Thesis

Setting clinical parameters in association to pathogenesis and prognosis of chronic lymphatic leukemia

Submitted by

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Graz, 01. Dezember 2025

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Graz, 01. Dezember 2025 Florian Johannes Schubert, m.p.

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Zusammenfassung in Deutsch

Einleitung: Die chronisch lymphatische Leukämie (CLL) stellt die häufigste Form der Leukämie dar. Trotz einer im Durchschnitt hohen 5-Jahres-Überlebensrate zeigen Betroffene ein stark unterschiedliches Krankheitsverhalten, das von einer Vielzahl klinischer, zytologischer, histopathologischer und genetischer Faktoren beeinflusst wird.
Ziel dieser retrospektiven Studie war die Analyse klinischer und genetischer Parameter in einer umfangreichen, gut dokumentierten Kohorte von CLL-Patient*innen, die an der Abteilung für Hämatologie der Medizinischen Universität Graz behandelt wurden.

Material und Methoden: Erfasst wurden klinische Daten wie die Anzahl der befallenen Lymphknotenregionen oder das Auftreten einer Richter-Transformation (RT). Ergänzend wurden genetische Veränderungen untersucht, darunter Deletionen von 17p und 11q, Trisomie 21 sowie der Mutationsstatus des Immunglobulin-Schwerkettenvariablen-Gens (IgHV). Die Auswertung umfasste insgesamt 124 Patient*innen, von denen alle zum Diagnosezeitpunkt bereits eine Lymphknoteninfiltration aufwiesen.

Ergebnisse: Von den 124 untersuchten Personen benötigten 88 eine Behandlung, entweder in Form einer Immunchemotherapie oder einer zielgerichteten Therapie (z. B. mit Ibrutinib oder Venetoclax). Bei 24 Patient*innen kam es im Krankheitsverlauf zu einer RT. Eine hohe Anzahl (>2) betroffener Lymphknotenstationen zum Diagnosezeitpunkt sowie das spätere Auftreten einer RT standen mit einem verkürzten Zeitraum bis zur Therapienotwendigkeit in Zusammenhang. Zudem zeigten sich unmutierte IGHV-Gene, das gleichzeitige Vorliegen von del17p und del11q sowie das Auftreten einer RT als signifikant ungünstige Prognosefaktoren für das Gesamtüberleben. In der Subgruppe der Patient*innen mit Entwicklung einer Richter Transformation traten eine hohe Lymphknotenbeteiligung (>2) und del17p häufiger auf, als bei Patient*innen ohne RT. Weiterführende Analysen ergaben, dass neutropenische Infektionen bei etwa der Hälfte der Patient*innen sowohl unter Immunchemotherapie als auch unter

zielgerichteter Therapie auftraten. Kardiovaskuläre Nebenwirkungen wurden bei 8,9 % der mit Ibrutinib behandelten Personen beobachtet.

Schlussfolgerung: Die Ergebnisse verdeutlichen, dass insbesondere die Anzahl betroffener Lymphknotenregionen und der del17p-Status entscheidenden Einfluss auf Krankheitsverlauf, Auftreten einer Richter-Transformation und Überlebenswahrscheinlichkeit bei CLL haben. Die Kombination dieser Parameter könnte zur Entwicklung neuer Modelle zur Risikostratifizierung beitragen.

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Abstract in English

Introduction: Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia. Although the average five-year survival rate is relatively high, disease progression varies widely among patients and is influenced by numerous clinical, cytological, histopathological, and genetic factors.
This retrospective study aimed to evaluate both clinical and molecularbiological parameters in a comprehensive, well-documented cohort of CLL patients treated at the Division of Hematology, Medical University of Graz.

Materials and Methods: Materials and methods: Clinical data such as the number of affected lymph node regions or the occurrence of Richter transformation (RT) were recorded. In addition, genetic changes were examined, including deletions of 17p and 11q, trisomy 21, and the mutation status of the immunoglobulin heavy chain variable gene (IgHV). The evaluation included a total of 124 patients, all of whom already had lymph node infiltration at the time of diagnosis.

Results: Among the 124 patients, 88 required treatment with either immunochemotherapy or targeted agents, such as Ibrutinib or Venetoclax, and 24 developed RT during the course of their disease. A higher number of affected lymph node regions (>2) at diagnosis, as well as subsequent development of RT, were associated with a shorter time to treatment initiation. Furthermore, unmutated IGHV genes, the co-occurrence of del17p and del11q, and the presence of RT were all linked to reduced overall survival. Patients who developed RT at any time during the disease showed >2 affected lymph node regions and a del17p mutation more frequently compared to those without RT. Additional analysis revealed that neutropenia and infections occurred at similar rates (~50%) in patients treated with immunochemotherapy or targeted therapies. Cardiac adverse events were observed in 8,9% of patients receiving Ibrutinib.

Conclusion: Our findings suggest that specific clinical and genetic parameters—particularly the number of involved lymph node regions and del17p status—have a substantial impact on disease progression, Richter transformation, and overall

survival in CLL. The integration of these factors may facilitate the development of refined risk stratification models for CLL management.

Already published

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List of Abbreviations

CLL	chronic lymphocytic leukemia
BCR	B-cell receptor
VDJ	variable diversity joining
Ig	immunglobulin
GC	germinal center
SHM	somatic hypermutation
NHL	Non-Hodgkin lymphoma
CSR	class switch recombination
AID	activation-induced cytidine deaminase
DLBCL	Diffuse large B-cell lymphoma
CD	cluster of differentiation
IgHV	immunoglobulin heavy variable
MBL	monoclonal B-cell lymphocytosis
CK	complex cariotype
RT	Richter transformation
iwCLL	International Workshop on Chronic Lymphocytic Leukemia
FISH	fluorescence in situ hybridization
NGS	Next generation sequencing
BTK	Bruton tyrosine kinase
FCR	Combination of fludarabine cyclophosphamide rituximab
PFS	progression free survival
GFR	glomerular filtration rate
CR	complete remission
PR	partial remission
PD	progressive disease
SD	stable disease
BCL2	B-cell leukemia/lymphoma 2
BSC	Best supportive care
OS	overall survival
CKD	chronic kidney disease

DLBCL-RT	Diffuse large B-cell lymphoma type Richter transformation
R-CHOP	rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone
R-EPOCH	rituximab, etoposide, prednisone, vincristine, cyclophosphamide, hxdroxydaunorubicine
PET-CT	positron emission tomography
M-CLL	mutated CLL
U-CLL	unmutated CLL
RB	Rituximab -Bendamustin
IR	ibrutinib rituximab

