APPLICATION OF MOLECULAR CYTOGENETIC TEST IN HAEMATOLOGICAL MALIGNANCIES FOR THE FIRST TIME IN ALBANIA

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Abstract

Genetic Characterization and changes in genome organization are crucial in the clinical evaluation of almost every form of hematological malignancy. Studies has shown that Fluorescence In Situ hybridization has made possible to determine the genetic abnormalities and estabilish their and frequency. After great efforts and challenges, this technology was applied for the first time in the Molecular Cytogenetic Laboratory, Clinical Genetic Laboratory Service, University Hospital Center "Mother Teresa." The aim was to use proper probes as a diagnostic tool to understand the pathophysiology, diagnosis, treatment, prognosis, and monitoring of the disease activity. Thanks to simple procedure is possible to recognize hematological malignancy abnormality. Screening by in situ hybridization plays a supportive role in personalized medicine. The images captured using fluorescently labeled probes allow the confirmation of genetic abnormalities such as deletions, translocations, or break-apart rearrangements using Meta Class software. According to the European recommendations for probes in malignant hemopathies, these probes are available for diagnosis and classification of the diseases, providing important prognostic and therapeutic information, monitoring disease response to treatment, Assessment of minimal residual disease, and identification of an early relapse stage of the disease. The use of this technology for the first time in Albania in the molecular cytogenetics laboratory is an important achievement that contributes to the advancement of diagnostic and personalized medicine for hematology patients. It can provide a powerful tool for diagnosing these diseases with a very high level of accuracy and precision. This also increases the quality of health care and brings opportunities for further progress in scientific and medical research in the country. Accurately knowing the genetic characteristics of a hematological disease can help determine the best treatments for patients.

Keywords: Fluorescence in situ hybridization, probes, personalized medicine; targeted treatment, prognostic factor.

APLIKIMI I TESTIT TË CITOGJENETIKËS MOLEKULARE NË PATOLOGJITË HEMATOLOGJIKE MALINJE PËR HERË TË PARË NË SHQIPËRI

Abstrakt

Ndryshimet gjenetike në organizimin e gjenomës janë vendimtare në vlerësimin klinik të patologjive hematologjike. Hibridizimi me fluoreshencë In Situ ka mundësuar një përcaktim më të saktë të pranisë dhe shpeshtësisë së anomalive gjenetike. Pas përpjekjeve dhe sfidave të mëdha, aplikimi i kësaj teknologjie ka filluar të aplikohet për herë të parë në Laboratorin e Citogjenetikës Molekulare, Shërbimi i Laboratorit të Gjenetikës Klinike, Qëndra Spitalore Universitare "Nënë Tereza". Qëllimi është përdorimi i sondave të duhura si një mjet diagnostikues për të përcaktuar

patofiziologjinë, diagnozën, trajtimin, prognozën dhe monitorimin e aktivitetit të sëmundjes. Ekzaminimi me hibridizimin in situ luan një rol mbështetës në mjekësinë e personalizuar. Imazhet e kapura duke përdorur sondat e markuara me fluoreshencë, lejojnë konfirmimin e anomalive gjenetike si delecionet, translokacionet ose thyerjet duke përdorur softuerin MetaClass. Sipas rekomandimeve evropiane për hemopatitë malinje, këto sonda janë të disponueshme për diagnostikimin dhe klasifikimin e sëmundjeve, duke ofruar informacione të rëndësishme prognostike dhe terapeutike, monitorimin e përgjigjes së sëmundjes ndaj trajtimit, vlerësimin e mbetjeve minimale dhe identifikimin e një faze të hershme të rikthimit të sëmundjes. Përdorimi i kësaj metode për herë të parë në Shqipëri në laboratorin e citogjenetikës molekulare është një arritje e rëndësishme që kontribuon në avancimin e mjekësisë diagnostike dhe të personalizuar për pacientët. Ai mund të sigurojë një mjet të fuqishëm për diagnostikimin e këtyre sëmundjeve me një nivel shumë të lartë saktësie. Ky test gjithashtu rrit cilësinë e kujdesit shëndetësor dhe sjell mundësi për përparim të mëtejshëm në kërkimin shkencor dhe mjekësor në vend. Njohja e saktë e karakteristikave gjenetike të një sëmundje hematologjike mund të ndihmojë në përcaktimin e trajtimeve më të mira dhe të personalizuara për pacientët.

