease.

In patients without a deletion, the triple-probe system can distinguish M-*bcr* and m-*bcr* breakpoints by virtue of different signals on the derivative chromosome 9 (Figure 1A). In the current series 54 of 66 patients lacking a deletion had an m-*bcr* breakpoint while the remaining 12 patients had an M-*bcr* breakpoint. However, if a patient has a deletion of the derivative chromosome 9, then no hybridization signals will be present on this chromosome and so breakpoint position cannot be assessed using the triple-probe system. The MBCR/ABL system (QBiogene) was therefore used to study the single patient with a deletion. As shown in Figures 1A and D, the green BCR signal was absent from the Ph chromosome consistent with an m-*bcr* breakpoint. This result demonstrates that derivative chromosome 9 deletions are not restricted to patients with an M-*bcr* breakpoint.

Several mechanisms may account for the rarity of chromosome 9 deletions in Ph-positive ALL relative to CML. First, deletions are more common in patients with variant Ph translocations,[8,10,11] and whereas variant translocations occur in 10% of patients with CML they are rare in ALL.[13] None of the 67 patients presented here had a

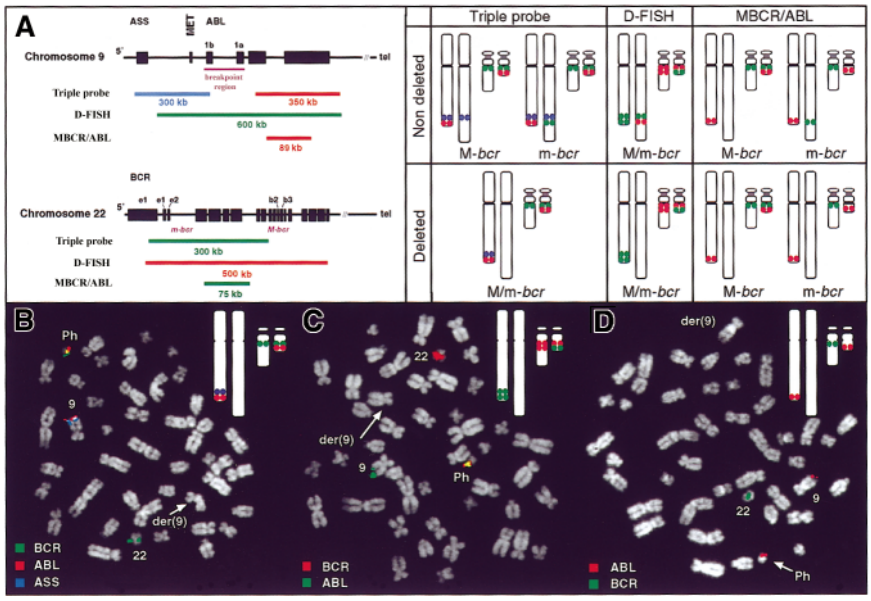

Figure 1. FISH analysis of bone marrow metaphases from Ph-positive ALL patients. (A) Structure of *ABL* and *BCR* genes with composition of the indicated probe systems together with expected hybridization patterns using all 3 probe systems on Ph-positive metaphases. Chromosome 9 homologues are shown on left (normal 9 and derivative 9, respectively) while chromosome 22 homologues are shown on right (normal 22 and Ph, respectively). (B-D) FISH analysis of Ph-positive bone marrow metaphases from ALL patient with deletion of derivative chromosome 9. Each panel shows a metaphase together with an image of the expected hybridization signals (inset). (B) Triple-probe analysis. The blue ASS signal is absent from the derivative chromosome 9 indicating a deletion of chromosome 9 sequences from this chromosome. (C) D-FISH analysis with absence of colocalized signal on der(9) revealing deletion of 9q and 22q sequences from this chromosome. (D) MBCR/ABL analysis. Lack of a BCR signal on the Ph chromosome indicates an m-*bcr* breakpoint. This signal does not appear on the derivative chromosome 9 consistent with a deletion of this region. Images were captured using an epifluorescence microscope (Axioplan 2, Zeiss, United Kingdom), and SmartCapture 2001 software (Digital Scientific, United Kingdom).

variant Ph translocation and this may account in part for the paucity of deletions.

