"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY IN BUCHAREST
GRADUATE SCHOOL OF MEDICINE STUDIES
MEDICINE

PhD THESIS

SUMMARY

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"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY IN BUCHAREST
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IDENTIFICATION OF MOLECULAR PROGNOSTIC FACTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA: IMPLICATIONS IN RISK STRATIFICATION AND TAILORED THERAPEUTIC APPROACH

SUMMARY OF THE PhD THESIS

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INTRODUCTION

Chronic lymphocytic leukemia (CLL), far from being considered merely a chronic condition, exhibits complex biology, the mechanisms of which have been recently elucidated. The clinical course of CLL is highly heterogeneous, reflecting significant cellular and molecular diversity. A detailed and in-depth understanding of the underlying cellular biology has spurred research into biomarkers that provide prognostic information.

The management of CLL has undergone significant transformations with the introduction of targeted therapies. Current therapies for CLL patients include Bruton tyrosine kinase (BTK) inhibitors such as ibrutinib or acalabrutinib, alone or in combination with anti-CD20 monoclonal antibodies, or the BCL-2 inhibitor venetoclax, administered in combination with anti-CD20 antibodies. These novel therapies target the B-cell receptor, or the anti-apoptotic proteins involved in CLL and have gradually replaced standard immunochemotherapy (CIT).

The prospective controlled randomized trials (RCTs) represent the gold standard in evidence-based medical research but may not always be representative of patients encountered in daily practice. Therefore, the importance of real-world evidence studies (RWE) has increased. RWE provides additional information on the safety and efficacy of new drugs, as well as predictive and prognostic factors. RWE analysis allows for the generalization of results from controlled trials and provides insights into the natural course of the disease, the effectiveness of therapies, and their safety profile, addressing important questions and uncertainties in real-time.

The aim of this study is to analyze the outcomes of CLL patients diagnosed and treated at a reference center such as the Hematology and Bone Marrow Transplant Center of the Fundeni Clinical Institute. Through retrospective analysis of data from current clinical practice regarding treatment with novel agents, we sought to provide practical guidance and support in selecting the optimal therapy considering patient profiles and disease characteristics.

Real-world experiences significantly contribute to a better understanding of diagnosis, prognosis, therapeutic approaches, and long-term outcomes of patients treated with novel targeted agents. However, it should be noted that RWE studies have limitations, such as the lack of standardization of data sources. Nonetheless, observational studies remain indispensable and powerful tools in analyzing a rapidly evolving field like CLL.

I. GENERAL PART

1. Risk Stratification in Chronic Lymphocytic Leukemia

1.1. Introduction

In the era of chemo-immunotherapy (CIT), the selection of treatment for patients with Chronic Lymphocytic Leukemia (CLL) was primarily based on age and comorbidities. Currently, prognostic stratification based on genetic characteristics plays a crucial role in managing CLL patients and establishing a personalized treatment algorithm. The variability in the survival of CLL patients is attributed to the molecular heterogeneity of the disease. The CLL genome exhibits its own clonal evolution dynamics, leading to the emergence of clones or subclones, often more aggressive. The proportion of patients with TP53 gene abnormalities increases from 5-15% at diagnosis [1] to 40% in the case of patients refractory to CIT [2]. Over the past two decades, rapid advances in genomic technology have significantly contributed to the molecular understanding of CLL. This progress has led to the identification of an increasing number of prognostic markers based on chromosomal aberrations or genetic mutations. Risk indicators can be divided into two categories based on their impact. Prognostic indicators refer to biomarkers that can provide information about the patients survival, regardless of the treatment applied. These markers represent intrinsic characteristics of patients or the disease, such as markers associated with overall survival (OS) or time to initiation of first therapy (TIFT). On the other hand, there is also the category of predictive indicators, which are biomarkers linked to extrinsic factors, such as therapeutic interventions. Predictive markers are used to predict the benefit of treatment and are evaluated at the time of therapy initiation to guide therapeutic decisions. Some markers can have both a prognostic and predictive role.

1.2 Epidemiology

CLL is a frequently encountered malignant hematological disorder in adults. It is the most common form of leukemia in Western Europe and the United States but rare in Asia [3], suggesting a significant impact of genetic factors on disease occurrence. CLL primarily affects elderly individuals, with a median age at diagnosis of 72 years. Recent data from the United States for the period 2012-2018 indicate an estimated 5-year survival rate of 87% [4].

1.3 Etiopathogenesis

The etiology of CLL is complex and still not fully understood, involving age-related risk factors, genetic factors, as well as exposure to toxic substances and infection with the

hepatitis C virus [5]. The cellular origin of CLL is controversial. Differentiation is based on the presence or absence of mutations in the variable region of the immunoglobulin heavy chain (IGHV), indicating an extrafollicular origin (unmutated IGHV) or involvement of the germinal centers in lymph nodes (mutated IGHV). The conceptual framework of CLL biology has undergone significant changes in recent decades. It is now known that the dynamic interplay between cell proliferation and cell death plays an important role in the pathogenesis of CLL. Complex processes of proliferation and accumulation are involved in the development and progression of the disease, and the homeostatic balance of these processes contributes to the clinical activity of CLL[6]. Signaling through the B-cell receptor (BCR) is crucial in CLL, and the specific structural pattern of the BCR and the dependence of leukemic cells on this receptor for proliferation and survival place it in a central role in signal transduction within the cell. The overexpression of BCL-2 proteins, known as "arbiters" of apoptosis, which regulate and mediate the intrinsic apoptotic pathway, confers resistance to apoptosis in malignant lymphocytes [7].

1.4 Diagnosis, staging, clinical presentation

CLL is characterized by the accumulation of dysfunctional B lymphocytes in the blood and secondary lymphoid organs. Diagnosis is typically incidental. To establish a diagnosis of CLL, immunophenotypic analysis is performed to identify an increased number of lymphocytes expressing specific antigens such as CD19, CD5, CD23, and clonality [8]. Clinical staging is a useful tool in managing patients considering the variability of CLL clinical course. The staging systems proposed by Rai and Binet consider clinical and biological factors, providing relevant information about patient survival in different stages. However, these systems have limitations in identifying patients in early stages but with potential for aggressive progression. In order to improve the discriminatory power of classical staging, a prognostic index for CLL (CLL-IPI) has been developed, combining genetic, biochemical, and clinical parameters [9]. CLL can be asymptomatic in approximately 50% of cases. In other cases, it most commonly manifests as lymphadenopathy (87%), splenomegaly (30-54%), and hepatomegaly (10-20%). Patients may also experience constitutional symptoms such as fatigue, night sweats, weight loss, and fever in the absence of infection. Patients with CLL can develop complications associated with the disease, including predisposition to infections, autoimmune phenomena, Richter transformation, and an increased risk of developing a second cancer.

Risk markers can be divided into two categories based on their impact: prognostic and predictive.

1.5 Prognostic markers

Table 1.1 Prognostic markers in CLL

Category	Variable
Patient characteristics	Age, comorbidities, male gender
Serology	LDH, β2M, TK, TDL, Lf
Flow cytometry	CD38, CD49d, ZAP-70
Cytogenetics	Del 13q, trisomy 12, CC, del 17p, del 11q
BCR Biomarkers	IGHV, stereotype subsets #1, #2, #8; VH1-69; VH3-21
Genetic mutations	TP53, ATM, BIRC3, NOTCH1, SF3B1, MYD88
Other	BMR, epigenetic factors, micro-ARN, III/IV Rai and C Binet

Prognostic markers are listed in ***Table 1.1***, with the most important ones being IGHV, TP53 mutations, abnormalities detected by FISH, and CD49d. They can provide information about patient survival regardless of the applied treatment.

1.6 Predictive markers

Predictive markers are presented in ***Figure 1.1***. They allow the prediction of response to specific treatment and form the basis of therapeutic decision-making strategies [10].