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1. Introduction

1.1. Definition.

Chronic lymphatic leukemia (CLL) is a non-Hodgkin lymphoma defined by the accumulation of small and mature appearing lymphocytes in various human tissues. These include the bone marrow, blood and lymphoid tissue(1). Due to the latest WHO classification scheme, there is no distinction between CLL and small lymphocytic lymphoma(2).
Among adults in western world chronic lymphocytic leukemia represents the most common form of leukemia and one of the most frequently arising forms of non Hodgkin lymphoma with an incidence rate of 4 -6/100.000 per year and a median age at diagnosis of 72 years. Despite the incident growing with age, the percentage of younger people being diagnosed with CLL has increased in recent decades. Still, over 70% of patients being diagnosed with CLL are older than 65 years of age. In terms of gender, men are more often diagnosed with CLL than women, at a ratio of 1,5-2:1.(3, 4)

1.2. Pathogenesis of CLL

1.2.1 Normal B-cell development

The development of a B cell begins in the primary lymphoid tissue and continues in the secondary lymphoid tissue, resulting in a highly differentiated plasma cell that produces antibodies.(5) In the human bone marrow, progenitor B-cells arise from hematopoietic stem cells.(6) Only the pre-B cell that develops from the progenitor cell expresses the pre-B-cell receptor on its surface.(5) Finally, immature B-cells that already possess a B-cell receptor (BCR) leave the bone marrow and head towards secondary lymphoid tissue for further maturing. During the development in the bone marrow, an important progress of the BCR takes place.(6) The BCR is composed of two subunits, a surface immunoglobulin (Ig) and a signalling subunit. The Ig is made up of four protein chains, two heavy

chains and two light chains held together by a disulfide bond. The surface Ig has the task of binding antigens. Thus, a very high variability is necessary. This is achieved through VDJ rearrangement. The heavy and light chains of the IG consist of constant and variable regions.(6, 7) The genes that define the variable regions are named variable (V), diversity (D) and joining (J) genes, whereas on the variable regions of light chains there are only V and J genes. Rearrangement of VDJ genes during the B-cell development in the bone marrow achieves an enormous diversity of antigen binding BCRs.(8)

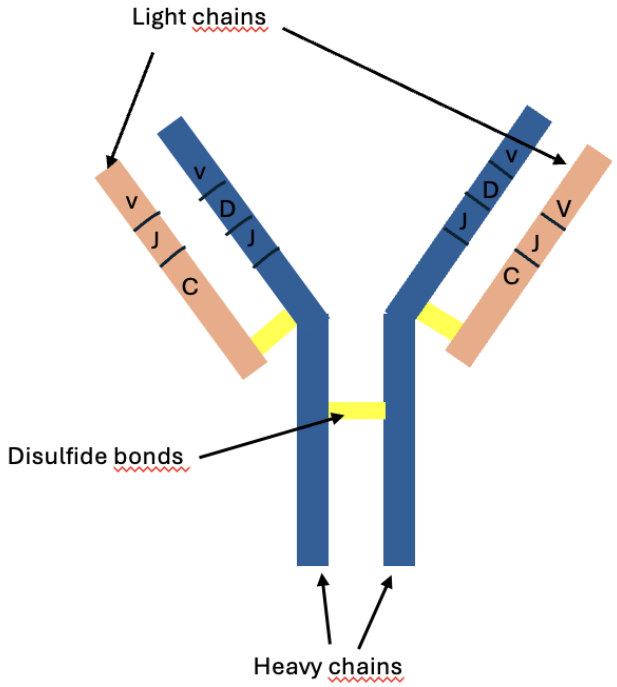

Figure 1: normal B-cell receptor

After leaving the bone marrow, immature B-cells enter secondary lymphatic tissues, such as the spleen and lymph nodes.

Mature naive B-cells then migrate to the border between the T-cell zone and the B-cell follicle. There, antigens are presented by T-cells. Depending on the affinity of the BCR to the antigen, either a germinal centre response or an extrafollicular response is triggered. The extrafollicular response induces the development and differentiation into short-living plasma cells with lower affinity. Those entering the germinal center (GC) undergo development into highly differentiated, long-living plasma cells.(5, 9)

Further genetic remodelling occurs here through somatic hypermutation (SHM) and class switch recombination (CSR).(5, 8) The class switch reaction describes the transition of the surface Ig into a different antibody class through a deletion recombination event induced by a specific enzyme, which is known as activation-induced cytidine deaminase (AID). This results in an alteration of the Ig heavy chain. (5, 8-10) AID also initiates SHM. SHM describes the creation of mutation in the variable domains of the surface Ig. This especially adjust the affinity for the fitting antigen.(8) However, SHM and CSR are not only fundamentally important components of normal B-cell development, but are also associated with the development of lymphatic neoplasms. So-called off-target mutations, which are unintended mutations outside the Ig genes, can promote the evolution of malignant lymphomas.(11)