Second, it is possible that a deletion is more likely to accompany a translocation with an M-*bcr* breakpoint. Since M-*bcr* breakpoints occur in the vast majority of patients with CML but in only a minority of patients with Ph-positive ALL, this could account for the rarity of deletions in the latter disease. Although the numbers are small our data are consistent with this idea since deletions were observed in only one of the 54 patients with an m-*bcr* breakpoint compared with 25 of 212 CML patients with a classical Ph translocation[11] ($P = .036$).

Third, the rarity of deletions in Ph-positive ALL may reflect features of the target cell in which the translocation occurs. CML results from transformation of a multipotent stem cell,[3] whereas ALL more often results from transformation of a committed B-cell progenitor.[14] Lymphoid cells undergo antigen receptor rearrangements that require accurate joining of double stranded DNA breaks[15] and may therefore employ mechanisms that minimize the concomitant occurrence of large deletions.

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To the editor:

Does the P_{2X1del} variant lacking 17 amino acids in its extracellular domain represent a relevant functional ion channel in platelets?

In a recent issue of *Blood*, Greco et al[1] reported on the expression of a novel structurally altered P_{2X1} receptor in platelets and in megakaryocytic cell lines. This P_{2X1} variant lacks 17 amino acids in its extracellular domain due to a deletion within exon 6 of the *P_{2X1}* gene (GenBank accession no. 17481172). The authors showed that, after heterologous expression in the 1321N1 astrocytoma cell line, P_{2X1del} subunits constitute a channel preferentially activated by adenosine diphosphate (ADP). In reverse transcriptase–polymerase chain reaction (RT-PCR) analyses, they described this variant as the major P_{2X1} mRNA of platelets, thus claiming that P_{2X1del} may play an important role as an ADP-activated ion channel in these cells. These conclusions are in contradiction with other studies[2,3] that show that the functional platelet P_{2X1} receptor is an adenosine triphosphate (ATP)–gated ion channel that is unresponsive to high-performance liquid chromatography–purified ADP. Indeed, the activation of the P_{2X1} receptor by ATP or by its stable analogs, α,β-methylene ATP and L-β,γ–methylene ATP, produces a rapid, quickly desensitized Ca^{2+} influx[4] that is responsible for reversible platelet shape change,[3,5] and that also plays a pivotal role during platelet aggregation induced by collagen.[3] These platelet responses to ATP were found to be antagonized by ADP similarly to the inward current produced by ATP in *Xenopus* oocytes expressing wild-type P_{2X1} receptors (P_{2X1wt}).[3] In addition, platelet receptors for ADP have been well characterized and are identified as 2 P_{2Y} receptors: P_{2Y1} and P_{2Y12} (reviewed in Gachet[6]). Both receptors are required for normal platelet responses to ADP, a conclusion recently corroborated in $P_{2Y1}-/-$ and $P_{2Y12}-/-$ knock-out mice.[7,8] Thus, the existence in platelets of an ADP-activated variant of the P_{2X1} ion channel as the major platelet P_{2X} representative, as hypothesized by Greco et al,[1] can be questioned.

In this letter, we present data that argue against the possibility for a role of P_{2X1del} in platelet function. First, RT-PCR analyses of independent platelet RNA samples followed by sequencing of the PCR products showed major abundance of the P_{2X1wt} mRNA (Figure 1A, lane 2; Figure 1B), whereas the platelet P_{2X1del} mRNA appeared as a minor product. In contrast to the findings of Greco et al,[1] we found comparable relative amounts of the P_{2X1del} mRNA in platelets and in the Dami megakaryocytic cell line (Figure 1A, lane 3; Figure 1B). Second, in order to further demonstrate the presence of P_{2X1del} transcripts in platelets the authors designed 2 different