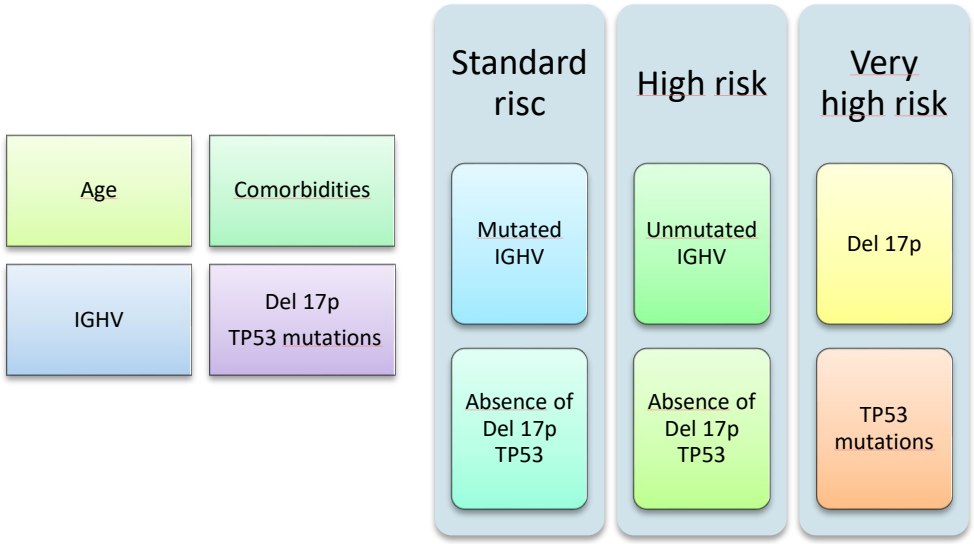

Figure 1.1 Predictive markers in CLL

The unfavorable prognosis of CLL patients is influenced on one hand, by advanced age and comorbidities, and on the other hand, by the genetic profile of the disease, particularly the presence of del 17p/TP53 abnormalities or the IGHV unmutated status.

1.7 IGHV mutation status

The analysis of the IGHV status involves classification into two categories: IGHV M (with mutations) and IGHV UM (unmutated), each being encountered in approximately 50%

of CLL clones. The presence of IGHV UM status is associated with shorter overall survival and a higher relapse rate after FCR treatment [11-13]. In the case of younger and eligible patients with IGHV M, the administration of FCR treatment can be considered, given its potential for inducing long-term remission. For patients with IGHV UM, the recommended approach is the use of targeted therapy with a pathway inhibitor, which can vary from patient to patient, depending on age and comorbidities.

1.8 TP53 abnormalities

The tumor suppressor gene p53 plays an essential role in maintaining genomic stability and is involved in the development of solid tumors and hematological malignancies. This gene, known as the "guardian of the genome," is located on chromosome 17p13.1, and TP53 gene abnormalities can result from either chromosomal deletions or genetic mutations. TP53 abnormalities can include both mutations and deletions, either mutations alone or deletions alone in equal proportions. TP53 abnormality remains the only recognized predictive biomarker for CLL, with a consensus recommendation to be detected before initiating treatment in all patients due to clear evidence of chemotherapy resistance. Cytostatic treatment does not benefit patients with TP53 abnormalities since progression-free survival and overall survival are short [14]. Specialized targeted therapies have significantly improved the survival of patients with TP53 mutations because they act independently of p53. Currently, new therapies based on the pathogenic mechanism are the gold standard for this subgroup of patients. However, no treatment has succeeded in completely reversing the negative prognosis associated with TP53 abnormalities [15,16].

2. Risk-Adapted Treatment in Chronic Lymphocytic Leukemia

Despite significant progress in understanding the molecular mechanisms and the introduction of new targeted therapies, CLL remains an incurable disease. Before treatment, the patient must present signs and symptoms of active disease. These have been defined by the iwCLL in 2018 and remain valid to this day. Over the past four decades, the therapeutic arsenal for CLL has expanded considerably. Treatment has evolved from using a single alkylating agent, such as chlorambucil, to combination therapies like FC and then to CIT combinations such as FCR or BR. Through the use of recombinant genetic technologies, two other molecules, ofatumumab and obinutuzumab, have been developed, contributing to impressive advances in managing CLL. In the last decade, several new oral agents have been approved for CLL treatment, including BTK inhibitors like ibrutinib and acalabrutinib, PI3K inhibitors, and venetoclax, a BCL-2 antagonist. These therapies, taken together, represent a

paradigm shift in approaching CLL patients. The role of allogeneic stem cell transplantation (ASCT) previously considered the only therapeutic option with a chance of curing the disease, has significantly decreased in the context of the new generation of drugs.

2.1 First-line therapy

The choice of therapy is based on stratifying patients according to the genetic risk of the disease along with the assessment of the patient health status. FCR-type CIT may have higher toxicity than targeted therapies and is not recommended for patients who meet one or more of the criteria: ECOG PS ≥ 2, CIRS > 6, severe hepatic impairment (Child-Pugh score B or C), and creatinine clearance < 70 mL/min [17].

2.2 Bruton tyrosine kinase (BTK) inhibitors

I. Ibrutinib (IB) is the first targeted therapy introduced in the treatment of CLL. Its efficacy has been demonstrated in no less than 5 randomized studies. IB is effective in the frontline setting in elderly patients with CLL, showing an improvement in OS and PFS compared to both chlorambucil [18], and BR [19]. In elderly patients, IB plus obinutuzumab has shown an advantage in terms of PFS over O-C [20]. In younger patients (i.e., ≤ 70 years), IB plus rituximab has demonstrated a benefit in PFS and OS compared to FCR [13].

II. Acalabrutinib (AB) is a second-generation BTK inhibitor with similar proven efficacy but reduced cardiovascular adverse effects compared to ibrutinib [21].

III. Zanubrutinib (ZB) is a novel generation BTK inhibitor that has shown promising results in the treatment of CLL [22,23].

2.3 BCL-2 antagonist

Venetoclax (Ven) is a BCL-2 antagonist that provides the advantage of fixed-duration therapy. When administered in the frontline setting for 1 year, in combination with 6 cycles of obinutuzumab, it produces advantages in PFS and OS compared to O-C in elderly patients [24]. Ven has demonstrated promising results in inducing deep and durable responses in patients with R/R CLL, including those with high-risk features such as del 17p or TP53 mutations. The median PFS was 53.6 months compared to 17 months with BR in the MURANO study [25] .

2.4 Combination of novel inhibitors

The highly effective synergistic effects and safety of the IB-Ven combination have recently been demonstrated in clinical studies for the treatment of frontline CLL patients regardless of age. Notably, the rates of CR (55%) and, particularly, undetectable minimal residual disease (MRD) in both peripheral blood (77%) and bone marrow (60%) are remarkable [26,27].

2.7 Management of relapsed or refractory CLL

It is important to histologically confirm the diagnosis of CLL before resuming treatment. Asymptomatic progression should be monitored. Richter transformation must be excluded. The treatment depends on the previous treatment used, duration of response, and the occurrence of resistance or toxicity to that treatment. CIT has been excluded from current recommendations for R/R CLL, and standard options include ibrutinib, acalabrutinib, idelalisib, duvelisib, venetoclax +/- rituximab [28].

II. ORIGINAL PART

3. General Hypothesis and Objectives

The selection of therapy in CLL depends on several factors, such as the patient overall health status, disease stage, presence of genetic mutations, and other prognostic factors. In recent years, targeted therapies have been developed that act on specific proteins involved in CLL development, such as BTK inhibitors and BCL-2 antagonist. These therapies are generally well tolerated and have demonstrated increased efficacy compared to traditional chemoimmunotherapy.

The management of CLL has undergone a radical transformation with the introduction of molecular biomarker-based prognostication and new non-cytotoxic treatments. From the introduction of the first covalent BTK inhibitor in 2014 to the most recent combinations of targeted drugs on "tumor," the treatment paradigm in CLL has changed. For clinicians, the therapeutic management of patients with CLL involves significant challenges, such as selecting appropriate treatment options, applying evidence-based decisions, managing adverse reactions, and monitoring long-term clinical and hematological outcomes.

Clinical trials alone are not sufficient to address in real-time the multitude of issues and questions related to therapy selection, patient prognostication, management of adverse effects, treatment sequencing, specific toxicity, and treatment standards. Therefore, the importance of real-world evidence (RWE) and population-based studies has significantly increased. They can provide valuable and practical insights into unexplored aspects of CLL, including patient demographics, natural disease progression, therapy effectiveness in underrepresented subgroups in clinical trials, and treatment safety.

The aim of this scientific work is to establish a standard of care for patients with CLL by providing clinical support regarding aspects related to diagnosis, prognosis, and treatment

in CLL. Additionally, the paper aims to provide an overview of therapeutic approaches, management of adverse effects, and long-term outcomes in patients with CLL treated with targeted medications. Furthermore, this thesis aimed to study the impact of clinical, biological, and genetic markers on prognosis and risk in CLL using data collected in a real-world clinical setting.

4. General Research Methodology: Identification of Molecular-Genetic Factors with Prognostic Role in Chronic Lymphocytic Leukemia

In our study, we have highlighted the importance of prognostic genetic testing in CLL to guide clinical management. We have identified the main predictive and prognostic markers in CLL: the 17p deletion detected through FISH, TP53 gene mutations identified by Sanger sequencing, and the presence of IGHV mutations analyzed through molecular biology.