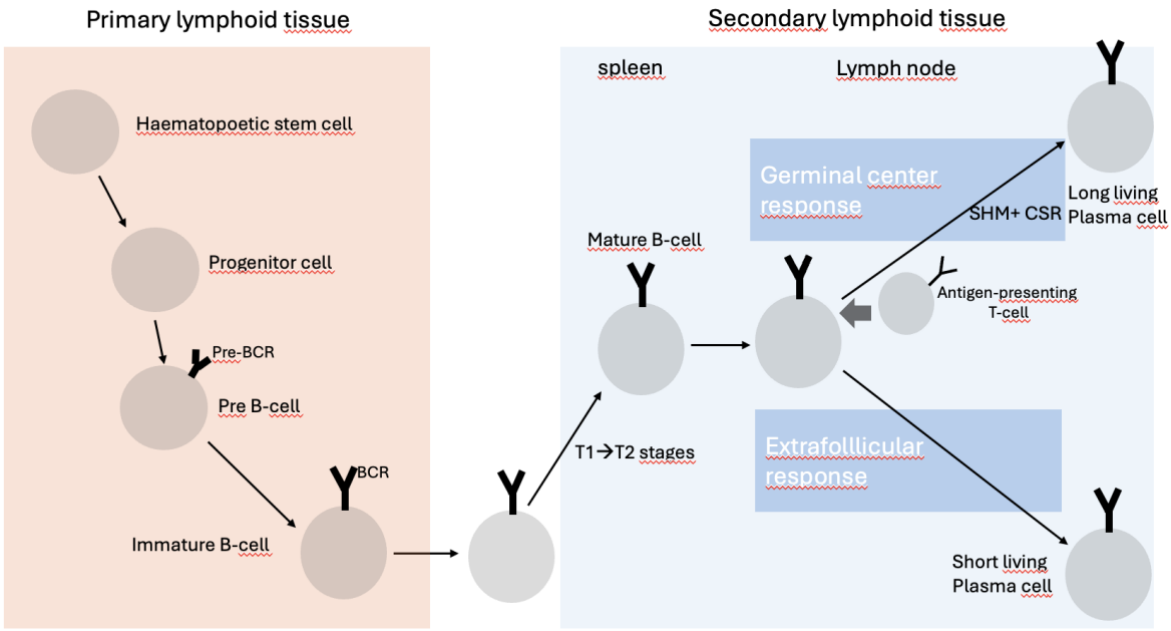

Figure 2: Overview of B-cell developement

1.2.2 Malignant lymphomas

'Neoplasms of the lymphatic system, also known as lymphomas, can arise from all stages of development, both B and T lymphocytes. They are generally divided into two groups: Hodgkin lymphoma and non-Hodgkin lymphoma (NHL). While

Hodgkin's lymphomas have a very good prognosis in most cases—the cure rate is approximately 80%—NHLs are significantly more heterogeneous in terms of outcome and disease development and are known to spread to extranodal sites more frequently. (12, 13) The most common form of NHL in Western societies is diffuse large B-cell lymphoma (DLBCL), followed by other common malignancies like marginal zone lymphoma, follicular lymphoma and chronic lymphatic leukemia.(14, 15)’1

CLL is defined by the clonal accumulation of mature CD5+ B-cells. CLL cells, like normal and functioning B-cells, also express the BCR. Antigen selection and stimulation seem to have an important effect on the development, expansion and disease progression of the disease. This is clearly indicated by the fact that certain properties of the BCR clearly influence the course of the disease. Of particular importance is the mutational status of the immunoglobulin heavy chain variable region genes (IgHV).(16, 17)
By looking into possible gene rearrangements of the IG gene in CLL B-cells compared to the germline sequence, it turned out, that around 50% of cases somatic mutations occurred in IgHV genes.(18) It was shown that patients with these somatic mutation in IgHV genes had a clearly less malignant disease and thus a longer survival rate.(19)

1.2.3 Monoclonal B-cell lymphocytosis (MBL)

MBL is defined as the presence of monoclonal B-cells at a count of less than 5000 per cubic millimeter. Besides, there must be no other signs of a proliferative disease, such as lymphadenopathy or organomegaly. Although this type of lymphocytosis does not warrant therapy, it is considered a precursor to CLL. One to two percent of patients with MBL develop CLL each year.(20, 21)

1 Translated using AI

1.2.4 Genomic abberations

Additionally, CLL cells often exhibit chromosomal abnormalities.
Modern molecular cytogenetic methods can discover such genomic aberrations in over 80 percent of CLL patients. Deletion 13q14 was found most often, in around 50 to 60 percent of patients, followed by deletion 11q and 12q trisomy.
There is a clear and strong influence of these genomic abberations on survival. Patients with del17p had the worst outcome.(17, 22) One reason for this is that the important tumor suppressor gene p53 is located in band 17p13. The greater part of cases with loss of the p53 gene are attributable to a large 17p deletion.(23) Patients with del11q and trisomy 12q also showed a worse survival outcomes than those with a normal karyotype.(22)
If there are three or more genomic aberrations, this is referred to as a complex karyotype (CK).(24) CK is also an independent prognostic marker associated with poorer survival, shorter time to treatment and a worse response to treatment, including ibrutinib.(25, 26) CK is said to be present in 11-18% of CLL patients who have not undergone treatment so far, but it is even present in up to 40% of CLL patients with a refractory disease.(27)

1.3 Richter transformation (RT)

Richter transformation describes the development of CLL into a more aggressive lymphoma through clonal evolution, which is most frequently a diffuse large B-cell lymphoma.(28) Approximately 2 to 10% of CLL patients undergo RT. RT is usually first recognized by a sudden clinical deterioration of the patient. Often, the first signs of development are the appearance of new lymphadenopathy, night sweats or a sudden increase in serum lactate dehydrogenase.(29) Patients who have undergone RT are known to have an overall poorer outcome due to a faster disease progression and restrained therapeutic strategies. There have been found a number of genetic aberrations that seem to have an important influence on the development of RT.(30) The loss of TP53 and CDKN2A seem to be two of the most important ones as wells as the presence of trisomy 12.(31)

1.4 Risk factors

Despite no causative factors for the development of CLL being determined so far, it is verified that a genetic predisposition exists and leads to a higher risk of falling ill with CLL.(3) The risk for individuals with a family history of CLL is 8,5 times higher compared to individuals who do not have relatives with CLL(1). Additionally, lifestyle factors appear to influence the risk as well. People who had spent a longer period of time living on a farm or had worked as a hairdresser were found to be at a higher risk of illness while a higher dose of sun exposure seem to increase the risk.(3) Furthermore, studies have showed that having received blood transfusions in the past is an associated risk factor.(14)

1.5 Clinics

1.5.1 Clinical presentation and diagnosis

According to the WHO classification, CLL is a lymphocytic lymphoma that is distinguished exclusively by hematogenous dissemination from small lymphocytic lymphoma(32). Various diagnostic measures, such as blood smears, immunophenotyping, and in some cases, determination of the genetic characteristics of circulating lymphoid cells, should ensure that it is not a CLL-like lymphoma. “These could include mantle cell lymphoma, hairy cell leukemia, splenic marginal zone lymphoma and peripheral T-cell malignancies”(33).(33)

Typically, newly occurring lymphocytosis (> 5000 per cubic milliliter) raises the first suspicion of CLL. 70% of patients are asymptomatic at the time of diagnosis. ‘The remaining 30% of patients present with symptoms, which are most often caused by the lymphadenopathy, followed by splenomegaly and hepatomegaly and non-specific B symptoms.

However, lymphocytosis can have many possible causes, which can be either reactive or malignant.'2 Therefore, it is important to first exclude the reactive causes. Thus, the next diagnostic step is flow cytometry with immunophenotyping which is necessary to secure the diagnosis.(20)

For a diagnosis of CLL, a leukocytosis of at least 5x10^9/L B lymphocytes in the peripheral blood is necessary for at least three months. A characteristic blood smear shows small mature lymphocytes. Other characteristics are a narrow border of cytoplasm, a dense nucleus lacking discernible nucleoli, and Gumbrecht nuclear shadows which are cells that have ruptured during peripheral blood smear examination.(32, 34) These morphological changes can also be seen in monoclonal B-cell lymphocytosis.

