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Review article

Mechanism, detection and significance of some chromosomal rearrangements in chronic myeloid leukaemia (CML) and acute lymphoblastic leukaemia (ALL)

Dariusz ŁADOŃ , Michał WITT

Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland

Abstract. Fluorescence *in situ* hybridization (FISH) allows detection of specific chromosomal aberrations in abnormal cells. In chronic myeloid leukaemia (CML) and acute lymphoblastic leukaemia (ALL), chromosomal abnormalities have been found in the bone marrow of children and adults. Detection of a number of malignant cells carrying specific aberrations after bone marrow transplantation is of great importance. FISH techniques with the use of specific probes for CML and ALL could detect a minimal residual disease and mixed chimerism after bone marrow transplantation.

Key words: acute lymphoblastic leukaemia, chronic myeloid leukaemia, fluorescence *in situ* hybridization, minimal residual disease, mixed chimerism.

Introduction

Recently novel approaches to detection of specific nucleotide sequences in complex genomes have been developed, non-isotopic *in situ* hybridization (NISH) including. This technique makes possible efficient detection of various numerical and structural chromosomal abnormalities (LICHTER et al. 1988, PINKEL et al. 1988, CREMER et al. 1990).

From the technical perspective two types of non-radioactive *in situ* hybridization protocols are used.

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Correspondence: D. ŁADOŃ, Institute of Human Genetics, Polish Academy of Sciences, Strzeszyńska 32, 60-479 Poznań, Poland.

– Direct procedure. The probes are labelled with nucleotides modified with a reporter molecule, which can be directly detected after hybridization. This method is used when chromosome painting is performed and large chromosome regions are targeted.

– Indirect procedure. This is based on modified-labelled probes, which are detected using sandwich techniques. The nucleotides used to label the probe are conjugated with biotin, digoxigenin, and acetylaminofluorene. When the probe is labelled with biotin, the detection can be achieved through a reporter linked to an antibody or avidin. The reporter groups include enzymes, like horseradish peroxidase and alkaline phosphatase, and their respective insoluble substrates, fluorescent molecules, such as fluorescein, rhodamine and colloidal gold particles. Due to higher detection sensitivity, widely used procedures are biotin-avidin and digoxigenin-antidigoxigenin systems, combined with fluorochromes.

Because of the use of fluorescent system in NISH this method was named fluorescence *in situ* hybridisation (FISH). The method has several important advantages:

1) high signal resolution – the detection takes place exactly at the site where the probe is hybridised;

2) possibility of amplification of signal intensity by using an immunological method;

3) potential to combine several different fluorochromes for simultaneous detection of different target regions;

4) short duration of the whole procedure.

The sensitivity of the FISH method allows for detection of specific chromosomal aberrations and for their quantitative assessment in cells at the stage of mitotic metaphase and also in interphase nuclei (CREMER et al. 1988). Double interphase FISH is of special interest in detecting structural chromosomal aberrations, particularly when the quality of metaphase spreads is not sufficient for classical cytogenetic analysis of banding patterns (e.g. in cancer cytogenetics) (ARNOLDUS et al. 1990)

Chronic myeloid leukaemia (CML)

CML is a clonal bone marrow disease with incidence of 1 case per 100,000 people per year. Chromosomal abnormality occurring in the bone marrow cells in 90% of CML adult and children patients is a reciprocal translocation between chromosomes 9 and 22 t(9;22)(q34;q11). NOWELL and HUNGERFORD (1960) first described this chromosomal abnormality in 1960. They observed an abnormally small chromosome in peripheral blood cells from two patients with CML. It was recognised that this chromosome named the Philadelphia chromosome (Ph), was a translocated chromosome containing the small chromosome 22. In 1973, with the development of chromosome banding techniques (RASKIND et al. 1998),

Ph chromosome was finally recognised as a translocation between chromosomes 9 and 22. This chromosomal abnormality results in the fusion of a part of the Abelson oncogene (*ABL*) from 9q34 with the breakpoint cluster region (*BCR*) at 22q11.2 (WESTBROOK et al. 1992). The origin of this fusion is described below. The fusion creates a tumour-specific marker and results functionally in an increased tyrosine kinase activity (RABBITS 1994).