4.1 Material and method

4.1.1 FISH analysis for deletion p53 (17p13) detection

The analysis was performed on peripheral blood cells collected with heparin as an anticoagulant. The cells were spread on a slide after processing using the direct method. We used the XL P53 probe (MetaSystems), which included the following probes: the LSI TP53-Spectrum Orange probe with specific sequences for the TP53 gene region (17p13) and the 17cen-Spectrum Green probe with specific sequences for the D17Z1 region (17cen). Preparations were evaluated using an Olympus microscope with fluorescence filters and CytoVision Software. A total of 200 cells were evaluated, and in all cells, 2 signals were observed for the TP53 probe (17p13) and 2 signals for the centromere 17 probe (D17Z1), representing a normal hybridization pattern. The interpretation of results was conducted in accordance with International Standards (ISCN 2016).

4.1.2 TP53 mutations detection by Sanger sequencing

The analysis was conducted on genomic DNA extracted from a peripheral blood sample to identify the mutational status of exons 3-10 of the TP53 gene. The reference sequence NM_000546.5 (Human GRCh38.p10) was utilized. A library was prepared using a panel designed with Twist technology, which targeted the capture of TP53 gene exons and the flanking splicing regions (5-20 bp).

Library sequencing was performed using the MiniSeq sequencer (Illumina). The obtained sequences were aligned to the reference genome (GRCh38/hg38) and filtered

according to specific quality criteria. The analysis aimed to identify variants within exonic regions or splicing regions (at least 5 bp), including nonsense or missense mutations, synonymous mutations, indels, small insertions, or deletions found at an allelic frequency (VAF) greater than 10%. Variant nomenclature and classification are based on the guidelines of the Human Genome Variation Society (HGVS) (http://varnomen.hgvs.org/) and the American College of Medical Genetics and Genomics (ACMG).

4.1.3 The molecular test to detect somatic hypermutation in the IGHV region

The analysis was performed on RNA isolated from peripheral blood collected with EDTA anticoagulant. RNA molecules isolated from the patient sample were reverse transcribed into complementary DNA and then amplified using primers for VH leader and constant region. After Sanger sequencing, the obtained results were compared with the database (IMGT/V-QUEST) for all known sequences of germ line variable regions. The sequence of the VH segment closest to the germ line is reported, along with the percentage of homology with it. Homologies of ≥ 98% are described as unmutated, while homologies < 98% are described as mutated.

4.2 Patients

In the context of this research work, we conducted genetic and molecular analysis to identify potential prognostic markers on a total sample of 88 patients over a period of 6 years, from 2016 to 2021. This analysis was carried out at the Hematology Clinic of the Fundeni Clinical Institute. We presented the results obtained in three main studies aimed at analyzing the impact of these factors on prognosis in three distinct situations:

1. Assessment of targeted therapy (such as ibrutinib) in CLL patients, either as a first-line treatment or at the time of disease relapse.

2. Comparison of the effectiveness of ibrutinib with that of standard chemotherapy in CLL patients who had not received prior treatment.

3. Evaluation of the outcomes of allogeneic stem cell transplantation in patients identified with 17p deletion.

5. STUDY I: Real-world experience with ibrutinib therapy in Chronic Lymphocytic Leukemia patients [29]

5.1 Working hypothesis and specific objectives

In recent years, the management of CLL has undergone a significant transformation due to the use of molecular biomarkers for prognostication and the introduction of new non-

cytotoxic treatments. Therapeutic strategies are individualized based on the patient profile and disease features, but there are no clear models for selecting treatment options. Additionally, in addition to patient preferences and collaboration, each medication can have its own adverse reactions, which can affect treatment adherence and the achievement of therapeutic goals.

Ibrutinib is the oldest "new" molecule in the therapeutic arsenal for CLL. Numerous studies have highlighted the very good efficacy of ibrutinib in treating CLL, and it is now widely used as a first and second-line therapy [13,15,16,19,20,30]. Many studies have evaluated the use of ibrutinib in the real-world population of CLL patients, and the results generally support the findings from clinical trials. Data from over 1,000 CLL patients treated with routine ibrutinib in over 40 centers in the United States have shown that ibrutinib was associated with high rates of therapeutic response and durable remissions, as well as improvements in OS and PFS, both independently and compared to standard chemoimmunotherapy [31-33]. In Europe, experiences from countries such as Spain, Sweden, and Italy have suggested that ibrutinib is an effective and well-tolerated treatment for CLL in routine clinical practice [34-36]. On the other hand, several studies conducted in the real-world population have reported lower rates of therapeutic response and higher rates of discontinuation of ibrutinib compared to the results obtained in clinical trials [33,36-38].

The aim of this study was to perform a retrospective analysis of patients diagnosed with CLL at the Fundeni Clinical Institute who received ibrutinib treatment between 2016 and 2021, in order to identify a standard of care for patients with CLL based on real-world data. The primary objective of this research was to evaluate the management of CLL patients in the daily clinical practice in the context of modern therapies, with a focus on the following aspects: patient and disease characteristics, treatment responses, drug tolerance, spectrum of adverse reactions, and survival.

Another important objective of this study was to explore clinical, biological, genetic, and molecular factors with potential prognostic value and establish their role in risk stratification for relapse, survival, and poor treatment tolerance in the context of new therapeutic molecules. Other objectives of this research include comparative evaluation with other real-world studies and major clinical trials of patient and disease characteristics, treatment responses, drug tolerance, spectrum of adverse reactions, and survival of CLL patients in a university center, as well as recording and detailing adverse events associated with the applied therapies through comparison with known data from clinical trials or other real-world experiences.

5.2 Patients and method

5.2.1 Data collection

The research was conducted retrospectively using the printed and electronic medical records of patients diagnosed and treated at the Hematology and Bone Marrow Transplant Center of the Fundeni Clinical Institute, between January 1, 2016, and June 30, 2021.

The study population consisted of 123 patients over the age of 18 diagnosed with CLL or small B-cell lymphocytic lymphoma (SLL) who received ibrutinib treatment. The study population included 107 patients with relapsed or refractory CLL (R/R) and 16 treatment-naive CLL patients (TN) [29]. All patients provided informed consent to participate in the study. The local Ethics Council approved the study through decision number 748 on August 1, 2018.

5.2.2 Methods

The ECOG score was recorded as noted in the medical records or calculated if not explicitly documented. Patient follow-up occurred until death or study discontinuation, whichever occurred first. Data regarding treatment efficacy included treatment duration and reasons for definitive discontinuation. The best response was assessed according to the iwCLL 2018 criteria [8]. The overall response rate (ORR) included rates of partial remission (PR) and complete remission (CR), defined by clinical and hematological criteria. Temporary discontinuation was defined as a minimum period of 14 days without ibrutinib. PFS was measured from the start of treatment to disease progression or death, and OS was measured from the start of treatment to death from any cause. Treatment response was classified according to the iwCLL 2018 criteria [8].

5.2.3 Statistical Analysis

The demographics of patients and disease characteristics were analyzed and reported descriptively. Quantitative variables were reported as medians with interquartile range (IQR), while qualitative variables were described using numbers and percentages.

Comparison between categorical variables was performed using the chi-square test or Fisher's exact test. Differences between two groups of continuous variables were assessed using the Mann-Whitney test. Variables with a significance level of $p < 0.2$ in the univariate analysis were included in a multivariate model using the enter method. Multivariate logistic regression was performed to evaluate factors associated with treatment discontinuation, dose modifications, temporary interruptions, and adverse effects. Kaplan-Meier method and log-rank test were used to estimate the duration of ibrutinib treatment, PFS, and OS. Multivariate Cox proportional hazards regression was performed to assess the association between

demographic factors (age), clinical factors (ECOG performance status, CIRS comorbidity score, disease stage), and biological factors (genetic and molecular abnormalities) with PFS and OS. A p-value < 0.05 was considered statistically significant for all comparisons. All statistical analyses were performed using IBM SPSS Statistics 20.0 for Windows (IBM Corp., Armonk, NY, USA).

5.3 Results

5.3.1 Study population

The present study includes 123 patients diagnosed with CLL/SLL who received ibrutinib as treatment between January 2016 and June 2021 at the Hematology and Bone Marrow Transplant Center of the Fundeni Clinical Institute [29]. he patients were enrolled consecutively, with 105 patients diagnosed with CLL and the remaining 18 diagnosed with SLL (***Figure 5.1.A***).

At the initiation of ibrutinib, 16 of them had no prior treatment (TN), while 107 had disease relapse or refractory disease (R/R) to previous therapy lines (***Figure 5.1.B***).