Fjalë kyçe: Hibridizimi In Situ me fluoreshencë, sonda, mjekësi e personalizuar; trajtim “target”, faktor prognostik.

Introduction

Malignant hemopathies are a group of neoplasms of hematopoietic cells, characterized by their disrupted differentiaon and multiplication (1). Hematological malignancies have historically been a pioneer among cancers in the use of genetic analysis, particularly for diagnosis and classification. Genetic Characterization and changes in genome organization are crucial in the clinical evaluation of almost every form of hematological malignancy. Molecular diagnosis is an important diagnostic tool in the diagnosis and their management (2).

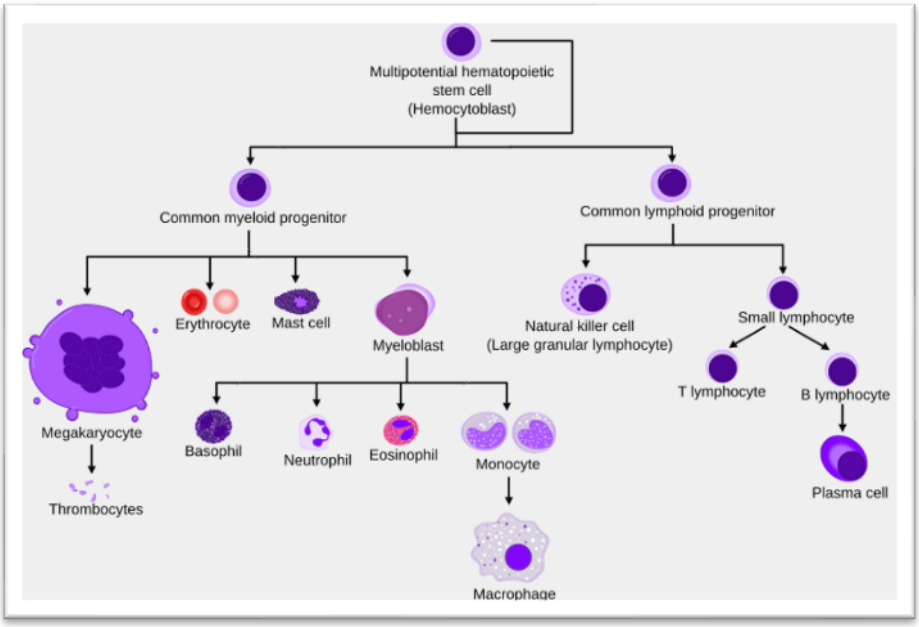

Figure 1. The development from hematological stem cell to mature cell of different blood cell lines(A. Rad and M. Häggström. CC-BY-SA 3.0 license." Image: Hematopoiesis (human) diagram)

The hematological diseases have been shown to be associated with a variety of genetic aberrations,

from a single base-pair substitution to whole chromosomal abnormalities. This characterisation at the molecular level of those recurrent nonrandom cytogenetic abnormalities has specifically identified different disease-related genes being involved in myeloid and lymphoid malignancies. Molecular cytogenetic analysis has become essential for disease diagnosis, classification, prognostic stratification, and treatment guidance (3). Fluorescence In Situ hybridization (FISH) has augmented the enhancement in this field making more precise to determine the presence and frequency of genetic abnormalities. After great efforts and challenges, the application of FISH technology has started to be applied for the first time in the Molecular Cytogenetic Laboratory, Clinical Genetic Laboratory Service, UHC "Mother Teresa ."The aim was to use proper FISH probes such as BCR/ABL, AML1/ETO, PML/RARA, IGH/MYC, P53 etc, as a diagnostic tool to understand the pathophysiology, diagnosis, treatment, prognosis, and monitoring of the disease activity in Chronic Myeloid Leukemia (CML), Acute Myeloid Leukemia (AML), Chronic Lymphoblastic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL), Myelodysplasia (MDS), Multiple myeloma and Lymphoma cases. FISH has some advantages compared to standard cytogenetic analysis, such as being able to identify too small genetic changes difficult to be detected under a microscope, not requiring cell culture, and being able to be applied directly for a quick evaluation of interphase nuclei. The application of FISH involving an enormous variety of chromosome-specific DNA probes helps to further determine the molecular subclasses and also estabilish the cytogenetic risk categories for the patients with some particular hematologic malignancies. It is also useful in identifying the undetectable by conventional chromosomal analysis of some genetic abnormalities and also it can monitor the residual disease during treatment and follow-up (4).

