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## SHORT REPORT

## Derivative chromosome 9 deletions in chronic myeloid leukaemia: interpretation of atypical D-FISH pattern

T S K Wan, S K Ma, W Y Au, L C Chan

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**Background/Aims:** New molecular cytogenetic techniques are increasingly applied as a routine investigative tool in haematological malignancies, both at diagnosis and subsequent monitoring. This report describes the interpretation of atypical signal patterns encountered using BCR–ABL dual colour dual fusion fluorescence in situ hybridisation (D-FISH) translocation probes in chronic myeloid leukaemia (CML).

**Methods:** Interphase FISH experiments were carried out using BCR–ABL D-FISH probes in 46 patients with CML at diagnosis and during subsequent disease monitoring. Atypical hybridisation signal patterns were characterised by molecular cytogenetic techniques and correlated with conventional karyotyping.

**Results:** Two patients showed atypical interphase D-FISH patterns with one orange, one green, and one fusion (1O1G1F) signal. The presence of BCR–ABL gene fusion was documented by a dual colour single fusion (S-FISH) probe. The submicroscopic deletion of the ABL–BCR fusion gene on the derivative chromosome 9 in these cases was subsequently characterised by metaphase FISH on relocated G banded metaphases.

**Conclusions:** Atypical interphase D-FISH patterns should not be interpreted in isolation and should be considered in conjunction with other cytogenetic or molecular genetic investigations.

The Philadelphia (Ph) chromosome, resulting from the balanced translocation, t(9;22)(q34;q11.2), which fuses the 5' sequences of the BCR gene on chromosome 22 with 3' sequences of the ABL gene on chromosome 9, is the diagnostic hallmark of chronic myeloid leukaemia (CML). This translocation generates two fusion genes, BCR–ABL on derivative chromosome 22q, known as the Ph chromosome, and a reciprocal ABL–BCR fusion gene on derivative chromosome 9q. The technique of fluorescence in situ hybridisation (FISH), using probes that bind to specific sequences at the Ph translocation breakpoints, has been applied extensively at initial diagnosis of CML to investigate cases with failed cytogenetics, to detect cryptic BCR–ABL gene fusion, and to decipher complex Ph rearrangements. The quantification of BCR–ABL positive cells is achievable through interphase FISH and hence facilitates the monitoring of disease response to treatment.

"The Philadelphia chromosome, resulting from the balanced translocation, t(9;22)(q34;q11.2), is the diagnostic hallmark of chronic myeloid leukaemia"

The conventional FISH strategy based on differentially labelled BCR and ABL probes, which gives a single fusion signal (S-FISH) and one signal each for the wild-type alleles is

accurate for metaphase analysis, but shows high false positive value as a result of coincidental juxtaposition of signals. This drawback severely limits its potential for the detection and quantification of minimal residual disease, because the normal cut off point for S-FISH has been set within the 7–10% range.<sup>1,2</sup> To tackle this problem, a dual colour dual fusion (D-FISH) BCR–ABL probe system has been developed.<sup>3,4</sup> These are large probes, which are designed to span the translocation breakpoints and are labelled with different fluorochromes. In addition to one signal each from the normal BCR and ABL genes, two fusion signals are created in a Ph positive cell: a BCR–ABL fusion signal on derivative chromosome 22q and an ABL–BCR fusion signal on derivative chromosome 9q. Variant signal patterns are encountered in three way Ph translocations and the addition of an extra Ph chromosome when investigated by the D-FISH system.<sup>5</sup> We describe the molecular cytogenetic characterisation of two patients with Ph positive CML, who both showed an identical atypical D-FISH pattern consistent with ABL–BCR deletion, and discuss its interpretation in the context of disease diagnosis and monitoring.

## MATERIAL AND METHODS

## Cytogenetic analysis

Cytogenetic analysis was performed on Giemsa banded (G banded) metaphases obtained through short term synchronised and unsynchronised cultures of bone marrow or peripheral blood cells based on standardised protocols. Details of the karyotype were reported in accordance with ISCN 1995.