With the progress of the disease, additional chromosomal aberrations can occur. These changes sometimes cause haematological and clinical manifestations of advanced phases of the disease with increased malignancy, and thus may serve as valuable prognostic indicators.

In the pathways of cytogenetic evolution in the blast crisis of CML, terms "major" (70% of blast crisis) and "minor" (30%) routes are used. Additional changes in the "major route" occur in the excess of the Ph chromosome (15% of CML cases), trisomy 8 (11%), trisomy 19 (1%), and/or the formation of an i(17q) (12%). The most common changes in "minor route" are trisomy 21 (7%) in combination with one or more "major route" abnormalities, and - Y (loss of the Y chromosome) (5% of CML cases) (MITELMAN 1994). The type and number of secondary aberrations seem to be correlated with the phenotypic features that dominate during blast crisis. Screening for these secondary chromosomal aberrations is of particular prognostic significance, and for this purpose the fast and sensitive FISH method is currently being used.

Translocation (9;22) resulting in *BCR/ABL* fusion

Analysis of the most frequent aberration t(9;22)(q34;q11), causing the *BCR-ABL* gene fusion in about 50% of patients with ALL, shows that the breakpoint is located in the M-*BCR* region (Major *BCR*). In the other 50% the breakpoint is in another region of the *BCR*, called m-*BCR* (minor *BCR*) (RODENHUIS et al. 1985). The same translocation occurs in neutrophilic-CML but in a different breakpoint region called μ-*BCR*. This suggests that the particular location of the breakpoint in *BCR* (and in *ABL*), and thus the composition of the fusion *BCR/ABL* protein, may determine the disease phenotype (PANE et al. 1996).

The breakpoint in the *ABL* gene can occur anywhere within an over 300-kb long segment at the 5' end of the gene, upstream of the first alternative exon I b, between exons I b and I a, or downstream of exon I a (MELO 1996). In the majority of CML patients and in one third of ALL cases the breakpoint in the *BCR* gene is found within a 5.8-kb region known as the major breakpoint cluster region (M-*BCR*) spanning five exons from 12 to 16 of the *BCR* gene, named b1 to b5. Processing of the primary *BCR/ABL* transcript usually results in hybrid *BCR/ABL* mRNA molecules with a b3a2 and/or b2a2 junction encoding a $p210^{BCR/ABL}$ fusion protein. In very rare cases of CML and one third of ALLs, the breakpoint in *BCR*