FISH methodology

FISH as a technique makes possible the detection of the DNA sequences in interphase nuclei starting from fixed samples. The technique uses DNA probes that hybridize into single unique sequences. After fixation and denaturation, target DNA is available for annealing to a similarly denatured, fluorescently labeled DNA probe, which has a complementary sequence. Following hybridization, an unbound and nonspecifically bound DNA probe is removed, and the DNA is counterstained for visualization with an anti-fade solution containing DAPI (4′,6-diamidino-2-phenylindole) applied to the slide, and a coverslip must be added. For FISH analysis, fluorescence microscopy with specific filters for identifying fluorochromes and a charge-coupled device (CCD) camera that captures the images allows the visualization of the hybridized probe on the target material (6).

Thanks to simple FISH procedure is possible to recognize hematological malignancy abnormality. Screening by in situ hybridization plays a supportive role in personalized medicine. The images captured using fluorescently labelled DNA probes, allow the confirmation of genetic abnormalities such as deletions, translocations or breakapart rearrangements using MetaClass software (5).

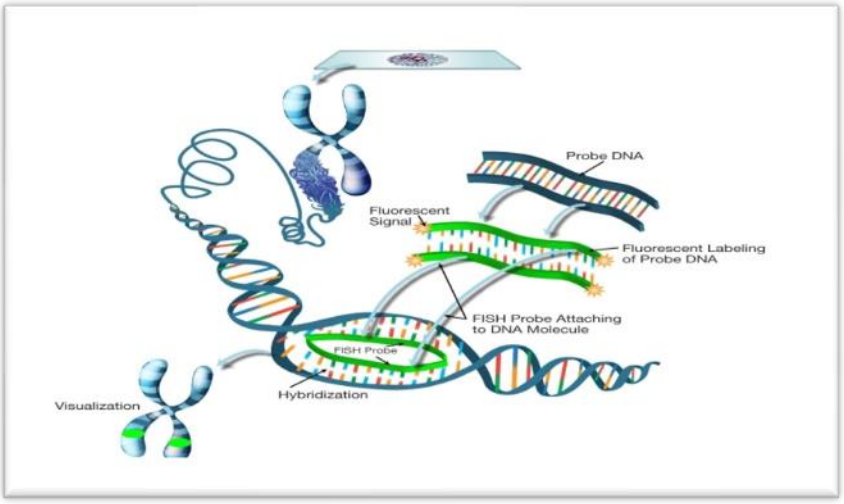

Figure 2. Sample and slide preparation, denaturation of DNA, hybridization and slide analyses (Arun Kumar, Mar 17, 2020)

FISH using specific probes that are capable of defining these stereotypic structural rearrangements has now become a routine diagnostic test in our molecular cytogenetic laboratory, and the technique has been shown to be useful in the management of our hematological malignancies patients. We use a range of FISH probes, specific for a number of hematological malignancies like Chronic Myeloid Leukemia (CML), Acute Myeloid Leukemia (AML), Chronic Lymphoblastic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL), Myelodysplasia (MDS), Multiple myeloma and Lymphoma cases. The names of the probes, chromosome regions, and probe types are shown in the table below.