However, in this case, there are fewer than 5x10 ^9/l lymphocytes visible in the peripheral blood and there must be no evidence of a lymphoproliferative disease.(21, 32)

Next, the immunophenotype must be defined. A characteristic feature is the restriction of either kappa or lambda light chains in flow cytometry. Cells of chronic lymphocytic leukemia express certain B-cell markers. Determining these markers is important to excluding other lymphoproliferative disorders mentioned above.(3) A co-expression of the surface antigen CD5 together with CD19, CD20 and CD23, which are B-cell antigens is found in CLL cells. Additionally, there is a rather low expression of CD20 and CD79b.(32)

"According to the guidelines of the international Workshop of CLL (iwCLL), the diagnosis of CLL is based on the following criteria"(32):

1. The existence of at least 5x10^9/l B lymphocytes over a time period of 3 months. If this criteria is not provided and additionally no clinical symptoms can be found, MBL can be diagnosed.
2. A blood smear examination shows typical findings
3. Immunophenotyping with light chain restriction and co-expression of specific antigens as described above.

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Additionally there are a few more diagnostic tests that can be performed to help determine the prognosis or tumor burden, which is important for evaluating treatment.(32)

With Fluorescence in situ hybridization (FISH) of peripheral blood lymphocytes, genomic aberrations can be detected. These are very important prognostic markers for progression and survival as already mentioned above.

Another diagnostic option that has provided completely new insights into the genetic influences on the prognosis of CLL is next generation sequencing (NGS). Genes that are typically analyzed with NGS are TP53, IgHV, NOTCH1 as well as other mutations with prognostic influence. This can be important for both therapy selection and prognosis estimation.(3)

Bone marrow biopsy or aspiration are not required for diagnosis of CLL but it can help to distinguish CLL-induced cytopenia from other causes. Furthermore, they help to assess the suitability of a therapy. Additionally bone marrow assessment takes place to verify a complete remission.(3)

1.5.2 staging

CLL appears to exhibit a high degree of variability in terms of both clinical presentation and clinical course. In fact, this variability is greater than that seen in all other forms of leukemia.(35) Therefore, a useful staging system is necessary to divide CLL into subgroups.
Currently, there are two popular clinical staging systems divide patients into risk groups. Both, the Rai and Binet classifications, are based on clinical and laboratory markers.(36)

1.5.3 Rai classification

The Rai classification includes stages 0 to IV. It is based on the view that inadequate lymphocytes progressively accumulate in patients with CLL over time. Stage 0 involves only an accumulation of leukemia cells in the bone marrow

and/or blood lymphocytosis. In stage I there are enlarged lymph nodes in addition to a blood lymphocytosis. Stage II impresses with a magnified liver or spleen and blood lymphocytosis. Beside the lymphocytosis which must be also present in stage III and IV, stage III is defined by a presence of anemia and stage IV by the presence of thrombocytopenia.
Several studies have shown that the rai classification is a solid predictor of survival.(37)

1.5.4 Binet classification

The Binet staging system is widely recognized as alternative main staging system for CLL. It is based on the results of survival analyses that showed the most important prognostic factors for survival. In Binet's analyses, these were thrombopenia and anemia. For patients without either of these risk factors the number of lymph node areas affected had an important influence on survival. The lymph node regions are defined as follows:
First, there is the cervical region, then the axillary region and finally the inguinal region. These regions are also staged as uni- or bilateral. Furthermore, there is an enlargement of the spleen and liver.
This is broken down into a three-stage classification. Patients with a Binet A CLL do not suffer from anemia or thrombocytopenia and have less than three affected lymph node areas. Binet B disease also shows no signs of anemia or thrombocytopenia but impresses with three or more involved areas. Binet C is defined by the presence of anemia and/or thrombocytopenia.(38)
However, the Rai and Binet classifications, both developed in the 1970s, are not suitable for forecasting the individual outcome, especially in early stage CLL.(22)
Over the decades new technology has enabled the discovery of more and more prognostic factors, such as FISH and NGS.(39)

Table 1 Overview of Rai and Binet classification

	Lymphozytosis (> 5x10^9/L)	Enlarged lymph	Affected lymph	Hemoglobin	Platelet count

		nodes/hepato-splenomegaly	node regions		
Rai 0	+	-		>11g/dl	> 100.0 G/L
Rai 1-2	+	+		>11g/dl	>100.0 G/L
Rai 3-4	+	+		< 11g/dl	< 100.0G/L
Binet A			0-2	>10g/dl	>100.0 G/L
Binet B			3-5	>10g/dl	> 100.0 G/L
Binet C			Any	< 10g/dl	< 100.0 G/L

1.6 Therapy

1.6.1 Indications for primary treatment

If patients in the early stages of the disease (considered to be Ray 0 or Binet A) are asymptomatic, they are monitored and receive no primary therapy as long as there are no signs of disease progression. (32) Studies have shown that a conservative treatment strategy is advantageous in terms of survival for most patients with early-stage CLL.(40) Patients who are treated using this “watch and wait” strategy should undergo a follow-up examination every 3-6 months, consisting of laboratory tests and a physical examination, in order to detect any possible progression of the disease at an early stage.(41)
Patients with intermediate (Ray I-II, Binet B)- and high-risk (Ray III, Binet C) stages can also benefit from a primarily conservative treatment strategy if there is no evidence of an active disease progression.
The criteria for an active disease are shown below. If at least one criterion applies, treatment should be initiated.

1. Bone marrow failure, which manifests itself by deteriorating thrombocytopenia and/or anemia.
2. Splenomegaly and/or lymphadenopathy, defined as enormous, progressive or symptomatic
3. Progressive lymphocytosis, meaning a doubling time of the lymphocyte blood count of less than six months or an increase of at least 50 percent over two months.
4. Autoimmune complications, poorly responsive to therapy.
5. Extronodal organ involvement.
6. Other symptoms related to the disease, such as uncontrolled weight loss, strong fatigue, fevers and night sweats.(32, 42)

1.6.2 Primary treatment strategies

‘In the new, revised guideline from 2024, primary therapy with a BCL-2 or Bruton's tyrosine kinase (BTK) inhibitor is recommended for all patients regardless of their genetic risk profile.(43) Various studies have shown that both acalabrutinib alone and venetoclax in combination with obinutuzumab or ibrutinib have a significantly better progression-free survival compared to chemoimmunotherapy with chlorambucil-obinutuzumab.(44-47) Therefore, conventional chemoimmunotherapy is of little value and should only be used at the patient’s request for patients under 65 years of age with a low-risk profile, meaning the presence of an IgHV mutation and the absence of a del17p/TP53 mutation and a complex karyotype. This group of patients in particular has been shown to benefit from chemoimmunotherapy with fludarabine-cyclophosphamide- rituximab (FCR) in terms of overall survival.(48)’3

