

## APPLICATION OF FLUORESCENCE IN SITU HYBRIDIZATION IN DEFINING A COMPLEX t(9;21;22) Ph FORMATION

Marco Mancini, Mauro Nanni, Michele Cedrone, Maria Rosaria De Cuia, Maria Beatrice Rondinelli, Francesco Malagnino, Giuliana Alimena

Section of Hematology, Department of Human Biopathology, University "La Sapienza", Rome, Italy

### ABSTRACT

We describe the application of fluorescence *in situ* hybridization (FISH) in a case of suspect chronic myelogenous leukemia (CML), cytogenetically characterized by a t(21;22) with no clear involvement of chromosome 9. The two color FISH technique, performed using specific painting probes for chromosomes 9,21,22 and BCR/ABL translocation probe, enabled us to confirm the diagnosis of CML by detecting the BCR/ABL rearrangement on chromosome 22q and the involvement of chromosome 9 in a variant translocation t(9;21;22).

*Key words:* CML, variant Ph translocation, FISH

Chronic myelogenous leukemia (CML) is characterized in more than 90% of cases by a karyotypic marker, the Philadelphia chromosome, originating from a reciprocal t(9;22)(q34;q11) translocation, and genetically resulting in a fusion BCR/ABL gene. Variant Ph translocations (vPhTs), derived through other rearrangements, occur in 5-10% of cases.<sup>1,2</sup> In some instances the correct interpretation on the nature of these changes may be difficult by standard cytogenetics, especially when the Ph chromosome does not show its usual morphology (*masqued* Ph), or when, even in good quality metaphases, chromosome 9 is apparently unaffected.<sup>1,3,4</sup> The application of fluorescence *in situ* hybridization (FISH) represents a powerful tool to improve the accuracy of cytogenetic analysis by rapidly and unambiguously delineating chromosome aberrations, both in metaphase and interphase cell nuclei.<sup>5</sup>

We report on the use of FISH technique in a case with a suspect diagnosis of CML and apparently carrying at standard cytogenetics a

t(21;22) translocation, with no evident involvement of chromosome 9 in a Ph-like chromosome formation.

### Materials and Methods

#### Case history

A 39-year-old male was referred to our Hospital on September 1993, because of an occasional blood test displaying a moderate leukocytosis (WBC  $30 \times 10^9/L$ ), with normal hemoglobin and platelet count, in the absence of any relevant symptom. At physical examination no spleen and liver enlargements were evident. The WBC differential as well as the bone marrow (BM) aspirate were consistent with a chronic phase of CML.

After cytogenetic and molecular results were obtained (October 1993) (see **Results** section), the patient was started on IFN therapy; and he is now in chronic phase (CP), at the fourth month of treatment.

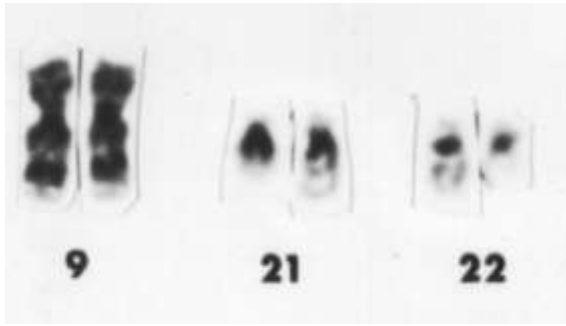


Figure 1 GTG-banded partial karyotype from BM cell showing the  $t(21;22)(q22;q11)$ .

#### Standard cytogenetics

Cytogenetic study by standard methods was performed on September 93, at the time of leukocytosis detection, on BM cells, by direct technique and short term culture (24h); GTG banding was applied, and karyotype was expressed according to standard nomenclature (ISCN,1991).

#### In situ hybridization

Dual color FISH was performed in metaphase and interphase nuclei, by using commercial probes from Oncor (Gaithersburg, MD), namely BCR/ABL translocation and chromosome 9,21, and 22 painting probes.

The BCR/ABL translocation DNA probe was a mixture of a digoxigenin-labeled cosmid DNA probe specific for the CML major (M) breakpoint cluster region of the BCR gene, and a single biotin-labeled cosmid specific for the ABL gene. Specific painting probes were libraries labeled with biotin (#21 and #22) and digoxigenin (#9), respectively. In situ hybridization and immunological detection of the probes were performed following the procedures described by Arnoldus et al.,<sup>6</sup> modified, and the Oncor instructions. Finally, the slides were dehydrated, embedded in a glycerol mixture containing DAPI as counterstaining and the antifade reagent DABCO (Sigma).

The analyses were performed in a fluorescence microscope (Zeiss, Axiophot) with the appropriate filter combination. Photographs were taken using a Kodak Ektachrome 800 film.

## Results

#### Standard cytogenetics

The bone marrow karyotype detected at standard cytogenetics was  $46,XY,t(21;22)(q22;q11)$  in all the 25 examined metaphases. In particular, the rearrangement resulted in a Ph-like chromosome (or  $22q^-$ ) and in a  $21q^+$  chromosome; apparently, both chromosomes 9 were unaffected (Figure1).

#### In situ hybridization

The dual color FISH was performed following cytogenetic results, in the suspect of a variant Ph translocation and in order to verify the presence of BCR/ABL rearrangement.

**Chromosome painting.** Hybridization with the three library probes resulted in an overall staining of chromosome 9 in red (digoxigenin-labeled) and chromosome 21 and 22 in green (biotin-labeled). Differently from standard cytogenetics evidence, specific libraries of chromosomes 9, 21 and 22 supported the hypothesis of a  $t(9,21;22)$ . In fact, the first sample, using probes for chromosomes 9 and 22 evidenced: a) chromosome 22 probe sequences associated with the uninvolved chromosome 22, the Ph-like chromosome, and the end of a G chromosome interpreted as being the  $21q^+$ ; b) chromosome 9 probe sequences joined to the unaffected chromosome 9 and to the  $22q^-$  chromosome (Figure 2a).