## FISH

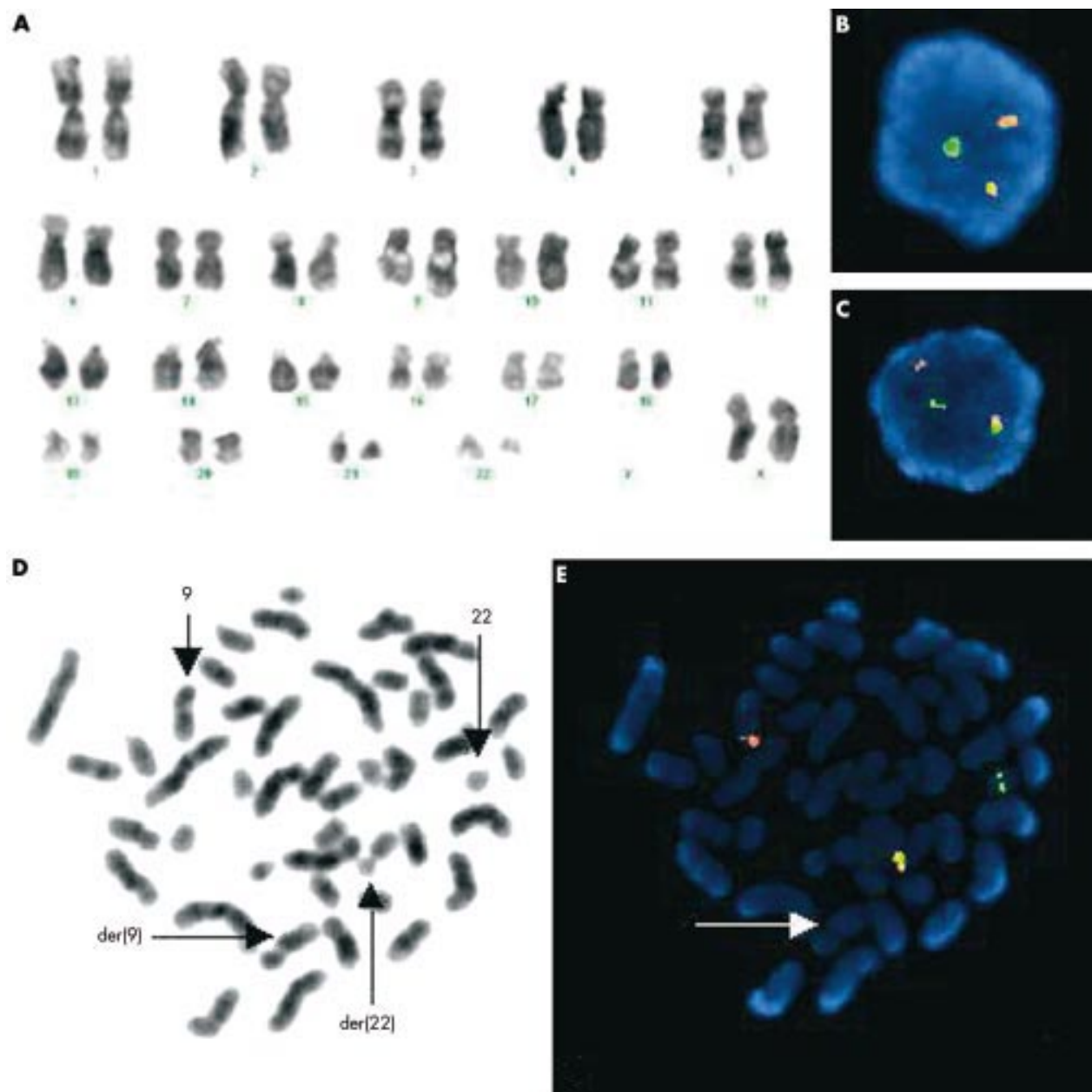
Detection of BCR–ABL gene fusion was performed using both BCR/ABL dual colour single fusion (S-FISH) and BCR/ABL dual colour dual fusion (D-FISH) translocation probes (Vysis, Downers Grove, Illinois, USA), according to the manufacturer's instructions. In total, 300 interphase nuclei on cytopsin smears were analysed for the presence of fusion signals. In addition, metaphase FISH was performed on G banded Ph positive metaphases relocated using microscope coordinates, as described previously,<sup>6</sup> thereby allowing direct morphological correlation of fusion signals and abnormal chromosomes.

## Case histories and results

## Case 1

A 35 year old Chinese woman presented with a one month history of weakness and bone pain. Complete blood counts showed: haemoglobin, 117 g/litre; white blood cell count,  $47.0 \times 10^9$ /litre (neutrophils, 80%; lymphocytes, 13%; metamyelocytes, 6%; blasts, 1%), and platelet count  $878 \times 10^9$ /litre. The

**Abbreviations:** CML, chronic myeloid leukaemia; D-FISH, dual colour dual fusion fluorescence in situ hybridisation; FISH, fluorescence in situ hybridisation; G, Giemsa; Ph, Philadelphia; S-FISH, dual colour single fusion fluorescence in situ hybridisation



**Figure 1** Case 2 (A) Complete karyotype showing  $t(9;22)(q34;q11.2)$ . G banding with trypsin/Giemsa. (B) Interphase fluorescence in situ hybridisation (FISH) with the dual colour dual fusion (D-FISH) probe, showing one orange, one green, one fusion (yellow) signal, and one missing fusion signal. (C) Interphase FISH with the dual colour single fusion (S-FISH) probe, showing the presence of BCR-ABL gene fusion (yellow). (D, E) Metaphase FISH analysis on a relocated G banded chromosome using the D-FISH probe. No ABL-BCR fusion signal is detected on the derivative chromosome 9 (white arrow).