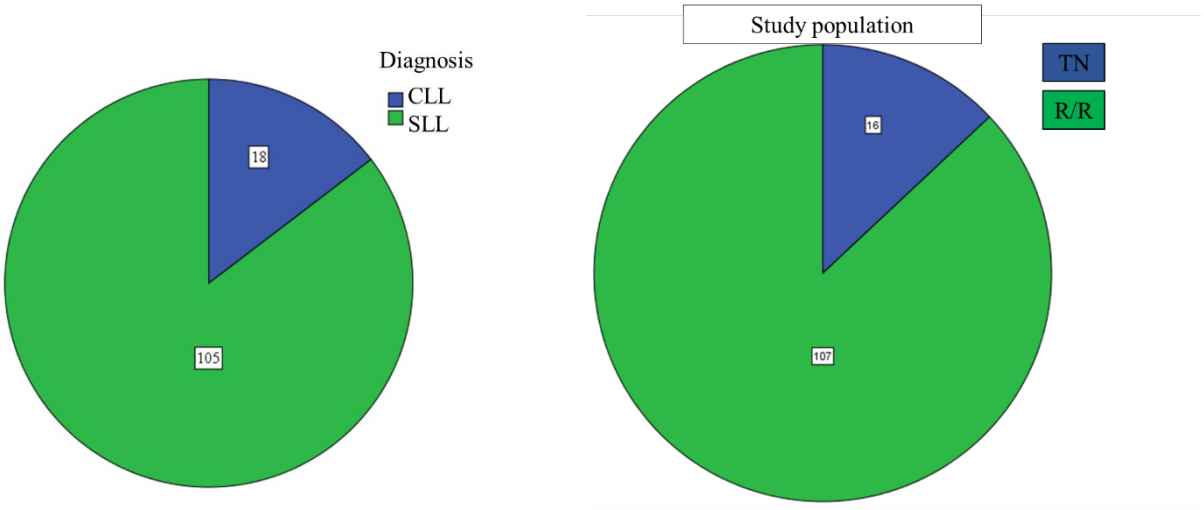

Figure 5.1.*A.* Study population: 18 (15%) patients were diagnosed with SLL, 105 (85%) with CLL. ***B.*** 16 (13%) patients were treatment naive (TN) while 107 (87%) had R/R CLL.

In summary, the main characteristics are as follows: the overall median age (IQR) was 65 (58-71) years, the majority of patients had a CIRS score of less than 6 points (89.4%), CLL staging showed that 47.7% and 36.6% of patients had Rai stage III/IV and Binet stage C disease, the molecular prognostic profile, including IGHV mutational status, del 17p, and TP53 abnormalities, was available for 35.8%, 42.3%, and 36.6% of patients, respectively.

Among the patients tested, 34.1% ($n = 42$), 10.6% ($n = 13$), and 12.2% ($n = 15$) were positive for IGHV unmutated, del 17p, and TP53, respectively.

5.3.2 Treatment efficacy - response rates and survival

The median follow-up (IQR) from the start of treatment was 37 (24-53) months for R/R CLL patients, and 19 (16-26) months for TN patients. At median follow-up, the median progression-free survival (PFS) for R/R patients was 50 months [95% CI: 42.3-57.7], while the median overall survival (OS) was not reached (NR) [95% CI: NR-NR]. The 24-month PFS and OS rates were 77.7% [95% CI: 69.5%-85.9%] and 87.9% [95% CI: 81.5%-94.4%], respectively. When ibrutinib was used as a first-line treatment, the median PFS and OS were not reached (NR) [95% CI: NR-NR]. The 24-month PFS rate was 75.8% [95% CI: 50.9%-100%], and the OS rate was NR [95% CI: NR-NR]. There were no statistically significant differences between TN and R/R regarding PFS and OS. The number of prior therapy lines before ibrutinib did not affect PFS and OS (***Figure 5.2***).

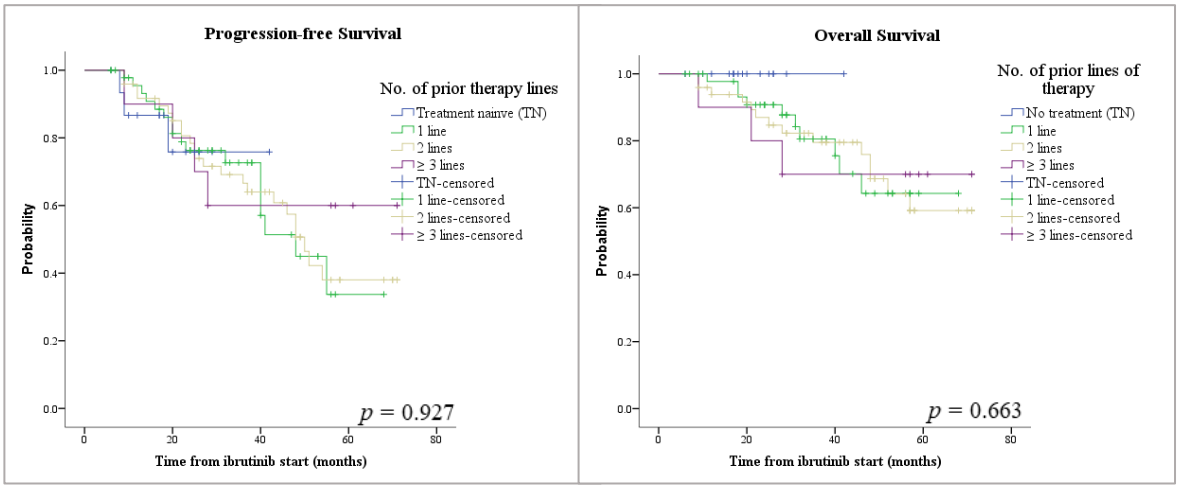

Figure 5.2 Progression-free survival (PFS) and overall survival (OS) according to the number of prior treatments received before ibrutinib (pre-I) in the entire study cohort.

Similarly, age (≥ 65 years) at the initiation of ibrutinib did not significantly influence PFS ($p = 0.6130$) and OS ($p = 0.489$). The subgroup analysis did not reveal any differences in PFS ($p = 0.073$) and OS ($p = 0.888$) between del 17p positive and negative patients. However, when patients with unknown FISH test results were included in the analysis, patients with del 17p had a hazard ratio (HR) for progression or death (PFS) of 3.26 [95% CI: 1.0-10.9]($p = 0.001$) (***Figure 5.3***).

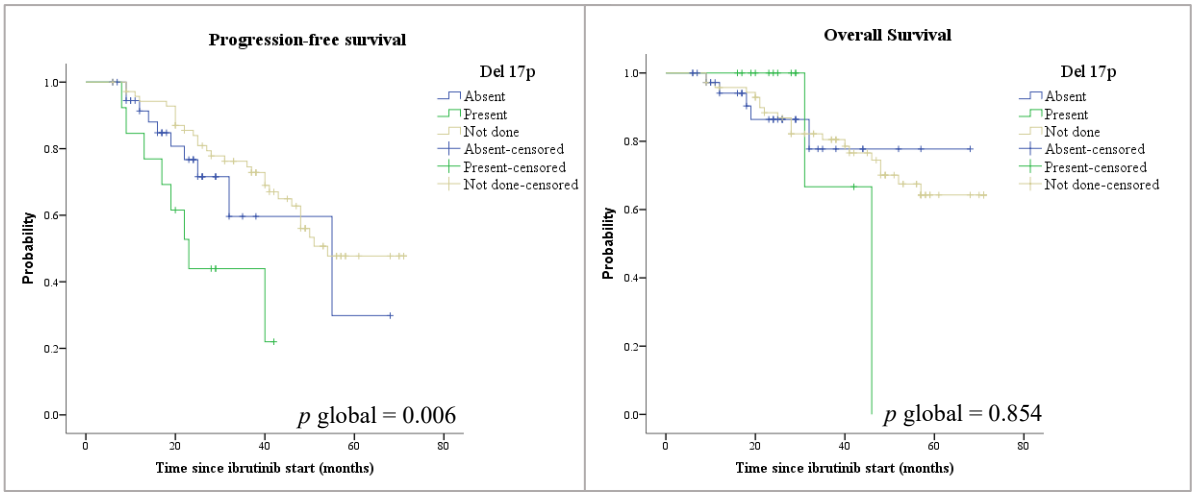

Figure 5.3 Kaplan–Meier estimated progression-free survival and overall survival curves according to the del 17p (present/absent/not done).

The median PFS and OS in the del 17p group were 23 months (95% CI: 17-40) and 46 months (95% CI: 41-46), respectively. Log-rank tests showed that PFS and OS were not significantly different in patients with IGHV non-mutated (NM) or TP53 mutations. After including patients with unavailable molecular markers, PFS became significantly shorter in those with IGHV NM compared to patients with unknown status ($p = 0.037$). On the other hand, patients expressing both markers, del 17p and TP53, had a shorter PFS with a 3.24-fold higher risk [95% CI: 1.06-9.94] of disease progression or death ($p = 0.028$), but OS was not affected. An ECOG status ≥ 2 significantly decreased both PFS and OS.