When choosing the substance for immunotherapies, age and comorbidities should be considered when choosing the substance and second-generation BTK inhibitors should be used instead of first-generation inhibitors if necessary.(43) Those two groups show a similar progression free survival (PFS) with the second-generation substances, such as acalabrutinib having a fewer number of adverse

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effects, particulary those affecting the cardiovascular system.(46) BTK inhibitors should also be favored if the patient suffers from a reduced kidney function (GFR >30).The various immunotherapies also differ in terms of therapy duration. BTK inhibitors alone are used for long-term oral therapy. For patients who prefer a time-limited therapy, treatment with venetoclax-obinutuzumab or venetoclax-ibrutinib is available.(43)

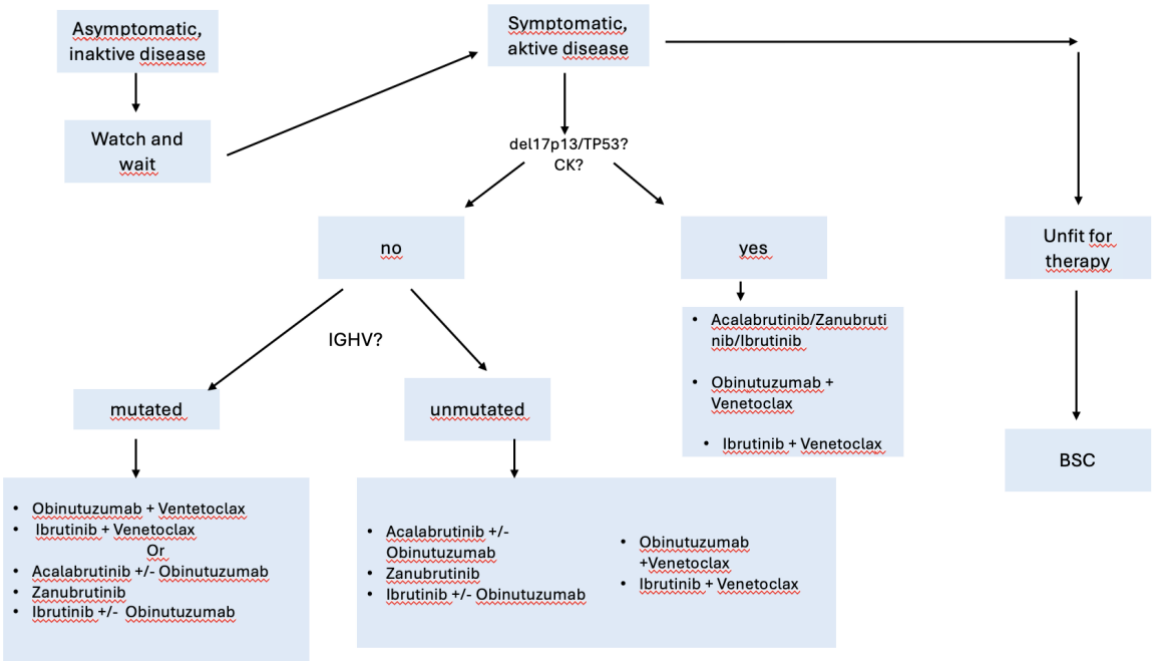

Figure 3 Algorithm of CLL first line therapy according to onkopeadia(49)

1.6.3 Response after treatment

In order to assess the response to treatment or during the maintenance phase of a treatment, two groups of parameters need to be examined. Firstly, there are parameters that evaluate the lymphoid tumor load as well as general symptoms. These include the size of the lymph nodes, liver and spleen, the lymphocyte count and as previously mentioned constitutional symptoms. Secondly, the hematopoietic system must be assessed. Parameters for this evaluation include the platelet and hemoglobin counts in peripheral blood and additionally a bone marrow assessment can also be performed.

The time point of performing the assessment should be as least 2 months after the finalization of therapy. For continued therapies the evaluation of response should

be performed at least two months after the maximum response, which is defined as the period of time during treatment when no further improvement is seen for at least two months of therapy.(32)
The response to treatment is divided into four different groups: Complete remission (CR), partial remission (PR), progressive disease (PD) and stable disease (SD).
CR is achieved when there is no clinical evidence of a present disease also including a bone marrow assessment with no marrow infiltrate and no B-lymphoid nodules.
Patients with PR still have clinical symptoms such as lymphadenopathy or anemia/thrombocytopenia, as well as bone marrow infiltration and B-lymphoid nodules but these are reduced by more than 50% from the original state.
A PD on the other hand, is defined as worsening of clinical symptoms by at least 50%.
If neither the PR criteria nor PD criteria are met, this is referred to as SD.(3, 32)

1.6.4 Second-line treatment

An immunotherapy with BTK Inhbitor or BCL2 Inhibitor should also be selected for second-line therapy.
In the case of early recurrence (within 2 years), the patient switch to the other substance class. In the event of a late recurrence after a time-limited therapy with venetoclax, a venetoclax-based therapy option can be used again.(43) With these new immunotherapeutic options, stem cell transplantation is now also used less frequently.
According to the new guidelines, allogeneic stem cell transplantation can only be considered for patients at genetic high-risk after at least two immunotherapies with different substance classes have failed.(43)

1.6.5 Best supportive care (BSC)

The term BSC describes a treatment that focuses mainly on maintaining and improving quality of life by controlling disease-related symptoms.(50) The supportive measures used in CLL include, above all, the prevention of infections

by administering immunoglobulins and vaccinations. Regular internal medical check-ups are therefore strongly recommended.(43)

1.6.6 Treatment of Richter transformation

RT has always had a very poor prognosis due to patients' poor response to chemoimmunotherapy. Complete remission was present in only 20% of cases and the median survival was determined to be between 6 and 12 months. However, prognostic markers have been identified that indicate a better response to therapy. Patients who were previously untreated for CLL seem to have relatively better survival rates.(51, 52) Furthermore, diffuse-non-large B-cell lymphomas that emerged following RT (DNLBCL-RT) and were clonally unrelated to the previous CLL clone showed a significantly longer survival and a less frequent TP53 mutations which are negative prognostic markers.(53) Initial chemoimmunotherapy with an R-CHOP regimen is recommended for these patients, followed by allogeneic stem cell transplantation to achieve a complete cure. Patients with a poorer prognosis should be enrolled in a clinical trial. Other chemoimmunotherapeutic options are available for these patients, such as R-CHOP or R-EPOCH combined with ibrutinib or venetoclax.(52)

1.7 Aim of checking clinical parameters for their prognostic relevance

The aim of our study was to identify prognostic factors in a clinical real world setting, to examine their relevance, and to compare them with the results of other centers. The special feature of our cohort was that all patients were diagnosed with CLL based on the detection of existing lymphadenopathy.
We tested various clinical and molecular genetic parameters for overall survival and time to therapy. We also attempted to find associations with potential prognostic value between the various factors.