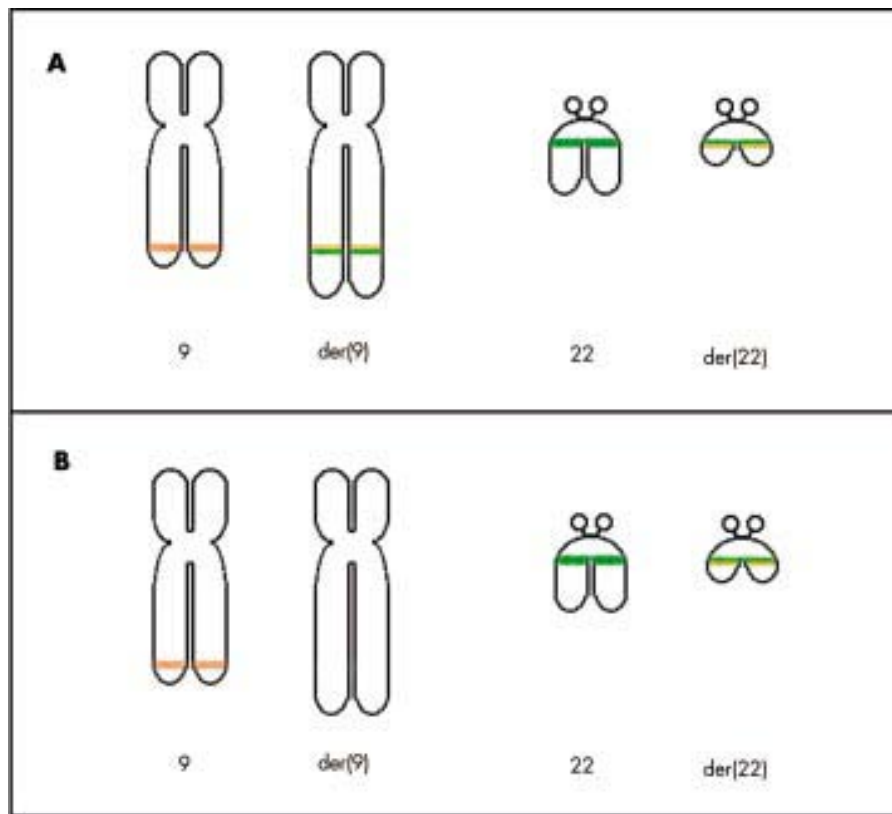
bone marrow aspirate showed an extremely hypercellular marrow with hyperplastic granulocytic series. All stages of myeloid maturation were represented and the blast count (2%) was within normal limits. However, basophils were increased (4%). Erythroid activity was relatively preserved and showed normoblastic maturation. Megakaryocytes were greatly increased with many small and hypolobulated forms. A diagnosis of chronic phase CML was made. A cytogenetic study of the bone marrow cells showed:  $46,XX,t(9;22)(q34;q11.2)$ .<sup>6</sup>

FISH was performed on interphase nuclei using the D-FISH system. Results showed single orange and green signals, indicative of normal BCR and ABL alleles, and one fusion signal in 95% of cells among 300 cells analysed, giving an atypical 1O1G1F D-FISH pattern. A normal clone (5%) with the 2O2G pattern was present. The presence of the BCR-ABL gene

fusion was confirmed on interphase nuclei using the S-FISH probe. Deletion of the ABL-BCR reciprocal fusion gene on the derivative chromosome 9 was further characterised by metaphase FISH on G banded Ph positive metaphases using the D-FISH probe.

#### Case 2

A 79 year old Chinese woman, who was known to have suffered from CML for 10 years, was investigated for suspected accelerated phase disease. An initial conventional cytogenetic study failed to yield metaphases for analysis. Detection of the BCR-ABL gene fusion was performed on interphase nuclei in peripheral blood leucocytes using the D-FISH probe. Results showed single orange and green signals, indicating the presence of normal BCR and ABL genes, and one fusion signal (1O1G1F) in 85% of cells among 300 cells analysed (fig 1B). A



**Figure 2** Signal pattern resulting from derivative chromosome 9 deletion as detected by dual colour dual fusion fluorescence in situ hybridisation (D-FISH). The ABL probe (chromosome 9) is labelled with an orange fluorochrome, whereas the BCR probe (chromosome 22) is labelled with a green fluorochrome, with both probes spanning their respective chromosomal breakpoints. (A) Typical D-FISH pattern in a BCR-ABL positive cell, showing one signal each for the wild-type alleles and two fusion signals (1O1G2F). (B) Atypical D-FISH pattern in a BCR-ABL positive cell with a reciprocal ABL-BCR deletion, showing one signal each for the wild-type alleles and one fusion signal only (1O1G1F).

normal clone (15%) with a normal D-FISH pattern (2O2G) was present. The presence of the BCR-ABL fusion gene was confirmed on interphase nuclei using the S-FISH probe (fig 1C). A repeat cytogenetic study by overnight culture of peripheral blood cells showed 46,XX,t(9;22)(q34;q11.2)<sup>12</sup> (fig 1A). The loss of the ABL-BCR fusion signal on the derivative chromosome 9 was further characterised by metaphase FISH on the G banded Ph positive metaphases using the D-FISH probe as described in case 1 (fig 1D,E).