The second sample, using specific painting probes for chromosomes 9 and 21, identified a small translocation, involving the terminal region of the long arms of chromosomes 21 and 9, by depicting in green both chromosomes 21 (the normal one and the  $21q^+$ ) in addition to the end of a chromosome 9 (Figure 2b).

**BCR/ABL rearrangement.** In both interphase and metaphase cells, hybridization with BCR/ABL probe resulted in two well distinct signals (one red and one green) and in one colocalized red-green signal, thus indicating the positivity for the M-BCR/ABL rearrangement.

## Discussion

Among variant Ph translocations, some origi-

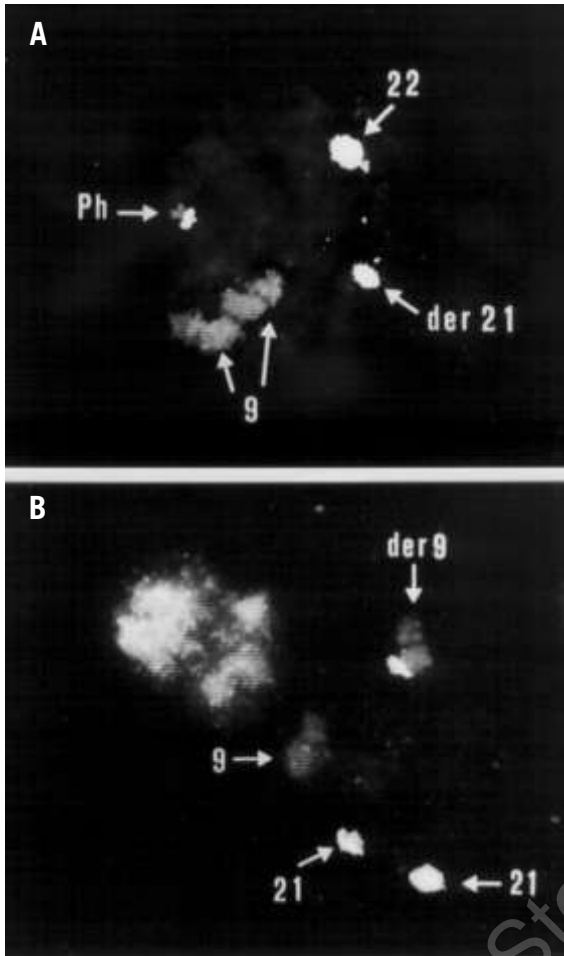


Figure 2. Translocation  $t(9;21;22)$  shown by two color CISS hybridization technique with chromosome 9, 21, and 22 painting probes. a) Painting of chromosomes 9 (in grey) and 22 (in white). Chromosome 22 probe sequences are associated with the chromosome 22, the Ph-like chromosome and the end of derived chromosome 21; chromosome 9 probe sequences are associated with the chromosomes 9 and the Ph chromosome; b) painting of chromosomes 9 (in grey) and 21 (in white). Chromosome 21 probe sequences identify the chromosomes 21, and a small segment translocated on the terminal region of the long arm of chromosome 9 (indicated as der 9).

nate from structural rearrangements which are at the limit of detection by standard cytogenetics. For instance, minimal changes in one of the chromosome 9 may be not evidenced and result in an apparent uninvolved of this chromosome in the Ph formation. In the past, *incorrect* interpretation of these cases gave rise to the subclassification of the vPhts in two major

types: a) simple vPht, involving chromosome 22 and a chromosome other than 9, and b) complex vPhts involving chromosomes 9, 22 and at least one other chromosome.<sup>3,7,8</sup> The application of radioactive *in situ* hybridization first displayed a substantial number of simple vPhts actually being undetected complex changes, also involving region 9q34.<sup>4,9-11</sup> Such findings can now be more easily evidenced by the application of FISH, which allows a faster and even more accurate detection of aberrations, by simultaneously painting specific nucleic acid target sequences with different fluorescent colors.<sup>12-14</sup>

In this report, we describe a case of myeloproliferative syndrome, marked by a moderate leucocytosis evoking a diagnosis of CML. Owing to the unexpected cytogenetic feature of a  $t(21;22)$  mimicking a Ph chromosome with no evident involvement of chromosome 9, we applied a dual color FISH. This included a first analysis with the specific painting probes, and a second analysis with the BCR/ABL rearrangement probe, aimed at 1) verifying the evenness of a complex vPht undetectable cytogenetically, 2) identifying the chromosomes affected in the change and, finally, 3) revealing the BCR/ABL rearrangement useful to unambiguously confirm the CML diagnosis.

Thus, a single technical approach enabled us to determine the involvement of the apparently unaffected chromosome 9 in a  $t(9;21;22)$ , as well as to detect the BCR/ABL molecular rearrangement. With present evidence, we furtherly support the idea that all variant Ph-producing changes in CML always involve both 22q11 and 9q34. In our case the BCR/ABL rearrangement was detected in the classic position, on the 22q- chromosome. Recent studies with FISH have demonstrated in similar cases of vPht or masked Ph that this chimeric gene can also be localized in unusual position, on different chromosomes.<sup>15,16</sup> This issue deserves to be better explored on a larger number of similar cases, in order to clarify whether different BCR/ABL locations may have different consequences at biological and clinical levels.<sup>17,18</sup>

Finally, involvement of chromosome 21 in a variant Ph translocation is a very rare event; in

fact to our knowledge, present case is the sixth mentioned so far<sup>1</sup> with this kind of aberration.

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