ISSN 3009-3848
ISSNe 3009-383X

Oncology Insights

Official Journal of the Serbian Association for Cancer Research

SDIR

Year 2023/No. 1

ISSN 3009-3848
ISSNe 3009-383X

ONCOLOGY INSIGHTS

Official Journal of
the Serbian Association for Cancer Research

Belgrade, Serbia
October, 2023

ONCOLOGY INSIGHTS
Official Journal of the Serbian Association for Cancer Research
Publishing annually

Publisher
Serbian Association for Cancer Research
Belgrade, Serbia

Editor-in-Chief
Dr Milena Čavić

Associate Editors
Dr Milica Pešić, Dr Ivana Z. Matić

Managing Editor
Dr Ana Damjanović Veličković

Technical Editors
Dr Marija Đorđić Crnogorac, Dr Milica Nedeljković

Editors
Dr Radmila Janković, Dr Marko Radulović, Prof. Katarina Zeljić, Dr Ana Krivokuća, Prof. Chiara Ambrogio, Dr Thomas Mohr, Prof. Engin Ulukaya, Prof. Vilma Petrikaite, Prof. Konstantinos Dimas, Dr Cristina Xavier, Dr Remond Fijneman, Prof. Ieronymos Zoidakis, Dr Sergi Castellvi-Bel, Dr Petar Ozretic, Prof. Sonja Levanat

Editorial Council
Dr Ana Đurić, Dr Marko Radulović, Dr Radmila Janković, Prof. Katarina Zeljić, Dr Lidija Todorović

Lector/Corrector
Jana Stefanović

Editorial Office
Serbian Association for Cancer Research

Printed by:
Connect Online Studio
Ćirila i Metodija 2a
Belgrade, Serbia

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

616-006-08

ONCOLOGY Insights : official Journal of the Serbian Associaton for Cancer Research / editor in chief Milena Čavić. - [Štampano izd.]. - 2023, no. 1- . - Belgrade : Serbian Associaton for Cancer Research, 2023- (Belgrade : Connect Online Studio). - 30 cm

Godišnje. - Drugo izdanje na drugom medijumu:
Oncology Insights (Online) = ISSN 3009-383X
ISSN 3009-3848 = Oncology Insights (Štampano izd.)
COBISS.SR-ID 125366281

L21

Significance of molecular diagnostics in therapy of chronic lymphocytic leukemia

Marija Denčić Fekete[1], Teodora Karan-Đurašević[2], Vojin Vuković[3], Jelica Jovanović[3], Senka Sanader[4], Darko Antić[3]
[1]*Institute of pathology, Medical faculty, University of Belgrade, Belgrade, Serbia*
[2]*Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 444a, Belgrade, Serbia*
[3]*Clinic of hematology, Koste Todorovića 2, University Clinical Center of Serbia, Belgrade, Serbia*
[4]*Clinical Center of Vojvodina, Hajduk Veljkova 1-9, Novi Sad, Serbia*