Univariate and multivariate Cox analyses on disease and patient characteristics at the initial time point showed an independent association with survival for LDH, ECOG PS, TILT, both in terms of PFS and OS, and for IGHV only with PFS.

5.3.3 The duration of ibrutinib therapy

Ibrutinib was administered for a median duration (IQR) of 32 months (20-48), 19 months (10-25), and 29 months (18-45) in the R/R, TN, and the entire study population groups, respectively. At median follow-up, the estimated Kaplan-Meier duration of ibrutinib treatment was 51 months [95% CI: 36.46-65.54] for R/R patients and NR [95% CI: NR-NR] for the TN group ($p = 0.386$).

54 (44%) patients permanently discontinued ibrutinib treatment after a median duration (IQR) of 22.5 months (12-41). The main reasons for the definitive discontinuation of ibrutinib were disease progression in CLL ($n = 21$), other causes/causes unrelated to treatment ($n = 14$), including 7 cases of death due to COVID-19, toxicity ($n = 11$), second cancer ($n = 6$), and Richter syndrome ($n = 2$) (***Figure 5.4***). Treatment discontinuation was

associated with a significant difference in overall survival (46 months vs NR, $p = 0.000$) between patients who discontinued and those who continued ibrutinib.

Toxicities leading to permanent discontinuation included atrial fibrillation ($n = 3$), infections ($n = 2$), bleeding ($n = 2$), reactivation of hepatitis B virus ($n = 2$), and ulcerative stomatitis ($n = 2$). Multifactorial logistic regression analysis found that an ECOG PS score ≥ 2 and a CIRS score ≥ 6 were independently associated with an increased risk of permanent discontinuation. Dose reductions were required in 35 (28.5%) patients. Ibrutinib was temporarily interrupted in 47 (38%) patients, with 36 (78%) requiring only a single interruption. Among the 21 patients (17%) who discontinued ibrutinib treatment due to disease progression, 16 received another line of therapy, including venetoclax in 13 cases.

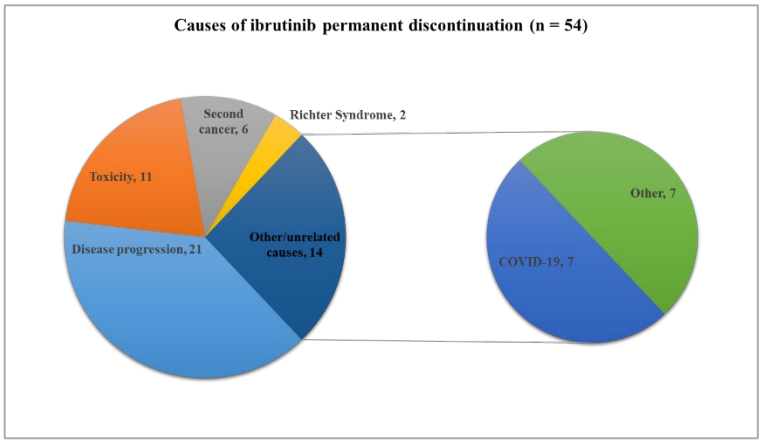

Figure 5.4 Causes for permanent discontinuation of ibrutinib therapy.

After a median follow-up of 9.5 months with salvage treatment, the estimated median PFS and OS were 18 [95% CI: 5.4-30.6] and 26 [95% CI: 2.1-49.9] months, respectively. Four out of the 11 patients who discontinued ibrutinib treatment due to toxicity required treatment after a median period of 7 months. Their median PFS was 37 months.

5.3.4 Adverse events

Adverse events (AEs) were recorded in 101 (82%) patients. The most common adverse reactions were infections (29.3%), anemia (27.6%), thrombocytopenia (26%), bleeding (24.4%), hypertension (23.6%), neutropenia (21.1%), and rashes.

5.4 Discussions

The present study explores the outcomes, tolerability, risk factors, and prognosis of patients with CLL who received ibrutinib outside of clinical trials. The study represents a retrospective analysis of real-world data from patients diagnosed with CLL or SLL and treated with ibrutinib between January 2016 and June 2021 at an experienced center in Romania, the Hematology and Bone Marrow Transplant Center of Fundeni Clinical Institute.

The study cohort included 123 patients, 16 of whom were in the frontline treatment group (TN group) and 107 with relapsed or refractory CLL (R/R group). Patients were followed for an average of 37 and 19 months in the R/R and TN subgroups, respectively. The clinical results of this study are in line with current reference studies [15,16,18,30,39,40]. However, TN patients had a slightly less favorable outcome [15,41].

The data from the presented study confirm that ibrutinib was beneficial for patients regardless of age and number of previous treatments. PFS and OS did not differ between patients in the frontline and relapsed settings, regardless of whether they had received one or multiple prior lines of treatment. Compared to clinical trials, the present study included younger patients with fewer comorbidities, less heavily pretreated individuals, but more patients with an ECOG performance status (PS) ≥ 2 and high-risk features (IGHV unmutated and del 17p). These patient characteristics reflect an early selection preference for ibrutinib in the clinical practice for treating CLL. Our datasets revealed a low level of genetic testing, especially in the relapsed/refractory situation. Therefore, the interpretation of the results should be done with caution. Although our data suggested a shorter median PFS of 23 (17-29) months and OS of 46 months in patients with del 17p, this observation did not reach statistical significance.

In our study, a poor ECOG PS (≥ 2) predicted shorter PFS and OS. This is consistent with the Italian experience demonstrating that a high ECOG PS predicts both inferior PFS and OS [42]. We could not demonstrate a difference in PFS or OS based on the CIRS score. However, a divergence between PFS and OS curves was observed after 50 months of treatment, confirming that the burden of comorbidities may increase and have an impact on outcomes with longer exposure to the drug. At a median follow-up of 32 months, the discontinuation rate of ibrutinib was 43.9% (54/123). Clinical trials and real-world data, including ours, have reported a wide range of discontinuation rates with variable overlap, largely due to differences in patient characteristics, median follow-up, treatment exposure, and management of adverse events.

In our study, 17% of patients permanently discontinued ibrutinib treatment due to CLL progression, while only 9% discontinued the medication due to toxicity. The reasons for this favorable tolerability may include younger age, fewer comorbidities, and fewer previous lines of therapy among the patients in the present study. Our findings are comparable to data from randomized clinical trials [15,16,18,30,40]. In contrast, real-world data either contradict [32,33,36,38,43], or confirm [34,35,42,44] a good tolerability profile.

In particular, our research highlighted a relatively high incidence (6.5%) of hepatitis B virus (HBV) reactivation associated with ibrutinib administration compared to other reports (1.9%) [45]. One-third of patients developed 63 infection episodes after a median treatment duration of 9 months (IQR 4-21). Eleven (9%) patients experienced a grade ≥ 3 infection. These findings are consistent with results obtained in a Spanish cohort [34], but differ from other reports that identified a higher overall incidence of infections (70%), with 50% of patients developing severe infections (grade ≥ 3) [15,36,38,46].

5.5 Conclusions

1. The present study aimed to investigate the outcomes, tolerability, risk factors, and prognosis of patients diagnosed with CLL/SLL and treated with ibrutinib in real-world settings. The results demonstrated the efficacy and safety of ibrutinib consistent with the main clinical studies.
2. The study cohort included 123 patients, of which 16 were in the frontline treatment group (TN group) and 107 had relapsed or refractory CLL (R/R group).
3. The median PFS in the R/R group, at a median follow-up of 37 months, was 50 months, while in the TN subgroup, the median PFS was not reached after a median follow-up of 19 months.
4. The study population included younger patients with fewer comorbidities and fewer previous treatments compared to the real-world CLL population.
5. The 24-month PFS rates were 77.7% for R/R patients and 75.8% for TN group patients. These results are consistent with other real-world studies.
6. TN group patients, predominantly consisting of high-risk patients (IGHV NM, del 17p/TP53), had worse outcomes compared to other studies.
7. Ibrutinib demonstrated therapeutic benefits regardless of age and the number of previous therapies administered to patients.
8. Patients with del 17p, TP53 mutation, and IGHV NM showed poorer outcomes in terms of time to disease progression and overall survival.
9. Poor ECOG performance status (≥ 2) was associated with shorter PFS and OS.
10. R/R patients with TILT under 24 months had worse outcomes (PFS and OS).
11. Discontinuations of ibrutinib treatment due to toxicity (9%) were less frequent than those caused by disease progression (17%), highlighting good tolerability.
12. The rate of discontinuation of ibrutinib treatment was similar to that reported in clinical trials (44%).