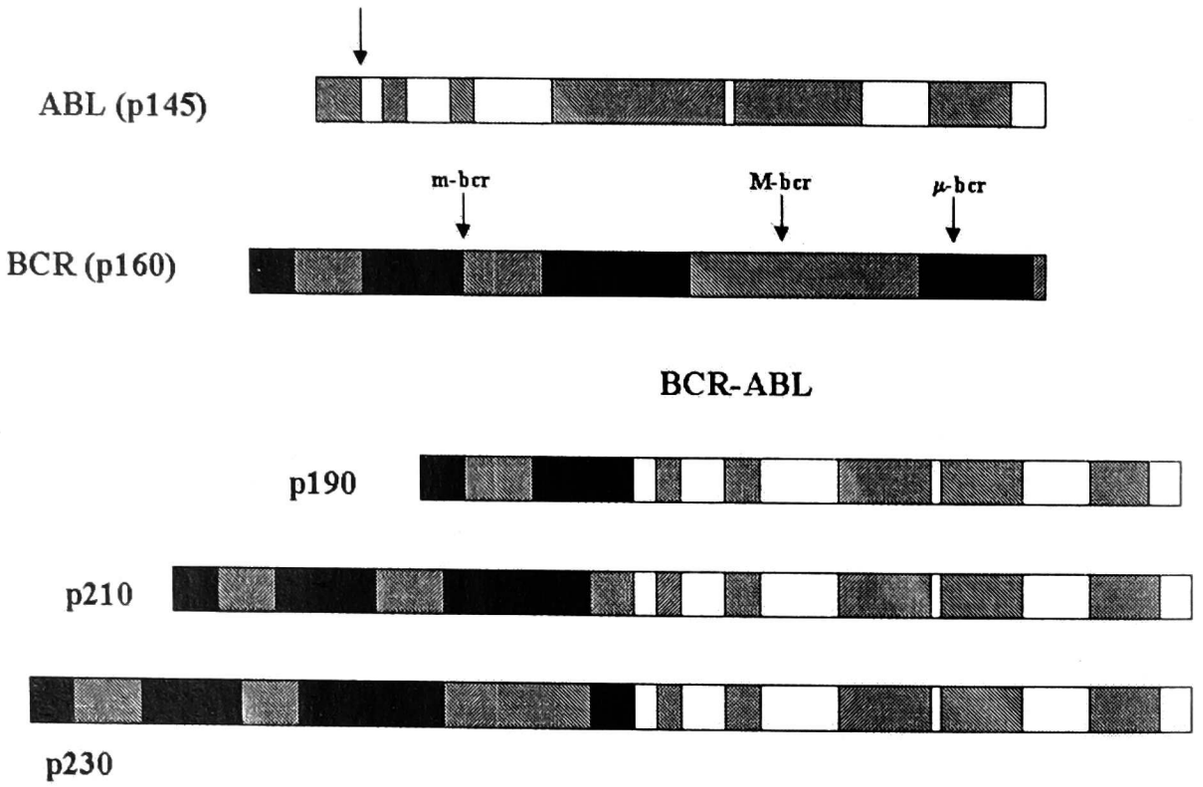

Figure 1. Translation products (chimeric proteins) of different fusion types occurring in CML and ALL (white=functional domains of *ABL* gene; black=functional domains of *BCR* gene; grey=fragments with no function assigned).

falls further upstream, in the long 54.4-kb intron between the two alternative exons e2' and e2, known as the minor *BCR* (m-*BCR*). In this event, exons e1' and e2' are removed by splicing, and the hybrid *BCR/ABL* transcript contains an e1a2 junction, and is responsible for translating into a smaller 190-kD *BCR/ABL* protein ($p190^{BCR/ABL}$) (Figure 1). Many researchers reported different *BCR* and

Table 1. Variable *BCR-ABL* transcripts in CML and ALL

Disease	M-*BCR* fusion	m-*BCR* fusion	μ-*BCR* fusion
Chronic myeloid leukaemia (CML)	b3a2 b2a2 b3a3 b2a3	e1a2	e19a2
Acute lymphoblastic leukaemia (ALL)	b3a2 b2a2 b3a3 b2a3	e1a2 e1a3	

ABL breakpoints, which resulted in various *BCR/ABL* transcripts in CML and ALL. In Table 1 different *BCR/ABL* transcripts present in ALL and CML are shown.

Acute lymphoblastic leukaemia (ALL)

ALL is a clonal malignant disease restricted to the lymphoid compartment. The incidence is 3.6 per 100,000 children under the age of 15 per year and it is diagnosed most frequently in children between 2 and 7 years of age with the frequency of 7 per 100,000.

In this malignancy the translocation t(9;22)(q34;q11), the same as in CML, was identified and considered a genetic marker of leukaemic cells. It occurs in 15-35% adults and in 2-10% of children with ALL (SURYANARAYAN et al. 1991). While an abnormal karyotype can be detected in the bone marrow and peripheral blood of the majority of children with ALL, in some of them no numerical and structural aberrations can be identified: a normal karyotype can be detected in about 20-30% of cases. A possible explanation for normal karyotype in ALL is the existence of some submicroscopic aberrations (HARBOTT 1998).

The most common translocations in leukaemic cells of children with ALL are t(1;19), t(4;11), t(8;14), and previously described t(9;22) and t(12;21). Other aberrations found in ALL are abnormalities of chromosome 14, with a breakpoint at q11, including translocations with various partner chromosomes as well as inversions and deletions. All these aberrations are specific for ALL (although some of them can also be present in AML) and have been found in the bone marrow cells of children and adults. The most common abnormalities attributing to immunological subtypes of ALL are shown in Table 2.

