



## A STUDY ON THE ROLE OF THE RB1 GENE AS A PROGNOSTIC FACTOR IN UNTREATED PATIENTS WITH B-CHRONIC LYMPHOCYtic LEUKEMIA

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### ABSTRACT

Chronic lymphocytic leukemia (CLL) is one of the most common leukemias in adults. After 2000, the FDA approved drugs for its treatment with mechanisms of action different from those of the then-known cytostatics. This new therapeutic approach, employing targeted therapy, is based on a better understanding of the biology and key pathogenetic mechanisms driving the development and progression of the disease. Deletion of the long arm of chromosome 13 is one of the most frequently described cytogenetic aberrations among CLL patients, the prognostic value of which is still debated, given that part of the tumor suppressor genes are located in this chromosome. Our study aimed to investigate the frequency of deletions affecting the retinoblastoma gene (RB1) and their impact on the time to first treatment (TTFT) in untreated B-CLL patients. The cohort included 40 patients with a confirmed diagnosis of CLL and deletion of the DLEU1 gene. Fluorescence in situ hybridization (FISH) was used to determine cytogenetic aberrations affecting the DLEU1 and Rb1 genes. The difference in time to first treatment in patients with/without deletion in the Rb1 gene was determined based on the log-rank test. In our study, we found a shorter time to initiation of first treatment in patients with a deletion in the Rb1 gene. Future clinical studies, including a more significant number of patients with an isolated deletion in the Rb1 gene, would confirm or reject the importance of the mentioned aberration as a prognostic factor and the necessity of its examination in routine practice.

**Keywords:** chronic lymphocytic leukemia, prognosis, tumor suppressor gene,

### INTRODUCTION

CLL is among the most common malignant blood diseases (30% of all leukemias), with an incidence of 3-5 per 100,000 population [1]. In most cases, the clinical course of the disease has the characteristic of indolent lym-

phoma, which usually has slow tumor growth; as a result, therapy can often be postponed. The use of modern technologies such as classic Sanger sequencing and next-generation sequencing (NGS) serves as a prerequisite for the more precise clarification of the factors related to the progression of the disease, as well as for creating prognostic models and risk-stratification systems. In hematological neoplasias, the determination of the cellular origin (the cell in which the first oncogenic event has occurred) and the normal counterpart of malignant cells (the ones in which the transformation occurred) is crucial for elucidating the mechanisms and natural history of the disease with implications for treatment [2]. To date, the question of the cellular origin of CLL remains unanswered. Although pathological changes at the hematopoietic stem cell level have been described, antigenic expansion of B-lymphocytes in the secondary lymphoid tissue microenvironment appears to be a driving force in CLL and lymphoma carcinogenesis [3]. Unlike other lymphomas originating from the germinal center, whose genetic hallmark is balanced reciprocal translocations, in CLL, this hallmark is absent in most cases [4]. The so-called hierarchical classification proposed by Dohner in 2000, based on the presence of cytogenetic abnormalities [3], is a turning point in the creation of risk assessment systems. Among the described five most frequently detectable cytogenetic disorders, graded according to negative predictive value, deletion of 13q14 [del(13q)] ranks last. Two types of 13q14 deletions have been described in scientific reports, distinguished by the size of the affected regions and accordingly the genes involved. Type I deletion affects the DLEU2 gene, MIR15A/MIR16-1 cluster as well as the first exon of the DLEU1 gene, and type II – a larger type of deletion, that includes the retinoblastoma gene (RB1) [ 5, 6 ] In a 2014 study by Y. Pekarsky et al., the authors described deletions in the 13q14 region affecting the tumor suppressor genes: miR - 15/ 16 and DLEU 7. Inactivation of DLEU 7 leads to activation of NF - kB and NFAT, while miR -15/16 inactiva-

tion causes constitutive activation of Bcl-2 and Mcl-1 [7]. The another mentioned tumor suppressor gene located on the long arm of chromosome 13 is RB1 gene. Tumor suppressor genes encode proteins that slow down or inhibit the progression of the cell cycle. They also encode proteins that arrest the cell cycle in cases of DNA or chromosomal damage, receptors for secreted hormones that suppress cell proliferation, and proteins that promote apoptosis and DNA repair enzymes [8]. The retinoblastoma gene is located on the long arm of chromosome 13 (13q14.2)[9,10]. It is one of the first identified tumor suppressor genes [11] whose protein product controls the cell cycle and growth, blocking the transition from G1 to S-phase [12]. RB1The gene is part of a large family of genes, including RBL1 and RBL2, both of which encode structurally related proteins such as pRB, p107, and p130 [13,14]. The protein products of these genes are the so-called ‘pocket proteins,’ possessing a binding region through which they bind viral oncoproteins and cellular factors, such as the E2F family of transcription factors [12]. Inactivation of RB1 by somatic mutation, deletion, or epigenetic silencing has been described in various cancers [15]. Based on genetic and molecular studies, specific activities of the proteins from the family of Rb in various tissues and cells have been identified, including the role of Rb in aging and p130 at rest [16]. In our country, there is not enough data from the studies conducted to follow up on the clinical significance of a deletion affecting the RB1 gene and its impact on the time to initiation of treatment in untreated B-CLL patients. Therefore, we set ourselves the goal of looking for a relationship between the mentioned cytogenetic aberration and the clinical course of the disease.