13. Younger age, fewer comorbidities, and fewer previous lines of treatment explain the favorable tolerability of ibrutinib treatment.
14. Patients who continued ibrutinib treatment showed superior net survival compared to those who discontinued the medication.
15. An increased incidence of hepatitis B virus reactivation associated with ibrutinib was identified.
16. An observed peculiarity in this study is the severe oral toxicity related to ibrutinib without neutropenia, which has not been highlighted in other experiences.

6. STUDY II: Chemo-immunotherapy versus ibrutinib as frontline therapy for 53 patients with Chronic Lymphocytic Leukemia

6.1 Working hypothesis and specific objectives

In Romania, starting from 2018, considerable efforts have been made to comply with Western standards regarding the diagnosis, prognosis, and treatment of CLL following the publication of the iwCLL guidelines. Integrating genetic prognostic markers and modern therapies into the management algorithm has posed a significant challenge for physicians. For example, 60% of CLL patients have unmutated IGHV status, which indicates a poorer prognosis compared to patients with mutated IGHV. For this category of patients, BTK inhibitors are the recommended first-line treatment option according to the guidelines. However, physicians had experience with the use of conventional immunochemotherapy, which yielded excellent short-term results. The new oral molecules promised improvements in outcomes but came with the price of indefinite administration and an unknown spectrum of adverse reactions. In these circumstances, physicians had to choose between using a new molecule proven to be effective, especially in high-risk patients, and traditional immunochemotherapy, considering the everyday patients, a general population quite different from those in clinical trials.

The objectives of this study were:

1. Comparing the effectiveness of targeted therapies with various chemoimmunotherapy regimens as first-line therapy in CLL in a sample of 53 patients.
2. Identifying patterns of therapeutic selection based on patient and disease characteristics.

3. Retrospective analysis of real-world data to aid in the development of more efficient strategies for selecting first-line therapy in CLL.

4. Improving the management of CLL patients and developing personalized therapeutic strategies based on real-time results from current practice.

6.2 Patients and method

6.2.1 Data collection

The research was conducted retrospectively using the printed and electronic medical records of patients diagnosed and treated at the Hematology and Bone Marrow Transplant Center of Fundeni Clinical Institute, during the period of June 1, 2018, to June 30, 2021. The study material consisted of 53 patients aged over 18 years diagnosed with CLL/SLL, who had not been previously treated and received specific first-line treatment during the mentioned 3-year period.

6.2.2 Methods were identical to those in the first study.

5.2.3 Statistical analysis was similar to that in the first study, and we used IBM SPSS Statistics 20.0.

6.3 Results

6.3.1 Study population

Fifty-three patients diagnosed with CLL/SLL who received first-line treatment between June 2018 and June 2021 at the Hematology and Bone Marrow Transplant Center of Fundeni Clinical Institute were included. The patients were divided into two main categories based on the treatment received: 16 (30%) received targeted therapy - ibrutinib, and 37 (70%) received immunochemotherapy (CIT) and were grouped into 5 therapeutic groups according to ***Figure 6.1***.

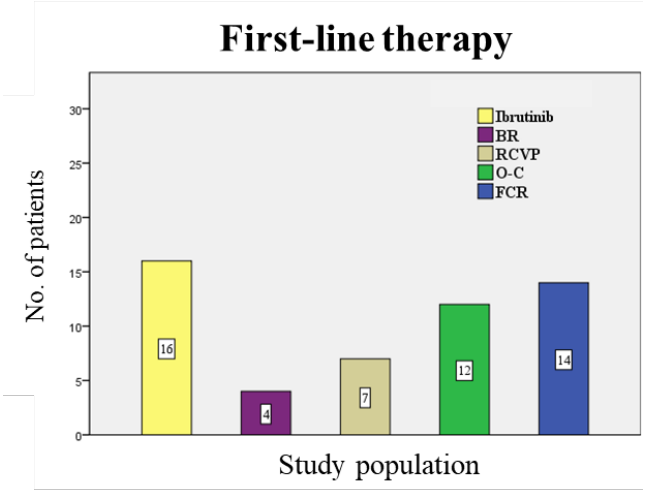

Figure 6.1 The distribution of patients according to the type of treatment received is as follows: IBR (*n* = 16), FCR (*n* = 14), O-C (*n* =12), RCVP (*n* = 7), BR *(n* = 4).

Demographic data

The median age (IQR) at the initiation of treatment for the entire study sample was 62 (53-68) years. There was a significant difference in the median ages among the 5 patient groups ($p = 0.009$). The median ages in the IBR, FCR, O-C, RCVP, and BR groups were 59, 57, 67, 55, and 71 years, respectively.

Performance status and comorbidities

Figure 6.2 shows the distribution of patients from the 5 groups based on ECOG PS and CIRS score. It can be observed that the majority of patients with good ECOG status and low CIRS score are in the FCR and IBR groups, while those with high CIRS score are predominant in the O-C and BR groups.

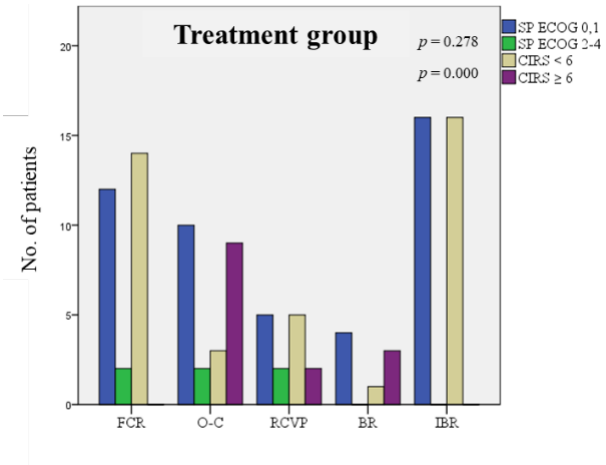

Figure 6.2 Patients distribution according to the ECOG PS and CIRS score.

Molecular and genetic profile

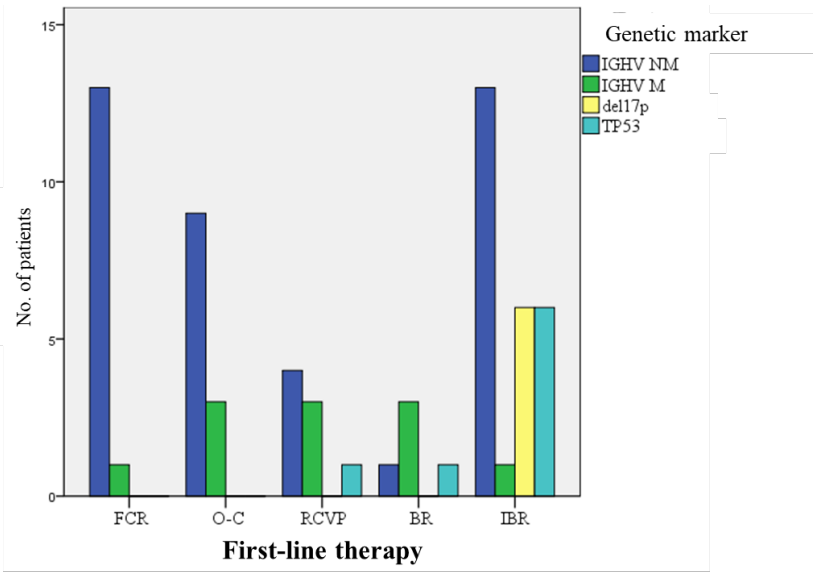

Figure 6.3 Patients distribution according to the genetic markers and type of therapy.

Out of the 51 patients investigated for IGHV status, 11 (21%) had mutations, while 76% (n = 40) had IGHV NM. FISH analysis for 17p deletion was performed on all patients, identifying a presence of 11% (n = 6). Regarding Sanger sequencing, it was performed on 52 patients, and TP53 mutation was identified in 15% (n = 8). In our study, we observed a significant association between IGHV status (p = 0.028), del 17p (p = 0.004), and the type of treatment administered (***Figure 6.3***).

6.2.3 Results – response rates and survival

The median follow-up for this study was 25 (17-30) months.