Table 2. Chromosomal abnormalities that attribute to the immunological subtype of ALL

B-lineage ALL				T-lineage ALL
Pro-B CD19	common CD10	Pre-B cylgM	B-ALL SlgM	
t(4; 11)	Hyperdiploidy > 50 t(9;22) t(12;21)	t(1;19)	t(8;14)	14q11 aberrations deletion TAL-1 (1p32)

Almost all patients with childhood ALL have chromosomal abnormalities, half of which are translocations. These translocations are nearly equaly divided between random and nonrandom rearrangements. Nonrandom chromosomal abnormalities have been correlated with leukaemic cell lineage, the degree of cell differentiation and the specific gene involved at the molecular level (Table 3.). For, clinical purposes such as a proper diagnosis and classification of the patients,

Table 3. Gene rearrangements and chromosome abnormalities in ALL

Abnormality	Gene rearrangements	Diagnosis of ALL
del(1)(p32)	*SIL/TAL1*	T-ALL
t(1;13)(p12;p21)	*TAL1/--*	T-ALL
t(1;7)(p32;q35)	*TAL1/TCRb*	T-ALL
t(1;7)(p34; 34)	*LCK/TCRb*	T-ALL
t(1;14)(p32;q11)	*TAL1/TCRd*	T-ALL
t(1;11)(p32;q23)	*MLL/AF1p*	pro-B ALL
t(1;19)(q23;p13)	*PBX1/E2A*	pre-B ALL
t(4;11)(q21;q23)	*MLL/AF4*	pro-B ALL
t(5;14)(q31;q32)	*IL-3/IgH*	pre-B/c-ALL
t(7;9)(q34;q32)	*TCRb/TAN*	T-ALL
t(7;10)(q34;q24)	*TCRb/HOX11*	T-ALL
t(7;19)(q34;p13)	*TCRb/LYL1*	T-ALL
t(8;14)(q24;q11)	*MYC/TCRa*	T-ALL
t(8;14)(q24;q11)	*PVT1/TCRd*	T-ALL
t(8;14)(q24;q32)	*MYC/IgH*	B-ALL
t(9;22)(q34;q11)	*BCR/ABL*	pre-B/c ALL
t(10;14)(q24;q11)	*HOX11/TCRd*	T-ALL
t(11;14)(p13;q11)	*TTG2/TCRd*	T-ALL
t(11;14)(p15;q11)	*TTG1/TCRd*	T-ALL
t(11;19)(q23;p13.3)	*MLL/ENL*	pro-B ALL
t(12;21)(p13;q22)	*TEL/AML1*	pre-B/c ALL

it is important to identify the specific chromosomal abnormalities and gene rearrangements in ALL. For many years, classical cytogenetic methods were the only techniques to identify chromosomal aberrations – this approach is still of great diagnostic importance but within the last decade the FISH technique became a routine procedure in this kind of analysis.

Detection of chromosomal aberrations in CML and ALL

The major aim of genetic testing in CML and ALL patients is an early detection of minimal residual disease and disease relapse following chemotherapy or bone marrow transplantation. The term minimal residual disease refers to the presence of detectable malignant cells in patients who are in conventional remission. Definitions of haematological remission include the presence of less than 5% of blasts in the bone marrow, with a normal peripheral blood count. Cytogenetic remission is defined as the finding of a normal karyotype in at least 20 bone marrow

metaphases for these patients in whom specific chromosomal abnormalities were identified (LIN, CROSS 1995)

Classical cytogenetics

Classical cytogenetic methods can be used to identify most of the chromosomal translocations occurring in bone marrow and peripheral blood cells in patients with ALL and CML. The detection of chromosomal changes has went through the evolution pathway from a simple description of abnormalities to the level of detection of important clinical markers; especially in childhood ALL it has an important clinical significance. The standard cytogenetic procedures are widely used for monitoring of the therapeutic response in Ph-positive patients.

Cytogenetic analysis is not feasible in some patients treated with interferon a (IFN-α) due to an inaspirable marrow and in 20-30% of patients with ALL due to an insufficient quality of metaphase spreads. Moreover, approximately 5% of CML patients harbour a *BCR/ABL* fusion gene that is undetectable by conventional cytogenetics, namely Ph-negative, *BCR/ABL* – positive CML (HAGEMEIJER 1987). The response to a therapy in these patients may be evaluated either by the still very useful and important standard cytogenetic techniques or by molecular cytogenetic methods for detection of leukaemic cells, such as fluorescence *in situ* hybridisation (TKACHUK et al. 1990). In CML patients the Ph chromosome became a marker of the malignant clone, enabling its detection and consequently monitoring of the course of the disease (AMIEL et al. 1994).