### MATERIALS AND METHODS

The study included 40 adult patients over 18, referred to the Hematology Clinic of Dr. Georgi Stranski “University Hospital -Pleven for six years (2016-2022). The patients had proven B-CLL based on results from flow cytometric immunophenotyping peripheral blood. A flow cytometer FACS Calibur (Becton Dickinson, Heidelberg, Germany ) and Cell Quest Pro software (Becton Dickinson) were used. Lymphocytes were separated by CD 45/SSC gating. The panel of monoclonal antibodies (Immunize, Salamanca, Spain) used included CD45, CD5, CD19, CD20, CD22, CD23, CD38, CD11a, CD 49d, CD 29, CD200. Locus-specific deletion DNA probes D13S319/13q34 (Vysis), XL RB1/DLEU/LAMP (MetaSystems ), and the FISH Probe Kit were used to study the DLEU1 and RB1 genes. Signal reading was performed on a fluorescence microscope.

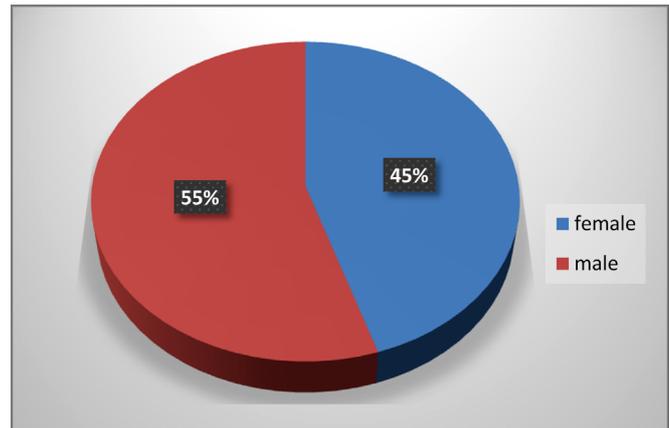
The staging of the patients was based on data from the physical status and imaging diagnostics. The European Binet staging system was used. The time to first treatment (TTFT) was defined as the interval from the diagnosis to starting the first therapy, the date of the last contact with the patient, or death (censored). In patients who met the criteria for active disease or progression of CLL, treatment was started according to the recommendations of the International Working Group on CLL. To compare time to initial treatment in patients with a deletion of the DLEU1 gene

and dependence on the presence or absence of RB1 gene deletion, the log-rank test (Kaplan– Meier) was used.

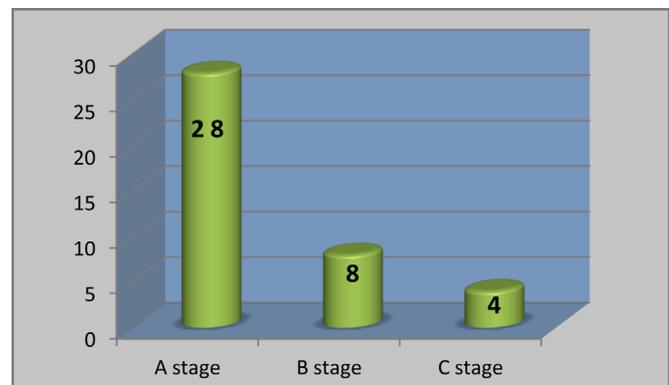
### RESULTS

It was determined that the average age of our patients at diagnosis of the disease was 69 years (range 37 - 83). The distribution of patients depending on the stage of the disease on diagnosis and the demographic gender indicator is shown in Fig.1 and Fig. 2.

**Fig. 1.** Distribution of patients with B-CLL by demographic indicator sex.

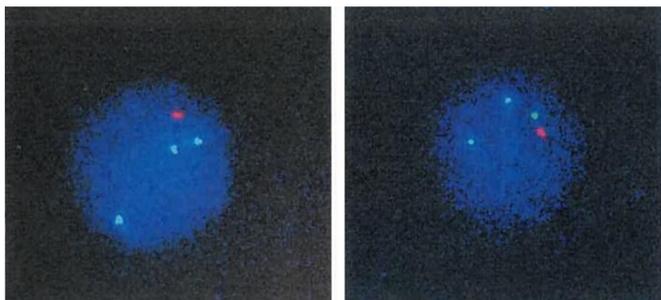


**Fig. 2.** Distribution of patients according to Binet disease stage.



The largest group was patients in the early A stages (n =28 ). Regarding gender, in the group we covered, men slightly predominated (n=23). Monoallelic deletion of the DLEU1 gene was found in 36 patients out of a total of 40 patients with DLEU1. In 4 of them, the loss of DLEU1 was in the form of a biallelic deletion. In 24 of the patients, no loss of the RB1 gene was detected. In 16 cases, monoallelic loss of RB1 was found in more than 10% of the malignant lymphocytes, and in two patients, the loss was d” 10%. Only one out of the 40 patients was found with biallelic loss of RB1. In one patient with trisomy, 13, 33% of the nuclei had lost one allele of the RB1 gene in disomy 13, and in 20% of the nuclei, there was a loss of two or three alleles of RB1 in trisomy 13 (Fig. 13).