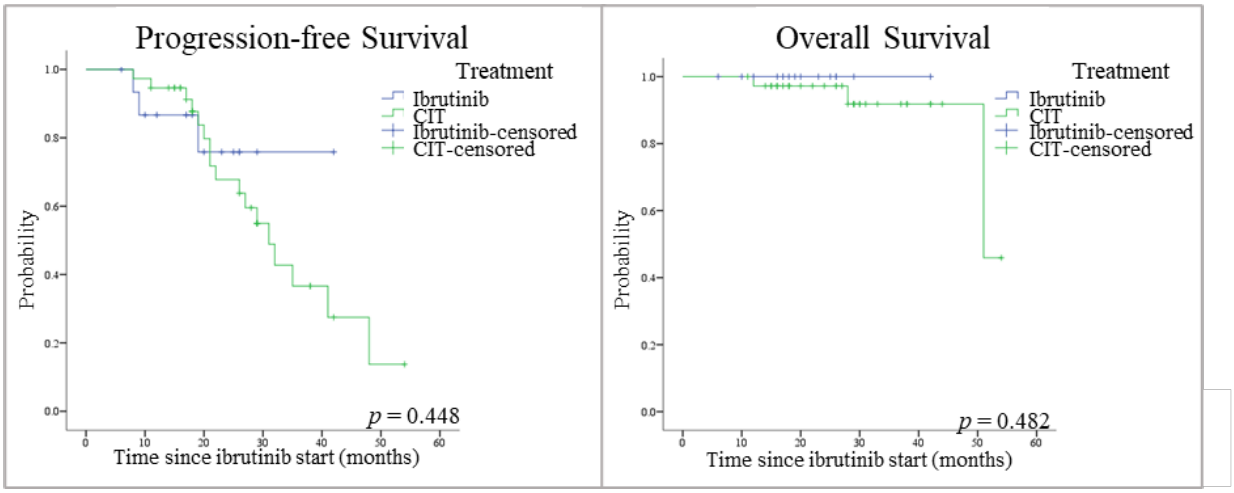

Figure 6.4 Kaplan-Meier plots showing the estimated PFS and OS for ibrutinib versus chemo-immunotherapy treated CLL patients.

The median PFS in the ibrutinib treated group was NR, while in the group receiving various CIT regimens, it was 31 [95% CI: 25-37] months. Similarly, the median OS was not reached in the ibrutinib treated group, but a median OS of 51 [95% CI: 29-82] months was estimated in the CIT group (according to ***Figure 6.4***). Patients treated with FCR experienced a median PFS of 48 (95% CI: 31-65) months, those treated with O-C had 21 (95% CI: 3-39) months of median PFS, and for RCVP treatment, the median PFS was 19 (95% CI: 10-28) months. The differences were statistically significant with p = 0.048.

6.3.4 Adverse events

AEs were more frequent in patient groups receiving FCR and RCVP regimens, as well as ibrutinib. The most common AEs were hematologic toxicities and infections. Severe AEs were observed in 30% (n = 16) of patients. The most frequent grade ≥ 3 AEs were neutropenia and infections. Patients treated with FCR exhibited a higher frequency of severe neutropenia and infections. At the same time, the RCVP regimen had a similar rate of grade ≥ 3 neutropenia compared to FCR, but severe infections were not as frequent.

6.4 Discussions

In this study, we compared the characteristics, prognosis, outcomes, and survival of patients with CLL in relation to five different frontline therapies. The age of patients had a significant influence on therapy selection. Younger patients received FCR (57 years) and IBR (59 years), while older patients predominated in the O-C (67 years) and BR (71 years) groups. Most patients with good ECOG performance status and low CIRS score were found in the FCR and IBR groups, while those with higher CIRS score predominated in the O-C and BR groups.

Patients with IGHV mutations (n = 11) were treated with CIT (O-C, BR, RCVP), while those with IGHV unmutated (n = 40) received FCR or ibrutinib. Patients with del 17p (n = 6) were exclusively treated with frontline ibrutinib, while the majority of those with TP53 mutation (n = 8) received targeted therapy. These findings highlight the preference of physicians for targeted therapy with ibrutinib or the FCR regimen in patients with high-risk factors. At the same time, it is noteworthy that patients with IGHV mutations, who could benefit the most from FCR according to clinical trial data [13,47], were treated with O-C, BR, or RCVP.

Compared to previously published studies, the study sample included younger patients with good physical condition and fewer comorbidities, a smaller number of patients with advanced disease, but more patients with unfavorable prognosis. Patients treated with ibrutinib had a longer time to progression or death compared to those treated with CIT. Comparative analysis among the five patient groups found unfavorable outcomes with RCVP, with lower values compared to the FCR regimen (p=0.001 for PFS and p=0.034 for OS). This finding confirms previous conclusions that CVP/CHOP is inferior to fludarabine in terms of efficacy [48].

AEs were more frequent in patients treated with ibrutinib (63%) compared to those treated with CIT (51%). The most common AEs were neutropenia and infections, especially in the FCR group. The most frequent severe AEs were again neutropenia and infections, with the FCR and RCVP groups being most affected in this regard.

Conclusions

1. This study represents a comparative analysis of the efficacy and safety of ibrutinib treatment with various CIT regimens used as frontline therapy in real-world clinical practice for CLL patients. The aim is to evaluate the outcomes achieved in a real clinical setting and discuss the benefits and limitations of different therapeutic approaches in CLL.

2. The research included 53 CLL/SLL patients treated with frontline therapy over a three-year period at the Hematology and Bone Marrow Transplant Center of the Fundeni Clinical Institute.

3. Patients received either ibrutinib (30%) or CIT (70%) and were categorized into five treatment groups: IBR (n = 16), FCR (n = 14), O-C (n = 12), RCVP (n = 7), and BR (n = 4).

4. Patient age influenced therapy selection, with younger patients receiving ibrutinib or FCR, while older patients received O-C or BR.

5. Initial therapy was not determined by clinical stage or tumor burden but by ECOG performance status and comorbidities.

6. We observed physician preference for targeted therapy with ibrutinib or the FCR regimen in patients with IGHV unmutated and del 17p. Meanwhile, patients with IGHV mutations were treated with different types of CIT, such as O-C, BR, or RCVP, rather than FCR.

7. Overall, our study sample included younger patients with good physical condition and fewer comorbidities, fewer patients with advanced disease stages, but a higher percentage with unfavorable prognosis compared to published population studies.

8. Patients treated with ibrutinib had a longer time to progression or death compared to those treated with CIT.

9. At a median follow-up of 25 months, patients treated with FCR had a median PFS of 48 months, those with O-C had 21 months, and for the RCVP group, it was 19 months. Median PFS was not reached in the groups that received ibrutinib or BR.

10. Comparative analysis showed unfavorable outcomes with RCVP compared to the FCR regimen, confirming that CVP/CHOP is inferior to fludarabine-based therapy.

11. AEs were more frequent in patients treated with ibrutinib (63%) compared to those treated with CIT (51%), but the majority of severe AEs (grade ≥ 3) were recorded in the FCR and RCVP groups, with neutropenia and infections being the most common.

12. One patient treated with FCR developed pure red cell aplasia during an acute Parvovirus B19 infection, leading to treatment discontinuation and subsequent CLL relapse.

7. STUDY III: The role of allogeneic stem cell transplant in high-risk Chronic Lymphocytic Leukemia patients [49]

7.1 Working hypothesis and specific objectives

Among the numerous prognostic and predictive biomarkers identified in previous decades, TP53 aberrations remain unquestionably the most important genetic lesion in CLL. The TP53 locus encodes the tumor suppressor protein p53, which plays a key role in cell division, apoptosis, and genomic stability. TP53 signaling can be affected by the deletion of the TP53 gene at the chromosomal locus 17p13.1, known as del 17p, or by genetic lesions including nonsense and missense mutations, deletions, insertions, or mutations at the binding site locus. Cumulatively, these aberrations are present in 4-8% of all CLL patients at diagnosis, 10% at the start of frontline therapy, and 30-40% in R/R CLL patients previously treated with chemotherapy-immunotherapy [50]. In a clinical context and for the sake of simplicity, the terms "del 17p" and "TP53 abnormalities" are used interchangeably.

TP53 abnormality remains the only predictive biomarker with consensus recommendations to be detected prior to treatment initiation in all patients due to clear evidence of chemotherapy resistance. CIT provides no benefit to patients with TP53 abnormalities as PFS and OS durations are short [14]. Targeted therapies have significantly improved the survival of patients with TP53 mutations, and currently, new molecular therapies represent the gold standard for this patient subgroup. However, no treatment has succeeded in completely reversing the negative prognosis associated with TP53 abnormalities.

Traditionally, allogeneic stem cell transplantation (allo-SCT) is considered the treatment option for all high-risk CLL patients eligible for transplantation at the time of R/R[51] being regarded as the only curative therapy available. However, given the emergence of highly effective targeted agents, its role needs to be reevaluated.