2. Material and Methods

Our retrospective study included 155 patients diagnosed with B-CLL by January 2024.

We excluded 31 patients due to a lack of data and poor documentation. Therefore, our final cohort consisted of 124 patients.

We collected basic, cytological and clinical data from the electronic medical records of the Styrian Association of Medical Institutions (KAGES). Date of last follow-up was the 20th of January 2024. All data collected was anonymized. This study was approved by the Ethics Committee of the Medical University of Graz (ethical application 28-516 ex 15/16).

Table 1 Baseline characteristics of patient cohort

Parameter	Number of patients	Range	Mean
Age	N= 124	31-95	74,06
Sex Fermale Male	N= 124 43 (34,7%) 81 (65,3%		
Survival Alive dead	n= 124 50 (40,3%) 74 (59,7%)		
Patients who received therapy Patients who suffered from advese effects Patients who received a dose reduction/discontinuation/delay	N=89 52 (58,4%) 63 (70,8%)		

Table 3 Collected clinical, cytological and genetic parameters

Clinical Parameters	Molecularbiological Parameters	Genetic Parameters
Groups of enlarged lymph nodes date of diagnosis overall survival <u>therapy:</u> • start date of therapy • time to therapy • substances used for therapy • Adverse effects • dose reduction/ therapy discontinuation/ delay <u>Comorbidities</u>: cardiovascular diseases • Diabetes Mellitus • Myocardial infarction • Cerebrovascular accident (CVA) or transient ischemic attack (TIA) • Peripheral vessel disease • Arterial hypertension • Atrial fibrillation COPD Other malignancies Dementia liver diseases medium/severe chronic kidney disease (CKD)	Immunglobulin locus	Complex karyotype del17p del11q trisomy 12

If a chromosome analysis of CLL cells was performed, del 11q, trisomy 12 and del 17p mutations were recorded. Additionally, the existence of a complex cariotype which is defined as three abberations or more.
Any cytological examinations for somatic hypermutations were recorded.
All 124 patients displayed lymph node infiltration at the time of diagnosis. To determine the number of affected lymph node groups, we primarily used the results of PET-CT examinations where they had been performed. In the absence of a PET-CT examination, we used the results of physical examinations. For our statistical analyses, we used the highest number of affected lymph node stations that a patient displayed at any time during their CLL treatment.
If CLL therapy was initiated at any time during the observation period, we identified the start date and the type of therapy administered.
By determining the time of diagnosis and the time at which therapy started, we could calculate the time to therapy.

We investigated adverse effects that appeared in patients who received any therapy for CLL or another lymphoma after undergoing Richter transformation. The following clinical manifestations were recorded as adverse effects: Infections of any kind; diarrhoea; atrial fibrillation; and secondary malignancies, which include any malignoma diagnosed after the start of therapy.

We recorded every instance of a dose reduction, discontinuation or delay that occurred during CLL therapy.

If patients died during the observation period, we identified and recorded the date of death. This enabled us to determine the period between diagnosis and death, which we then used to base our survival analysis.

3. Results

3.1 Patient cohort

Of our 124 patients, 43 were female (34,7%) and 81 were male (65,3%). The median age was 73, ranging from 31 to 95. Fifty patients (40,3%) were still alive at the time of data collection, while 74 patients (59,7%) had died. 19,4% (24) of patients developed Richter transformation (RT). Of those, 23 developed diffuse large B-cell lymphoma. In one case, it developed into Hodgkin's disease.

All 124 patients displayed lymph node infiltration upon diagnosis. In 25% (31 cases), the number of affected lymph node stations was determined by PET-CT examination. In the remaining 75% (93 patients), it was determined by physical examination or further examinations such as ultrasound or CT thorax/abdomen/pelvis scans, as shown in table 4. In the remaining 14,5% (18) of cases, it was not possible to determine the number of affected lymph node regions reliably. This was due to contradictory or incomplete documentation.

Table 2 Frequency and diagnostic modalities of lymph node involvement patterns by number of affected lymph node areas

Number of lymph node areas	1	2	3	4	5	No data
Occurence	16,1% (n=20)	14,5% (n=18)	23,4% (n=29)	24,2% (n=30)	7,3% (n=9)	14,5% (n=18)
Diagnosed by PET-CT	20% (n= 4)	22% (n=4)	27,6% (n=8)	30%(n=9)	66% (n=6)	
Diagnosed by physical examination/ ultrasound/CT	80% (n=16)	88% (n=14)	72,4% (n= 21)	60% (n=21)	33% (n= 3)	

Table 5 demonstrates the data from our cohort regarding the investigation of somatic hypermutations using IgHV sequencing.

Table 5 IgHV somatic hypermutation status among patients examined

Total number of patients	N= 124		
Examined for somatic hypermutations	Yes 19,4% (n=24)		No 80,6%(n=100)
IgHV	Mutated 41,6% (n=10)	Umutated 58,3% (n=14)	

‚Unmutated' means that the mutation ratio in those genes was under 2%.

Of all patients, 89 (71,8%) received therapy for CLL, including RT, while 35 (28,2%) did not receive any therapy at all. The most frequently administered therapies were bendamustine alone and in in combination with rituximab (n=41), and ibrutinib (n=39). Fludarabine and cyclophosphamide (FC) were given to 38 patients, either with or without rituximab (R-FC). Fourteen patients received venetoclax.
The therapies mentioned above are the ones we are going to focus on.

Of those who received therapy, 52 patients (58,4%) suffered adverse effects. These adverse effects included infections of any kind, including febrile neutropenia and diarrhoea, as well as cardiac adverse effects, such as atrial fibrillation, and the presence of secondary malignancies.