Fluorescent *in situ* hybridisation (FISH)

FISH is a sensitive quantitative method for detection of specific numerical and structural chromosomal aberrations, not only in cells arrested at the metaphase, but in the interphase nuclei as well. FISH using specific probes for leukaemia-related rearrangements may be used to analyse minimal residual disease (CAMPANA, PUI 1995). The FISH technique can be used in patients in whom chromosomal analysis by conventional cytogenetic methods is not available because of lack of cells undergoing metaphase due to technical problems such as lack of spreading or poor quality of banding. BARTRAM et al. (1983) described the first application of *in situ* hybridisation with the use of the *ABL* specific probe for detection of the translocation of *ABL* to the Ph-chromosome in CML.

A convenient method for detection of minimal residual is interphase FISH. Philadelphia chromosome detection by interphase FISH can also be used to monitor CML patients after allogeneic bone marrow transplantation for residual leukaemic cells. An advantage of this method is a possibility to analyse a large number of cells at the same time.

Potentially, FISH could accurately establish the presence or absence of cells with a Ph chromosome and detect cells with more than one Ph chromosome. This information would have an important diagnostic and prognostic value in

the characterization of patients suspected of having a Ph chromosome, because metaphases with multiple Ph chromosomes have been associated with the more aggressive stages of CML: blast crisis and acceleration phase (DEWALD et al. 1998).

Use of two colour probes (red and green) of the *BCR* and *ABL* sequences for FISH allows detection of the t(9;22)(q34;q11) translocation in CML leukaemic cells at a frequency above 1% (ARNOLDUS et al. 1990, TKACHUK et al. 1990) (Figures 2a,b,c).

For detection of mixed chimerism after sex-mismatched bone marrow transplantation, X and Y-chromosome specific probes can be applied. Simultaneous use of the X and Y probes in the same cell can extremely reduce the number of false-positive and false-negative results (WESSMAN et al. 1993, JUNGERMANN et al. 1998). The studies confirm that FISH can detect host cells in sex-mismatched transplanted patients in Ph-negative CML with *BCR/ABL* rearrangement when disease-specific probes are used (PALKA et al. 1996, TKACHUK et al. 1990).

FISH on different specimens (bone marrow cells and/or peripheral blood cells)

In many early reports, FISH analyses were carried out on cultured and uncultured bone marrow (BM) cells of CML patients (BUNO et al. 1998, BENTZ et al. 1994). However, the use of various specimens depends on the stage of disease and has to be correlated with medical examination performed at the same time. Usually at the beginning of the therapeutic process a bone marrow aspirate is available as a specimen to be studied; afterwards peripheral blood can be drawn during regular haematological check-ups.

Some reports have shown a very good correlation between results obtained from BM and PB (peripheral blood) specimens, with a higher percentage of *BCR/ABL* fusion-positive cells in BM than in PB (BENTZ et al. 1994). This observation indicates that almost all of the circulating mature T lymphocytes in CML were derived from Philadelphia negative normal stem cells prior to the transforming event resulting in CML (NITTA et al. 1985, AMIEL et al. 1994). FISH experiments were carried out on BM and PB smears and samples as well. A good correlation between FISH results obtained for interphase cells of BM and PB samples and PB smears was confirmed (DIEZ-MARTIN et al. 1998), which demonstrated the usefulness of this kind of specimens for detection of Ph chromosome in leukaemic cells.