## DISCUSSION

As a result of the generation of two fusion signals and improved specificity, D-FISH is a useful diagnostic method for detecting low amounts of BCR-ABL gene fusion in interphase nuclei. In one study, the mean percentage of false positivity was 0.25% (SD, 0.39%; range, 0–1.5%) based on the analysis of 200 interphase nuclei.<sup>3</sup> When extended to the analysis of 6000 nuclei, the detection limit was improved to 0.079%, making this technique capable of detecting minimal residual disease.<sup>3</sup> With the system that we use, the typical D-FISH pattern in a BCR-ABL positive interphase cell is one signal each for normal BCR and ABL alleles and the presence of double fusion signals (1O1G2F). However, variant D-FISH patterns are produced in three way Ph translocations, in which the ABL-BCR fusion is split (2O2G1F), and when there is an extra Ph chromosome present, in which case three fusion signals would be expected (1O1G3F). These D-FISH patterns should also be interpreted as BCR-ABL positive.<sup>5</sup>

Deletions of the derivative chromosome 9 adjacent to the Ph translocation breakpoint have recently been recognised in CML.<sup>7</sup> These deletions, found in around 15% of patients with CML, are large and occur at the time of the Ph translocation.<sup>8</sup> Although the deleted segments show considerable heterogeneity, they often span the translocation breakpoint, thus involving the reciprocal ABL-BCR fusion.<sup>9</sup> The presence of these deletions predicts a poor prognosis for those patients with CML who are undergoing conventional treatment (hydroxyurea or

interferon),<sup>8–10</sup> and may be related to the loss of one or more genes within the deleted region on derivative chromosome 9.<sup>10</sup> It is not yet established whether the adverse outcome associated with derivative chromosome 9 deletions is also applicable to those patients who are undergoing treatment with imatinib (STI571). In contrast to the typical D-FISH pattern seen in BCR-ABL positive cells, these patients show one signal each for the wild-type BCR and ABL alleles and a single fusion signal only (1O1G1F) (fig 2). In addition, other derivative chromosome 9 deletions involving either deletion of ABL alone (1O2G1F) or deletion of BCR alone (2O1G1F) can occur.<sup>5</sup>

“The single fusion signal in the 1O1G1F pattern as detected by interphase D-FISH, unlike metaphase FISH, may represent either BCR-ABL or ABL-BCR gene fusion”

A review of our records showed that, of the 46 patients with CML in whom D-FISH was performed at diagnosis or subsequent monitoring, two showed an atypical hybridisation pattern (1O1G1F), indicative of derivative chromosome 9 or ABL-BCR deletion. Both cases did not show the typical D-FISH pattern (1O1G2F) in the interphase nuclei, indicating that all BCR-ABL positive cells harboured the reciprocal ABL-BCR deletion. The correlation of conventional cytogenetics and metaphase FISH results showed that, in accordance with previous observations,<sup>8,9</sup> such deletions were not associated with detectable morphological changes on the derivative chromosome 9, and hence were of a submicroscopic nature. However, we argue that the single fusion signal in the 1O1G1F pattern as detected by interphase D-FISH, unlike metaphase FISH, may represent either BCR-ABL or ABL-BCR gene fusion, and should not be interpreted as positive in the absence of other supportive evidence for BCR-ABL gene fusion. Without conventional cytogenetics or molecular genetics data, the simplest way would be to perform S-FISH to confirm the presence of BCR-ABL gene fusion, as illustrated in the second case.

**Take home messages**

- The interphase dual colour dual fusion fluorescence in situ hybridisation (FISH) system can detect the BCR–ABL fusion gene with improved specificity in minimal residual disease and can identify variant or atypical patterns, such as derivative chromosome 9 deletions
- However, in clinical practice results should be interpreted together with information gathered through conventional cytogenetics, FISH, and molecular genetic studies

In conclusion, although the interphase D-FISH system for BCR–ABL fusion gene detection shows improved specificity in minimal residual disease and allows the identification of variant or atypical patterns, such as derivative chromosome 9 deletions, in clinical practice results should not be taken in isolation. They should be interpreted in the light of information gathered through conventional cytogenetics, FISH, and molecular genetic studies.

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