Table 1. A range of FISH probes optimized for haematological malignancies in Albania

Probe name	Chromosome region	Probe type	Diagnoses
BCR/ABL (ABL1) Dual Fusion	22q11.22-q11.23/9q34.11-q34.12	Translocation	CML, ALL
AML1/ETO (RUNX1/RUNX1T1) Dual Fusion	21q22.12/8q21.3	Translocation	AML
TEL/AML1 (ETV6/RUNX1) Dual Fusion	12p13.2/21q22.12	Translocation	ALL
PML/RARα (RARA) Dual Fusion	15q24.1/17q21.1-q21.2	Translocation	AML
IGH/CCND1 Dual Fusion	14q32.33/11q13.3	Translocation	CLL, Limphoma
IGH/MYC Dual Fusion	14q32.33/8q24.21	Translocation	ALL, Limphoma
Del(5q)	5p15.31-5q13.2	Deletion	AML, MDS, MPN
Del(7q)	7q22.1-q22.2/7q31.2	Deletion	AML, MDS
Del(20q)	20q12/20q13.12	Deletion	AML, MDS, MPN
P53 (TP53)	17p13.1	Deletion	ALL, AML, CLL, Limfoma, MDS, MM
ATM	11q22.3	Deletion	CLL
D13S319	13q14.2-q14.3	Deletion	CLL
P16 (CDKN2A)	9p21.3	Deletion	ALL, AML
E2A (TCF3)	19p13.3	Breakapart	ALL
MLL (KMT2A)	11q23.3	Breakapart	ALL, AML

Interpretation of results

When we use a translocation probe such as: BCR/ABL (ABL1) Dual Fusion, AML1/ETO (RUNX1/RUNX1T1) Dual Fusion, TEL/AML1 (ETV6/RUNX1) Dual Fusion, PML/RARα (RARA) Dual Fusion, IGH/CCND1 Dual Fusion, IGH/MYC Dual Fusion expected results of a normal and abnormal signals are shown below.

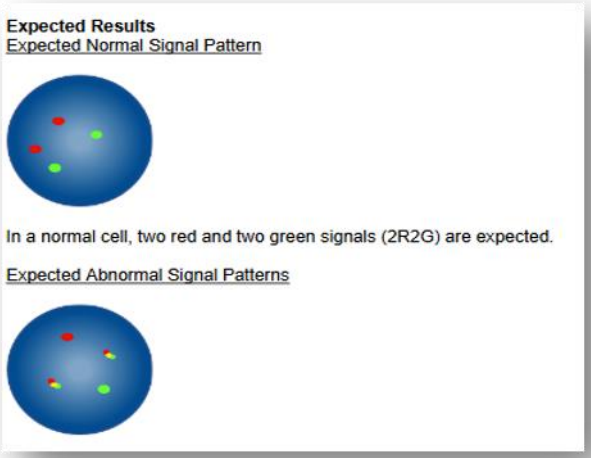

Figure 3. Analysis guidelines of one translocation probe (55)

When we use a deletion probe such as: Del(5q), Del(7q), Del(20q), ATM deletion, D13S319 deletion, P16 (CDKN2A) deletion and P53 (TP53) deletion expected results of a normal and abnormal signals are shown below.

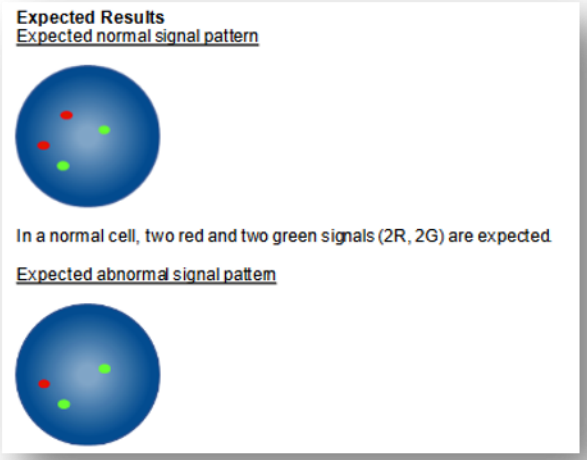

Figure 4. Analysis guidelines of one deletion probe (55)

When we use a breakapart probe such as: E2A (TCF3) or MLL (KMT2A) expected results of a normal and abnormal signals are shown below (55).