63 (70,8%) of patients undergoing therapy experienced a reduction in CLL therapy dosage. Under the term 'dose reduction', we also included patients with treatment delay or discontinuation of any therapy given against CLL.
With regard to table 6, 21,8% (27) of patients had diabetes mellitus type 1 or 2, and 6,2% (8) had experienced a heart attack at some point in their lives. 7,3% (9) had experienced a stroke or transient ischemic attack. 3,2% (4) developed

dementia. 12,1% (15 subjects) had COPD. 24,2% (30) had peripheral vascular disease of any kind, mostly deep vein thrombosis, peripheral artery disease, and varicose veins. 18,5% (23) had a liver disease, mostly fatty liver disease. 20,2% (25) of subjects had moderate or severe renal insufficiency. 53,2% (66) had arterial hypertension. This was by far the most common comorbidity in our cohort. Other malignancies occurred in 48,4% (60) of cases. Atrial fibrillation was present in 18,5% of cases. In four cases, atrial fibrillation only occurred after ibrutinib was administered.

Table 6 Overview of comorbidites in the study population

Total number of patients	n= 124	
Comorbidity	yes	no
Diabetes Mellitus	21,8% (n=27)	78,2% (n=97)
Myocardial infarction	6,5% (n=8)	93,5% (n=116)
CVA or TIA	7,3% (n=9)	92,7% (n=115)
Dementia	3,2% (n=4)	96,8% (n=120)
Peripheral vessel disease	22,6% (n=28)	77,4% (n=96)
Live disease	18,5% (n=23)	81,5% (n=101)
Medium/severe CKD	20,2% (n=25)	79,8% (n=99)
COPD	11,3% (n=14)	88,7% (n=110)
Arterial hypertension	53,2% (n=66)	46,7% (n=58)
Other malignancies	48,4% (n=60)	51,6% (n=64)
Atrial fibrilation	18,5% (n=23)	81,5%(n=101)
At least 3 comorbidities	44,4% (n=55)	55,6% (n=69)

3.2 Prognostic models

3.2.1 Survival

It is well known that certain genetic and molecular biological characteristics of CLL have an influence on overall survival (OS). Therefore, we aimed to test how these parameters influenced our lymph node-positive cohort in terms of OS. Table 7 summarized all association with any prognostic impacts. The survival analyses were performed using an log rank test, which examines significant differences in survival time between two groups from the time of diagnosis to the time of death.

Table 7: Comparison of clinical and genetic parameters between groups

Parameter	Group one (n)	Group two (n)	P value
RT	Yes (24)	No (100)	P =0,0014
Lymph node regions	1-2 (67)	3-5 (56)	P= 0,24
Somatic hypermutations	Mutated (10)	Unmutated (13)	P= 0,029
Del17p	Mutated (9)	Unmutated (69)	P= 0,11
Del11 q	Mutated (22)	Unmutated (56)	P=0,33
Trisomy 12	Mutated (14)	Unmutated (62)	P=0,16
Del17+del11q	Both mutated (3)	Not both mutated (74)	P=0,00042
Dosisreduction	Yes (25)	No (63)	P=0,12

Starting with Richter transformation (RT) which usually leads to a more aggressive lymphoma with a significantly poorer prognosis.(51)
In our cohort, 19,4% (24) of patients developed RT. These patients had significantly poorer survival compared to those who did not develop RT (p = 0,0014). The median survival time for patients with RT was 7,52 years, compared to 9,68 years for patients without RT. The corresponding Kaplan–Meier curve is illustrated in Figure 4.

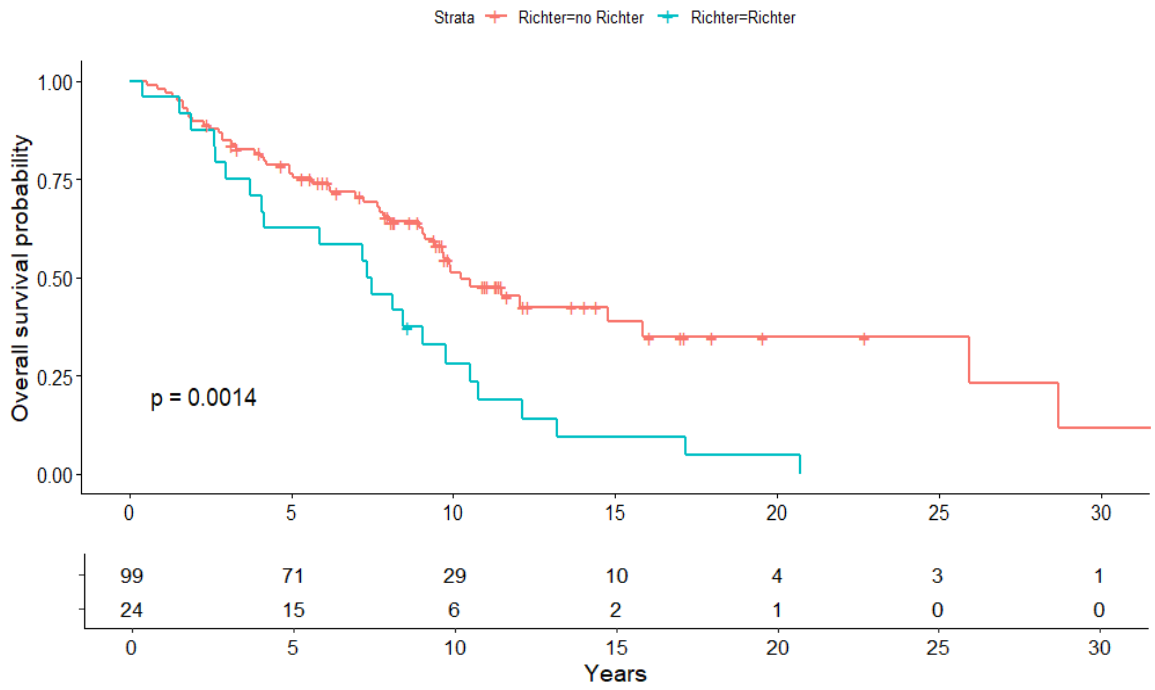

Figure 4 Kaplan-Meier analysis of overall survival in patients with and without Richter transformation. The red curve represents patients with no Richter transformation, while the blue curve represents patients with Richter transformation. Overall survival was compared between groups using the log-rank test (p=0,0014). The risk table below the plot indicates the number of patients at risk in each group at the respective time points (in years)

We also investigated the number of affected lymph node regions in relation to survival. In order to compare the effects of the number of affected lymph node regions, the patients were divided into two groups according to the number of regions affected. One group had a small number of affected lymph node regions. This group had one to two affected lymph node regions equivalent to a Binet A or low risk stage. The second group had several affected lymph node regions, representing a Binet B/C intermediate or high risk stage. This group had three to five affected lymph node regions. We retained this subdivision for all subsequent analyses.

There was no difference in survival rates between the two groups in our cohort. Figure 5 shows the survival rates of the above-mentioned groups.