False-positive and false-negative results in interphase FISH

The possibility of a false-positive result due to a random colocalization of the *ABL* and *BCR* signals in normal cells (used as a negative control applied for each analysis) is low, considering that the size of the signals is small compared to the size of the cell. Colocalization of the signal was shown in 0.5% to 2% of normal cells

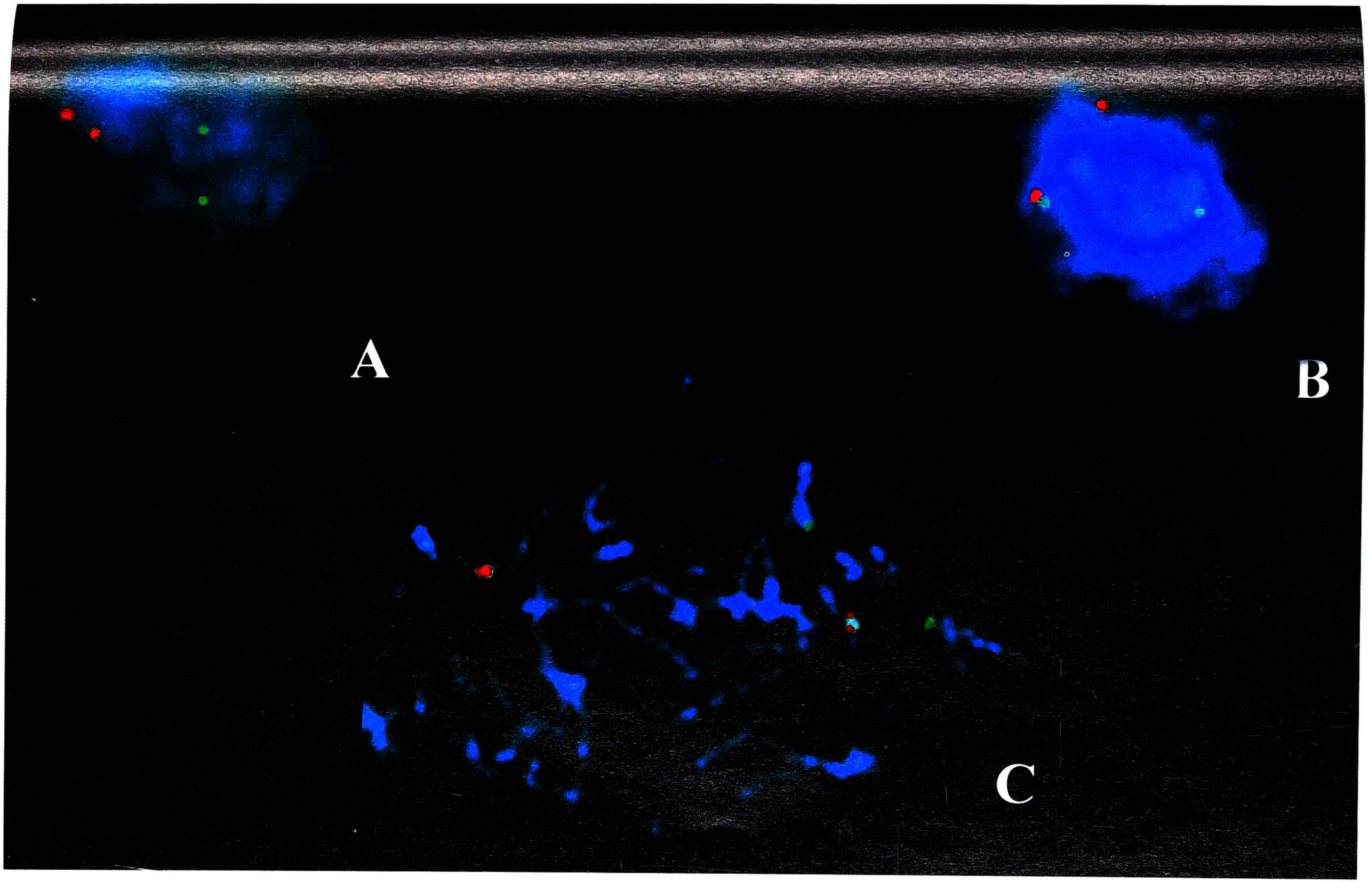

Figure 2. Hybridization patterns of ABL-specific probe (green) and BCR-specific probe (red) on: interphase nuclei of a healthy individual (A-negative control); interphase nuclei of a individual with CML (B); metaphase spread of an normal individual (C-negative control).

depending on the technique applied (BENTZ et al. 1994, AMIEL et al. 1994, DEWALD et al. 1998). The problem of false-negative results could be minimised with optimisation of the FISH procedure and of the detection system (BUNO et al. 1998, DEWALD et al. 1998).