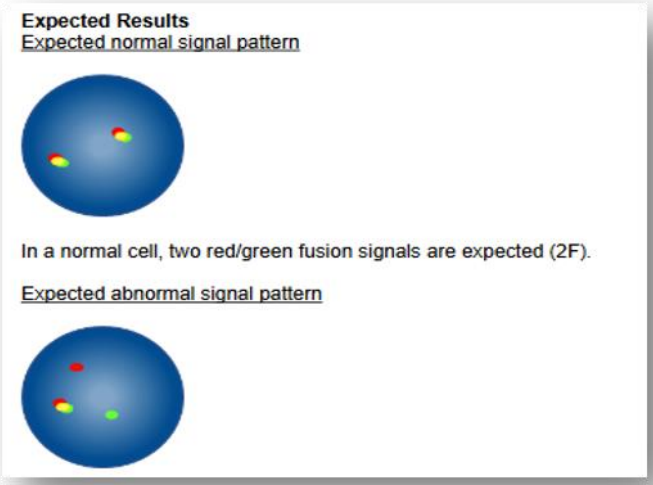

Figure 5. Analysis guidelines of one breakapart probe (55)

Discussion

According to the European recommendations for FISH probes in malignant hemopathies, these probes are available for diagnosis and classification of the diseases, providing important prognostic and therapeutic information, monitoring disease response to treatment, Assessment of minimal residual disease, and identification of an early relapse stage of the disease (7).
Below, we present some of the important information for each probe used in our service.

BCR/ABL (ABL1) Translocation, Dual Fusion Probe
Tthe location of the BCR (BCR activator of RhoGEF and GTPase) gene is found at 22q11.2, and speaking of the ABL1 (ABL proto-oncogene 1, non-receptor tyrosine kinase, his gene is located at 9q34.1. Translocation between these two genes gives rise to the BCR-ABL1 fusion gene and produces a Philadelphia chromosome, the visible result of this translocation. The translocation t (9;22) (q34.1;q11.2) is the hallmark of the diagnosis of the chronic myeloid leukemia and is found in around 90-95% of these cases. The remaining 5-10% of patient cases diagnosed with CML present a variant translocation or a cryptic rearrangement involving 9q34.1 and 22q11.2 that cannot be identified by routine cytogenetic analysis. The BCR-ABL1 fusion can also be found in 25% of adult acute lymphoblastic leukemia and in 2-4% of childhood ALL. The presence of a BCR-ABL1 fusion has been shown to confer a poor prognosis in ALL in both adults and children (8, 9). The detection of abnormalities is, therefore, of high importance for risk stratification, which will influence treatment and management decisions (9). In a small number of ALL cases, the translocation [Philadelphia chromosome] is not cytogenetically visible. In these cases, FISH is essential for detectig the presence of the fusion gene (10).
This rearrangement is also seen in some rare cases of acute myeloid Leukemia, characterized by its resistance to conventional standard chemotherapy and poor prognosis, so accurate and rapid identification of this chromosomal abnormality is vital (11).

AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion
The AML1/ETO (RUNX1/RUNX1T1) translocation, also known as the dual fusion test, is a FISH (Fluorescence In Situ Hybridization) test used to detect rearrangements involving the AML1 (RUNX1) region on chromosome 21 at location 21q22.1 and the ETO (RUNX1T1) region on

chromosome 8 at location 8q21.3. Acute Myeloid Leukemia (AML) with a RUNX1-RUNX1T1 fusion, resulting from a translocation t(8;21)(q22;q22), is recognized as a distinct disease entity in the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. (12).

The cytogenetically cryptic t(12;21)(p13;q22) translocation occurs between ETV6 (ETS variant 6) at 12p13 and RUNX1 (RUNX family transcription factor 1) at 21q22, resulting in the formation of the ETV6-RUNX1 chimeric fusion gene (13).The cytogenetically cryptic translocation t(12;21)(p13;q22) involves a rearrangement between the ETV6 gene located at 12p13 and the RUNX1 gene at 21q22. This translocation leads to the formation of the ETV6-RUNX1 chimeric fusion gene. Both ETV6 and RUNX1 encode transcription factors. ETV6 is essential for proper transcription processes during hematopoiesis in the bone marrow. (13, 14). B-lymphoblastic leukemia and lymphomas with t(12;21)(p13;q22) translocations are recognized as a distinct disease entity according to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. This group represents approximately 25% of childhood B-acute lymphoblastic leukemia (B-ALL) cases. (15). As the t (12;21) (p13; q22) translocation is cytogenetically-cryptic, FISH is an important diagnostic tool for this type of leukemia (16). B-ALL with ETV6-RUNX1 is considered to have a favorable outcome with cure rates of more than 90%. Studies demonstrate late relapses of the disease is attributed to the presence of a persistent preleukaemic clone escaping chemotherapy (15, 17).