Abstract in extenso:
Chronic lymphocytic leukemia (CLL) is a malignancy of mature CD5+ B lymphocytes that is characterized by exceptional clinical and biological heterogeneity. The Rai and Binet staging systems, developed in the late 1970s to early 1980s, are used in clinical practice to stratify CLL patients into risk categories and to help guide clinical follow-up options: to treat or to watch and wait. However, in early-stage disease, these systems are unable to predict what patients will face the progression to a more aggressive disease. That means, a number of molecular markers with prognostic and/or predictive impact exist and their assessment is strongly recommended in all patients prior to treatment initiation. One of the first recognized prognostic genomic aberrations in CLL include those detected by fluorescence in situ hybridization (FISH): del(17p), del(11q), trisomy 12 and del(13q), and the immunoglobulin heavy variable (IGHV) gene somatic hypermutation (SHM) status. Moreover, the rapid development of genomics techniques greatly expanded the understanding of CLL at the molecular level in the past decade. This resulted in the discovery of many newer prognostic markers based on chromosomal aberrations or gene mutations. For instance, next-generation sequencing (NGS) studies have led to the discovery of recurrently mutated genes in CLL, such as *NOTCH1*, *SF3B1*, *BIRC3*, *XPO1*, *POT1*, *NFKBIE* and *EGR2*, that are associated with poor clinical outcome. Among all of these biomarkers, the distinction between markers of prognostic and predictive values should be made. Prognostic markers refer to biomarkers that can provide information regarding the patient's outcome regardless of treatment. They are often assessed before treatment to help guide decisions on to treat or not. Markers associated with overall survival (OS) or time to first treatment (TTFT) represent such examples. On the other hand, predictive markers are related to therapeutic interventions with the ability to predict treatment response to a drug. These markers are normally assessed when patients receive the particular therapy. Some markers can be both prognostic and predictive. The National Comprehensive Cancer Network guideline recommends testing of *TP53* genetic alterations, *IGHV* mutation status, and several well-established cytogenetic markers for CLL prognostication. Of these, *TP53* mutations, *IGHV* unmutated status, del(17p), and del(11q), as well as complex karyotype (the presence of three or more unrelated clonal chromosomal abnormalities in a sample), are associated with poor prognosis. Normal karyotype and trisomy 12 are considered as intermediate prognostic factors, whereas del(13q) is associated with a favorable prognosis. The higher frequencies of the previously mentioned unfavorable markers (except for IGHV) found in the treated population usually imply the clonal evolution during disease progression or change in clonal dynamics induced by therapies, especially chemotherapies. Different molecular and genomic techniques are employed for detecting molecular biomarkers in CLL. For IGHV mutation status, the preferred method is Sanger sequencing to detect mutations in genomic DNA or cDNA following PCR, and align the resulting sequences to the germline IGHV using the IMGT/V-QUEST analytic tool, where ≥ 98% homology to the germ line is interpreted as unmutated, >2% nonhomology as mutated, and 97.0% to 97.9% is interpreted as borderline. Prognostically significant chromosomal abnormalities are frequently detected using fluorescence in situ hybridization, array comparative genomic hybridization or conventional karyotyping. Fluorescence in situ hybridization, although offers a high sensitivity and specificity, requires prior knowledge of chromosomal lesions for the probe designs and are limited to the chosen panel genes. The technique has limitations in detecting possible complex cytogenetic abnormalities, as well. On the other hand, karyotyping and array comparative genomic hybridization provide genome-wide coverage. Despite the fact that array comparative genomic hybridization does not effectively detect balanced chromosomal rearrangements, it uncovers more genomic abnormalities than karyotyping as the probe-based technology examines the chromosomal structure at a much higher resolution. Development in NGS technology in the past two decades, made the technique, especially targeted sequencing of gene panels, much less costly and accessible. Currently, in Serbia, genetic techniques such as FISH, conventional karyotyping, Sanger sequencing and NGS are available for detection of CLL biomarkers. Advances in the understanding of CLL pathogenesis have consequently led to the development of several highly effective targeted therapies, including Bruton tyrosine kinase (BTK), phosphatidylinositol 3-kinase, and BCL2 apoptosis regulator (BCL2) directed inhibitors. B-cell survival and proliferation is regulated by the BCR signaling pathway. In normal B cells, BCR is triggered by antigen ligation, leading to activation of a cascade of tyrosine kinases, including BTK. BCR signaling is aberrantly activated in many B-cell malignancies, including CLL. Ibrutinib has demonstrated high clinical efficacy acting as an irreversible potent inhibitor of Bruton's tyrosine kinase and targets several key components of the BCR pathway. However, despite having 80% to 90%

response rate, 10% to 15% of CLL patients, who respond initially, develop ibrutinib resistance and disease relapse in 2 to 3 years on ibrutinib treatment, mainly because of the acquisition of a BTK C481S mutation. The mutation prevents the drug from forming a covalent bond with the C481 residue that weakened the drug-BTK binding by 500-fold. As a result, BCR signaling and cell proliferation were restored in the tumor cells. BTK mutations may be found in approximately 70% of CLL patients who progressed on ibrutinib treatment. Another resistance mechanism is through acquired activating mutations in PLCG2, which is found in approximately 10% of the cases. Given these evidences, the current National Comprehensive Cancer Network guideline recommends testing for BTK and PLCG2 mutations for CLL patients receiving ibrutinib who are suspected of having disease progression. NGS has become the optimal method for detecting BTK or PLCG2 mutations in the setting of ibrutinib treatment, as multiple mutations in both genes may occur in the same specimen. Currently, approximately 20% of CLL patients who progressed on ibrutinib do not have either BTK or PLCG2 mutations; thus, with NGS, it is possible to uncover other less common but yet undefined drug-resistance mutations. In addition to BTK and PLCG2 mutations known to confer ibrutinib resistance, other molecular markers have been associated with an upfront high risk of relapse on ibrutinib treatment. It has been reported that complex karyotype, del(17p)/TP53 mutation, and del(18p) at baseline before ibrutinib treatment are strongly associated with disease relapse. Other approved targeted agents for CLL treatment include the phosphatidylinositol 3-kinase inhibitors idelalisib and duvelisib and BCL2 inhibitor venetoclax. For venetoclax, a novel BCL2-G101V mutation was identified to prevent drug activity through drug-protein interaction. Each patient with CLL may have several clinical and molecular markers of conflicting prognostic significance simultaneously, making the precise prognostication challenging. Today is of the greatest importance to apply ultrasensitive techniques to reveal molecular relapses after therapy initiation and to detect minimal residual disease after patients achieve complete responses.
Keywords: Chronic Lymphocytic Leukemia, Molecular Biomarkers, Molecular Techniques, Target Therapy