Conclusions

The crucial pathogenetic consequence of the translocations occurring in ALL and CML patients is the creation of a novel chimeric gene. For a long time classical cytogenetic evaluation was the only method used for, detection and monitoring of patient's response to, therapy applied. At present faster, more convenient and more sensitive molecular methods, such as FISH, are widely used as well. Their combination with other molecular screening methods, such as PCR and RT-PCR (not described in this article), give a detailed picture of disease diagnosis and progression, and could lead to a better, risk adapted therapy for the patients with CML and ALL.

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REFERENCES

AMIEL A., YARKONI S., SLAVIN S., OR R., LORBERBOUM-GALSKI H., FEJGIN M., NAGLER A. (1994). Detection of minimal residual disease state in chronic myelogenous leukemia patients using fluorescence *in situ* hybridization. Cancer Genet. Cytogenet. 76: 59-64.

ARNOLDUS E.P.J., WIEGANT J., NOORDERMEER J.A., WESSELS J.W., BEVERSTOCK G.C., GROSVELD G.C., van der PLOEG M., RAAP A.K. (1990). Detection of the Philadelphia chromosome in interphase nuclei (with 2 color plates). Cytogenet. Cell Genet. 4: 108-111.

BARTRAM C.R., de KLEIN A., HAGEMEIJER A., van AGTHOVEN T., GEURTS van KESSEL A., BOOTSMA D., GROSVELD G., FERGUSON-SMITH M.A., DAVIES T., STONE M., HEISTERKAMP M., STEPHENSON J.R., GROFFEN J. (1983). Translocation of c-abl oncogene correlates with the presence of Philadelphia chromosome in chronic myelocytic leukemia. Nature 306: 277-280.

BENTZ M., CABOT G., MOOS M., SPEICHER M.R., GANSER A., LICHTER P., DOHNER H. (1994). Detection of chimeric BCR-ABL genes on bone marrow samples and blood smears in chronic myeloid and acute lymphoblastic leukemia by *in situ* hybridization. Blood 83(7): 1922-1928.

BUNO I., WYATT W.A., ZINSMEISTER A.R., DIETZ-BAND J., SILVER R.T., DEWALD G.W. (1998). A special fluorescent *in situ* hybridization technique to study peripheral blood and assess the effectiveness of interferon therapy in chronic myeloid leukemia. Blood 92(7): 2315-2321.

CAMPANA D., PUI C.H. (1995). Detection of minimal residual disease in acute leukemia: methodologic advances and clinical significance. Blood 85: 1416-34.

CREMER T., LICHTER P., BORDEN J., WARD D.C., MANUELIDIS L. (1988). Detection of chromosome aberrations in metaphase and interphase tumor cells by *in situ* hybridization using chromosome-specific library probes. Hum. Genet. 80: 235-246.

CREMER T., POOO S., EMMERICH P., LICHTER P., CREMER C. (1990). Rapid metaphase and interphase detection of radiation-induced chromosome aberrations in human lymphocytes by chromosomal suppression *in situ* hybridization. Cytometry 11: 110-118.

DEWALD G.W., WYATT W.A., JUNEAU A.L., CARLSON R.O., ZINSMEISTER A.R., JALAL S.M., SPURBECK J.L., SILVER R.T. (1998). Highly sensitive fluorescence in situ hybridization method to detect double BCR/ABL fusion and monitor response to therapy in chronic myeloid leukemia. Blood 91(9): 3357-3365.

DIEZ-MARTIN J., LLAMAS P., GOSALVEZ J., LOPEZ-FERNANDEZ C., POLO N., SOLEDAD de la FUENTE M., BUNO I. (1998). Conventional cytogenetics and FISH evaluation of chimerism after sex-mismatched bone marrow transplantation (BMT) and donor leukocyte infusion (DLI). Haematologica 83: 408-415.

HAGEMEIJER A. (1987). Chromosomal abnormalities in CML. In: Bailliere's Clinical Haematology, London: Baillier Tindall 1(4): 963-981.

HARBOTT J. (1998). Cytogenetics in childhood acute lymphoblastic leukemia. Rev. Clin. Exper. Hematol. 5: 25-36.