**Fig. 3.** FISH analysis of a patient with trisomy 13 and deletion of the RB1 gene.

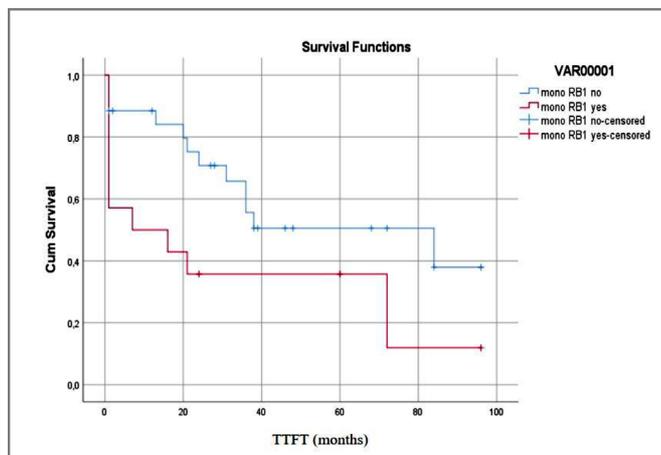


The signal pattern (3B0G1R) indicates loss of both alleles of DLEU1 gene and all three alleles of RB1 in trisomy 13 background.

The signal pattern (2B1G1R) indicates loss of one allele of the RB1 and one allele of the DLEU1 gene.

At an accepted threshold value of more than 10% of malignant lymphocytes, we found a difference between the time to first treatment in patients with loss of RB1 compared to the group without retinoblastoma gene deletion ( $p = 0.42$ ) (fig. 4).

**Fig. 4.** Time to first treatment of patients with B-CLL, depending on the presence of deletion in the RB1 gene.



### DISCUSSION

CLL is one of the variants of primary leukemic indolent lymphoma, characterized by a variable clinical course based on complex and changing pathogenetic mechanisms. The clinical significance of cytogenetic aberrations, called “moving targets” by some authors, is the subject of intensive study, given the increasingly widespread use of target therapy in the field of oncohematology. The interphase FISH analysis is a routine diagnostic method for investigating the most important clinically significant chromosomal abnormalities in CLL [17]. The 17p

and 11q deletions included in the risk stratification models are associated with an unfavorable prognosis, short patient survival, and resistance to therapy. The reason for the clinical heterogeneity observed in cases with isolated del(13q) is still unclear [18]. In recent years, two basic types of deletions at 13q14 have been established: del(13)(q14) type I (short), where the breakage is near the miR16/15a locus and does not include RB1; and del(13)(q14) type II (larger), which includes RB1 and suggests more significant genomic complexity and a more aggressive course of the disease [19]. Since the majority of clinical trials have focused on DLEU genes [20] in order to evaluate how the size of the deletions relates to the time to first treatment we further investigated the RB1 gene. We found RB1 gene deletion in 40% of the patients with DLEU1 gene deletion. A similar result was reported by Grygalewicz B et al., who reviewed the clinical significance of mono- and biallelic deletions affecting 13q14 in patients with B-CLL. In a cohort of 40 patients (25% of patients were on treatment during the study), the authors summarized the results and found that larger deletions, including those affecting the RB1 gene, were not significant enough to conclude that the presence of deletion 13 is an unfavorable prognostic factor [19]. In 2011, Michele Dal Bo et al., based on a study on the influence of tumor volume (defined as the percentage of cells containing an isolated deletion 13) and the presence of a deletion affecting the RB1 gene, proposed a scheme for evaluating CLL based on FISH analysis. According to these authors, patients with the 13q deletion found in less than 70% of nuclei, including the RB1 locus, had a shorter time to treatment initiation [21]. In the same year, Ouillette P. et al. suggested that identifying the two subtypes of 13q14 deletions (involving or not involving the RB1 gene) should be introduced into routine practice [22], given the difference in the clinical course of the disease. Ten years later, in 2021, Durak Aras et al. did not confirm the prognostic significance of deletions affecting the RB1 gene and TTFT [18]. Despite the significant advances in technology in molecular biology and genetics, virtually, there are no established standards for risk stratification in untreated B-CLL patients.

### CONCLUSION

In our study, aiming to determine the impact of a deletion on the retinoblastoma tumor suppressor gene, we found a difference in the time to initial treatment in a comparatively small group of patients. Future clinical studies covering a larger cohort and additional analysis of the mutational status of IGVH genes would contribute to clarifying the clinical significance of the RB1 gene in B-CLL patients.

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