PML/RARa (RARA) Translocation, Dual Fusion

The PML (promyelocytic leukemia) gene is located at 15q24.1, and the RARA (retinoic acid receptor, alpha) gene is located at 17q21.2. The translocation t (15:17) (q24;q21) gives rise to the PML-RARA fusion gene and is the diagnostic hallmark of more than 90% of cases of acute promyelocytic Leukemia. PML and RARA have both been implicated in normal hematopoiesis. PML possesses growth suppressor and proapoptotic activity, whereas RARA is a transcription factor that mediates the effect of retinoic acid at specific response elements (18). PML-RARA fusion protein behaves as an altered retinoic acid receptor with the ability to transmit oncogenic signaling (19).

IGH/CCND1 Translocation, Dual Fusion

The t (11;14) (q13;q32) translocation involving the CCND1 (cyclin D1) gene at 11q13.3 and the IGH (immunoglobulin heavy locus) gene at 14q32.33 is associated with cell lymphoma. IGH-CCND1 translocation status would be important for clinical management in recognized diagnostic and clinical care pathways (20).

IGH/MYC Translocation, Dual Fusion

The IGH/MYC translocation, dual fusion is a FISH test used to detect rearrangement involving the IGH (immunoglobulin heavy locus) gene at 14q32.33 and the MYC (v-myc avian myelocytomatosis viral oncogene homology) oncogene at 8q24 is a recognized recurrent abnormality commonly seen in patients with B-cell malignancy. The (8,14) (q24,q23) translocation is the most common and is found in approximately 85% of patients with Lymphoma (21).

Del(5q) Deletion probe

Deletions in the long arm of chromosome 5 are among the most common genetic abnormalities observed in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) with myelodysplasia-related changes. The EGR1 gene (early growth response 1), a tumor suppressor

located at 5q31.2, has been shown to contribute to the development of MDS and AML through haploinsufficiency. Loss of the 5q31.2 region, which includes the EGR1 gene, is associated with a more aggressive form of MDS and AML. This deletion is often accompanied by additional cytogenetic abnormalities and correlates with a poorer prognosis. This probe can also detect some deletions that are associated with 5q- syndrome. However, the probe does not cover the critical deleted region for 5q33 and is not intended to detect all deletions associated with 5q- syndrome (22, 23).

Del 7q deletion Probe

Monosomy of chromosome 7 and deletions of the long arm of chromosome 7 are common chromosomal abnormalities often observed in myeloid disorders, such as myelodysplastic syndrome and acute myeloid leukemia. The presence of monosomy 7 or deletion of the long arm of chromosome 7 (del(7q)) as identified through karyotyping is associated with a poorer prognosis in myeloid malignancies. These deletions are typically extensive, and there is considerable variability in the breakpoints found in myeloid diseases, which complicates the mapping of common deleted regions.

Del 20q deletion probe

Deletions of the long arm of chromosome 20 are recognized as recurrent chromosomal abnormalities associated with myeloid malignancies, particularly myeloproliferative neoplasms, myelodysplastic syndromes, and acute myeloid leukemia. Deletion of the long arm of chromosome 20 occurs in 4% of Myelodysplastic Syndromes (MDS) cases and in 1-2% of Acute Myeloid Leukemia (AML) cases. When the deletion of chromosome 20q is the only abnormality present in MDS, the prognosis is generally good. However, the presence of additional secondary abnormalities may indicate disease progression.

P53 deletion probe

The TP53 gene, located at 17p13.1, is a crucial tumor-suppressor gene that is often deleted in various types of human cancers. It is one of the most significant tumor suppressor genes, functioning as a powerful transcription factor that plays a vital role in maintaining genetic stability.