JUNGERMAN M., SĘDZIMIRSKA M., LANGE A., WITT M. (1998). Application of microsatellite markers for monitoring of reconstitution pattern in patients after bone marrow transplantation/peripheral blood progenitor cell transplantation. Arch. Immunol. Ther. Exper. 46: 39-43.

LICHTER P., CREMER T., BORDEN J., MANUELIDIS L., WARD D.C. (1988). Delineation of individual human chromosomes in metaphase and interphase cells by *in situ* suppression hybridization using recombinant DNA libraries. Hum. Genet. 80: 224-234

LIN F., CROSS N.C.P. (1995). Molecular methods for monitoring malignant disease after bone marrow transplantation. Curr. Opin. Hemat. 2: 460-467.

MELO J.V. (1996). The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood 88(7): 2375-2384.

MITELMAN F. (1994). Catalogue of chromosome aberrations in cancer (5th edn) New York: Willey-Liss,

NITTA M., KATO Y., STRIFE A., WACHTER M., FRIED J., PEREZ A., JHANWAR S., DUIGOU-OSTERNDORF R., CHAGANTI R.S.K., CLARKSON B. (1985). Incidence of involvement of the B and T lymphocyte lineages in chronic myelogenous leukaemia. Blood 66: 1053-1061.

NOWELL P.C., HUNGERFORD D.A. (1960). A minute chromosome in human chronic granulocytic leukemia. Science 132: 1497-1501.

PALKA G., STUPIA L., di BARTOLOMEO P., MORIZIO E., PEILA R., GUANCIALI F.P. (1996). FISH detection of mixed chimaerism in 33 patients submitted to bone marrow transplantation. B M T 17:231-236.

PANE F., FRIGERI F., SINDONA M., LUCIANO L., FERRARA F., CIMINO R., MELONI G., SAGLIO G., SALVATORE F., ROTOLI B. (1996). Neutrophylic-Chronic myeloid leuke-

mia: A distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). Blood 88: 2410-2414.

PINKEL D., LANDEGENT J., COLLINS C., FUSCOE J., SEGRAVES R., LUCAS J., GRAY J. (1988). Fluorescence *in situ* hybridization with human chromosome libraries: Detection of trisomy 21 and translocations of chromosome 4. Proc. Natl. Acad. Sci. USA, 85: 9138-9142.

RABBITTS T.H. (1994). Chromosomal translocations in human cancer. Nature 372 (10): 143-149.

RASKIND W.H., STEINMANN L., NAJFELD V. (1998). Clonal development of myeloproliferative disorders: clues to hematopoietic differentiation and multistep pathogenesis of cancer. Leukemia 12: 108-116.

RODENHUIS S., SMETS L.A., SLADER R.M., BEHRENDT H., VEERMAN A.J.P. (1985). Distinguishing the Philadelphia chromosome of acute lymphoblastic leukemia from its counter in chronic myelogenous leukemia (letter to editor). N. Engl. J. Med. 313: 51-52.

SURYANARAYAN K., HUNGER S.P., KOHLER S., CARROLL A.J., CRIST W., LINK M.P., CLEARY M.L. (1991). Consistent involvement of the bcr gene by 9;22 breakpoints in pediatric acute leukemias. Blood 77: 324-330.

TKACHUK D.C., WESTBROOK C.A., ANDREEFF M., DONLON T.A., CLEARY M.L., SURYANARAYAN K., HOMGE M., REDNER A., GRAY J., PINKEL D. (1990). Detection of bcr-abl fusion in chronic myelogeneous leukemia by *in situ* hybridization. Science 250: 559-561.

WESSMAN M.., POPP S., RUUTU T. (1993). Detection of residual host cells after bone marrow transplantation using non-isotopic *in situ* hybridization and karyotype analysis. B M T, 11: 279-284.

WESTBROOK C.A., HOOBERMAN A.L., SPINO C., DODGE R.K., LARSON R.A., DAVEY F., WURSTER HILL D.H., SOBOL R.E., SCHIFFER C., BLOOMFIELD C.D. (1992). Clinical significance of the BCR-ABL fusion gene in adult acute lymphoblastic leukemia: A cancer and leukemia group B study (8762). Blood 80: 2983-2990.