The present study [49] aims to analyze several aspects:

1. Comparison between allo-SCT and targeted therapeutic agents in high-risk CLL.
2. The optimal timing for allo-SCT and management of relapse after transplant.
3. Optimum therapy sequencing and strategies of achieving deep remission pre-SCT.
4. Current relevance of CIT in the context of the introduction of new therapeutic molecules and importance of including prognostic biomarkers in therapeutic strategies.

7.2 Material and method

Our center experience regarding patients with CLL/SLL who underwent hematopoietic stem cell transplantation in the Hematology and Bone Marrow Transplant Department at Fundeni Clinical Institute, the largest university center and tertiary referral hospital in Romania, is limited to two cases.

7.2.1 Case presentation I

We analyzed the case of a 40-year-old female patient diagnosed with SLL in January 2012 with the following treatment journey (***Figure 7.1):***

Figure 7.1 The sequence of treatment.

7.2.2 Case presentation II

The study also investigated the case of a 46-year-old man diagnosed with CLL in 2008. After several lines of treatment, including FCR-type CIT, CHOP, and alemtuzumab, the disease relapsed. We identified the presence of 17p deletion, and considering the relapse after three therapeutic lines, the decision was made to perform an allo-SCT from a compatible familial donor in March 2013. The patient achieved complete remission but developed severe complications, such as GVHD, and recurrent infections, which eventually led to his death three years later.

7.3 Discussions

The two cases presented represent specific, yet similar situations of CLL. The high risk and unfavorable prognosis derive from two characteristics: early relapse after purine analog-based therapy and the presence of del 17p. It is worth mentioning that the latter was detected 2 years after the initial diagnosis and following CIT. This delay can have significant consequences since del 17p can be intrinsically linked to CLL, where the administration of CIT is contraindicated, or chemotherapy itself can induce the acquisition of del 17p [52]. Indeed, the reported incidence of high-risk genetic abnormalities approaches 10% at the time of diagnosis and increases up to 50% at relapse [53,54]. A short-lived remission after FCR therapy suggests that del 17p may have been present from the beginning in the presented case. Timely confirmation of del 17p is crucial as it allows for the avoidance of unnecessary and potentially harmful treatments (e.g., FCR) and the prompt initiation of targeted therapy (e.g., BCR or BCL-2 inhibitors). Since the first hierarchical prognostic model based on FISH

analysis with four probes (i.e., del 17p, del 11q, del 13q, and trisomy 12), studies have established that del 17p is associated with the most unfavorable prognosis, with a median survival of less than 3 years [11,53,55].

Currently, allo-SCT remains the only potentially curative treatment option for CLL [51]. CLL is highly sensitive to graft-versus-leukemia (GVL) effects, achieving long-term remissions after reduced-intensity conditioning (RIC) or non-myeloablative allo-SCT [56-60]. Furthermore, the results have been similar in high-risk patients, indicating that allo-SCT with RIC can counteract the negative prognostic impact of del 17p or FCR resistance [56-60]. Furthermore, the results have been similar in high-risk patients, indicating that allo-SCT with RIC can counteract the negative prognostic impact of del 17p or FCR resistance [51,57,59].

The second case confirms what studies have shown before the emergence of new targeted therapies: in the case of high-risk CLL patients, allo-SCT leads to long-term survival with negative MRD in approximately 50% of patients, regardless of their genomic risk profile [57]. Despite long-term disease control and a low rate of early death, overall survival (OS; 62% at 2 years, 35% at 10 years) and EFS (49% at 2 years, 28% at 10 years) associated with allo-SCT decrease over time, while non-relapse mortality (NRM) increases to 40% at 10 years[61]. At the same time, long-term results show that new targeted therapies seem to provide high response rates and sustained tolerability, both in previously untreated CLL patients and those with R/R disease [25,62,63]. Allo-SCT needs to be weighed against new therapies, taking into consideration the risks associated with the procedure, comorbidities, disease characteristics, and high-risk factors, potential side effects, remission rates, and duration of response. An assessment of the benefit-risk ratio is always mandatory.

Several studies have investigated the use of ibrutinib for treating relapse after allo-SCT and have reported similar efficacy and safety profiles to those in high-risk CLL without transplantation [64,65]. Venetoclax, alone and then in combination with rituximab, marked the second stage of salvage therapy after transplant failure. Symptom improvement was rapid, but grade four pancitopenia occurred, as expected considering the published data [66]. Therefore, the doses were adjusted, leading to therapeutic underdosing and frequent interruptions.

7.4 Conclusions

1. The case studies highlight the high risk and unfavorable prognosis of CLL in the case of early relapse after CIT and the presence of 17p deletion.
2. Late detection of 17p deletion can have significant consequences as the effectiveness of chemotherapy is reduced in these cases.

3. Allo-SCT remains a potentially curative treatment option for CLL.

4. Administration of ibrutinib in a patient with R/R CLL and 17p deletion resulted in a good partial remission, and the treatment remained effective for 4 years.

5. Our first case aligns with literature observations regarding early relapse after allo-SCT, which appears to be more frequent in patients previously treated with ibrutinib.

6. Evaluation of the benefit-risk ratio is essential in the decision to perform allo-SCT, considering the individual patient characteristics and available targeted therapies.

7. The management of allo-SCT failure is not standardized and requires new strategies.

8. The use of DLI in cases of relapse or mixed chimerism post-transplant shows potential, but the evidence is anecdotal.

9. Venetoclax therapy represents an alternative salvage therapy after transplant failure but may involve side effects such as pancitopenia.

12. The two cases provide complementary information and reflect the results of allo-SCT in high-risk CLL: a 50% probability of achieving remission and a 50% risk of developing post-transplant complications.

8. Conclusions and personal contributions [29,49]

The three presented studies converge to create a monograph on the management of CLL in routine clinical practice.

The aim of this thesis is to provide useful, practical, and real-time information about the general population with CLL and address important clinical issues beyond standardized clinical research. This work represents the only comprehensive analysis conducted in Romania regarding recent data on the management of patients with CLL. The analyzed patient cohort (123 in Study I and 53 in Study II) is impressive, demonstrating that our center is a reference point nationally and internationally for the treatment and management of CLL patients.

These studies, through their results, contribute to a better understanding of essential aspects related to:

1. Risk stratification of patients: the utility of prognostic markers - IGHV, 17p deletion, TP53 - before treatment and optimization of therapies based on risk category (***Chapters 4, 5, 6, 7***). Age per se is not a predictor, but ECOG status and CIRS score are factors that can predict survival and tolerability to ibrutinib (***Chapter 5***).

2. Therapy selection: ibrutinib is preferred early in the disease course for young high-risk patients without comorbidities (***Chapters 5, 6, 7***), FCR-type CIT for young patients with standard risk, BR and O-C for elderly or comorbid patients without high-risk genetic abnormalities (***Chapter 6***).
3. Management of adverse events: patients treated with ibrutinib frequently experience manageable adverse events, with infections being the most common and concerning, while patients undergoing CIT more frequently experience severe adverse events including neutropenia and infections (***Chapters 5 and 6***).
4. Treatment sequencing: although the role of CIT has significantly diminished with targeted therapies, it is still used in practice; the presented data demonstrate the long-term superiority of ibrutinib regardless of age or number of previous therapies, and even if the results are poorer in patients with 17p deletion, it significantly improves their prognosis (***Chapters 6 and 7***).
5. Personalization of therapy: currently, there are multiple and varied therapeutic options, including ibrutinib as a targeted therapy, along with BCL-2 antagonists. The strategy is determined together with the patient, taking into account their preferences, physical condition, associated pathologies, and the risk factors based on genetic profiling (***Chapter 6***).
6. Role of allo-SCT: currently, targeted therapies are the preferred treatment in most cases, both in first-line and relapsed settings. Allo-SCT is considered a secondary option, typically used in cases of dual refractoriness after new molecules (BCR inhibitors plus anti-BCL-2) and requires good disease control pre-transplant (***Chapter 7***).
7. CLL with high risk continues to present an unfavorable prognosis and remains an unresolved issue despite therapeutic advancements and allo-SCT **(*Chapters 5 and 7)***.

The results highlighted in the presented work indicate that genetic testing for identifying prognostic markers is recommended for all CLL patients to guide disease management and predict survival and disease progression. This underscores the importance of identifying prognostic markers in CLL

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SCIENTIFIC PUBLICATIONS

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   The chapter from which the article was conceived: 4 and 5 (page 29-80)

2. **Moldovianu, A.M**.; Crisan, A.M.; Varady, Z.; Coriu, D. **The Difficult-to-Treat del 17 p Patient-A Case Report in Chronic Lymphocytic Leukemia**. Medicina (Kaunas) 2021, 58, doi:10.3390/medicina58010033.
   LINK: https://www.mdpi.com/1648-9144/58/1/33
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   The chapter from which the article was conceived: 7 (page 113-124).