Screening for the loss of TP53 is essential because deletions or losses on the short arm of chromosome 17, which encompasses the TP53 region, are frequently observed in many cancers. These changes are often linked to disease progression, a poorer response to treatment, and an unfavorable prognosis. Specifically, loss of TP53 is found in 10% of patients with chronic lymphocytic leukemia and is regarded as the most concerning prognostic marker for this disease. (27, 28). In acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), the loss of the TP53 gene is linked to a poor prognosis and is frequently considered an indicator of disease progression or the development of secondary disease. In patients with multiple myeloma, TP53 loss occurs later in the disease course and serves as a marker of progression, which is associated with a very poor prognosis. (32, 33).

ATM deletion probe

The protein kinase ATM (ATM serine/threonine kinase) gene at 11q22.3 is frequently deleted in cases of B-cell chronic lymphocytic leukemia. ATM is an important checkpoint gene involved in the management of cell damage. Its function is to assess the level of DNA damage in the cell and attempt repair by phosphorylating key substrates involved in the DNA damage response pathway (34). Screening for deletions of ATM and/or TP53 is vital to allow informed therapy choices for B-CLL patients, as deletions of TP53 and ATM confer poorer prognosis in this disease (37)

therefore, the use of FISH has proved to be a powerful tool in both the diagnosis and management of patients with B-CLL (34,35,36). Analysis of the ATM/TP53 interaction in B-CLL has shown that TP53 and ATM play an important role in the proliferation of lymphoid cancer (34). In the absence of ATM, damaged cells are allowed to continue to proliferate (38).

D13S319 deletion probe

Rearrangements leading to the loss of all or part of the long arm of chromosome 13 are seen frequently in a wide range of hematological disorders. Chromosome 13q aberrations occur in 16-40% of multiple myeloma cases. Historically, deletions of 13q have been associated with poor prognosis in MM, but it is believed that its prognostic relevance may be related to its association with other concurrent genetic lesions (39, 40). Deletions affecting 13q14 are also the most frequent structural genetic aberrations in chronic lymphocytic Leukemia (41, 42, 43). This region is found to be heterozygously deleted in 30-60% and homozygously deleted in 10-20% of CLL patients (44).

P16 deletion probe

The CDKN2A (cyclin-dependent kinase inhibitor 2A) gene at 9p21 is a tumor suppressor gene that has been shown to be deleted in a wide range of human malignancies. Loss of the CDKN2A gene results in cellular proliferation and dysregulation of proapoptotic pathways. Deletions of 9p that include the CDKN2A gene are frequently reported in patients with acute lymphoblastic leukemia in approximately 30% of adult B-cell ALLs, 30% of childhood ALLs, and up to 50% of T- cell ALLs. In adult B-cell ALL, CDKN2A deletions are frequently acquired in disease progression (45, 46, 47, 48). CDKN2A loss has been implicated with shorter overall survival in ALL patients.

E2A (TCF3) Break-apart Probe

The TCF3 (transcription factor 3) gene is located at 19p13.3. Translocations involving TCF3 are some of the most common rearrangements in childhood B-cell acute lymphoblastic leukemia.
According to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia, B lymphoblastic leukemia/Lymphoma with t (1;19) (q23;p13) TCF3-PBX1 is recognized as a distinct (49, 50).

MLL (KMT2A) breakapart probe

The KMT2A (lysine methyltransferase 2A) gene at 11q23.3 is commonly rearranged in acute leukemias, especially in infant leukemia and secondary leukemia (51). KMT2A rearrangements can be detected in approximately 80% of infants with acute lymphoblastic leukemia and in 5-10% of pediatric and adult ALLs (52, 53). They can also be found in 60% of infant acute myeloid leukemia in 3% of de novo and 10% of therapy-related adult AML cases (52, 54). Historically, KMT2A rearrangements in acute leukemia were associated with a poorer outcome, but recent studies have shown that the prognosis is highly dependent on the fusion partner, and it may differ between children and adults (51).

Conclusions:

The use of FISH for the first time in Albania in the molecular cytogenetics laboratory is an important achievement that contributes to the advancement of diagnostic and personalized medicine for hematology patients. It can provide a powerful tool for diagnosing these diseases with a very high level of accuracy and precision. This also increases the quality of health care and brings opportunities for further progress in scientific and medical research in the country. Accurately knowing the genetic characteristics of a hematological disease can help determine the best

treatments for patients..

Conflicts of Interest: No conflict of interest.

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