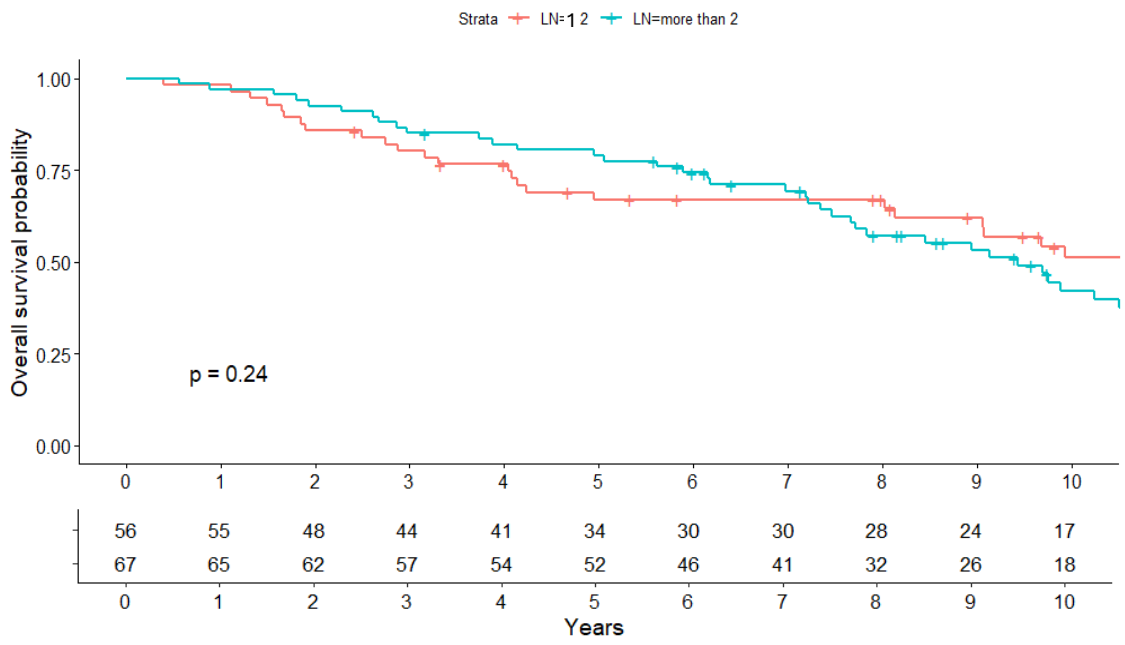

Figure 5 Kaplan-Meier analysis of overall survival in patients with 1-2 and patients with more than 2 affected lymph node regions. The red curve represents patients with 1-2 affected lymph node regions, while the blue curve represents patients with more than two affected lymph node regions. Overall surivival was compared between groups using the log-rank test (p=0,24). The risk table below the plot indicates the number of patients at risk in each group at the respective time points (in years)

Among molecular biological factors, somatic hypermutations in the form of IgHV mutations are a recognized prognostic factor.(54) Despite the small number of cases, we tested whether the presence of somatic hypermutations influences overall survival.

Of the 18,5% (23) of patients who underwent histopathological examination for somatic hypermutations, survival was significantly worse in those without somatic hypermutations compared to those with somatic hypermutations (p = 0,029). Figure 6 shows the survival differences of these groups using the Kaplan-Meier curve.

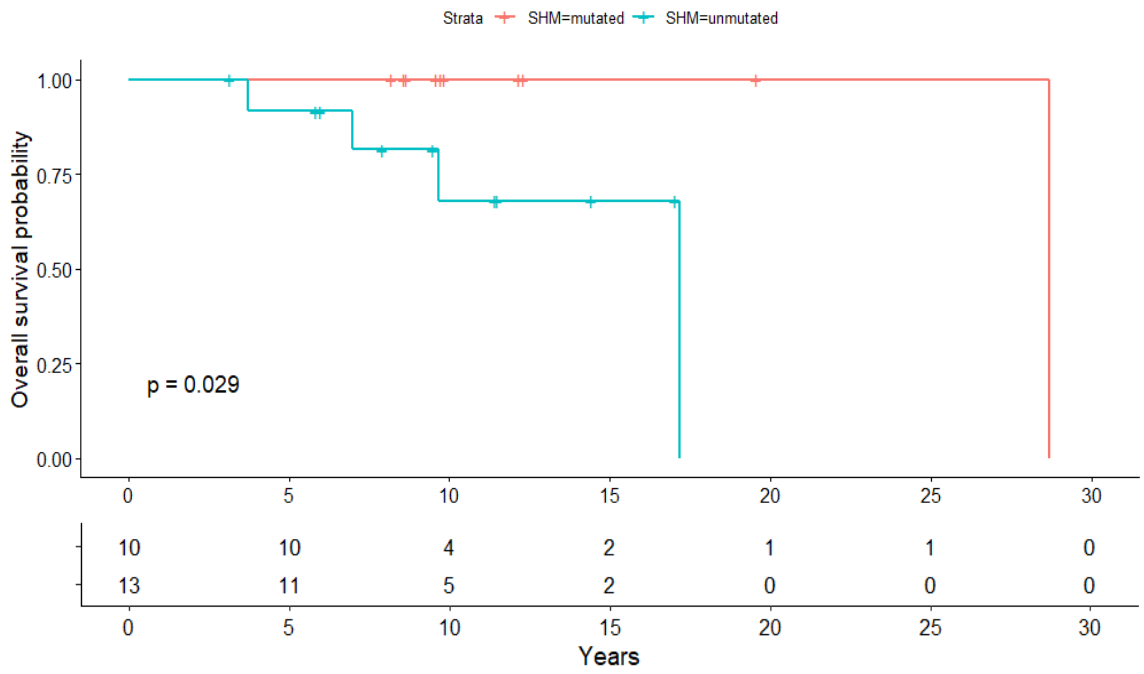

Figure 6: Kaplan-Meier analysis of overall survival in patients with and without somatic hypermutation. The red curve represents patients with existing somatic hypermutation, while the blue curve represents patients without somatic hypermutation. Overall survival was compared between groups using the log-rank test (p=0,029). The risk table below the plot indicates the number of patients at risk in each group at the respective time points (in years)

Genomic aberrations have an impact on the prognosis of CLL, with del17p, del11q, and trisomy 12 being the aberrations with the most negative impact.(22) Therefore, we investigated the OS for these in our cohort.

Del17p mutation in combination with a Del11q mutation shows significantly poorer survival (p = 0,00042). However, the small number of patients with both mutations should be noted here.

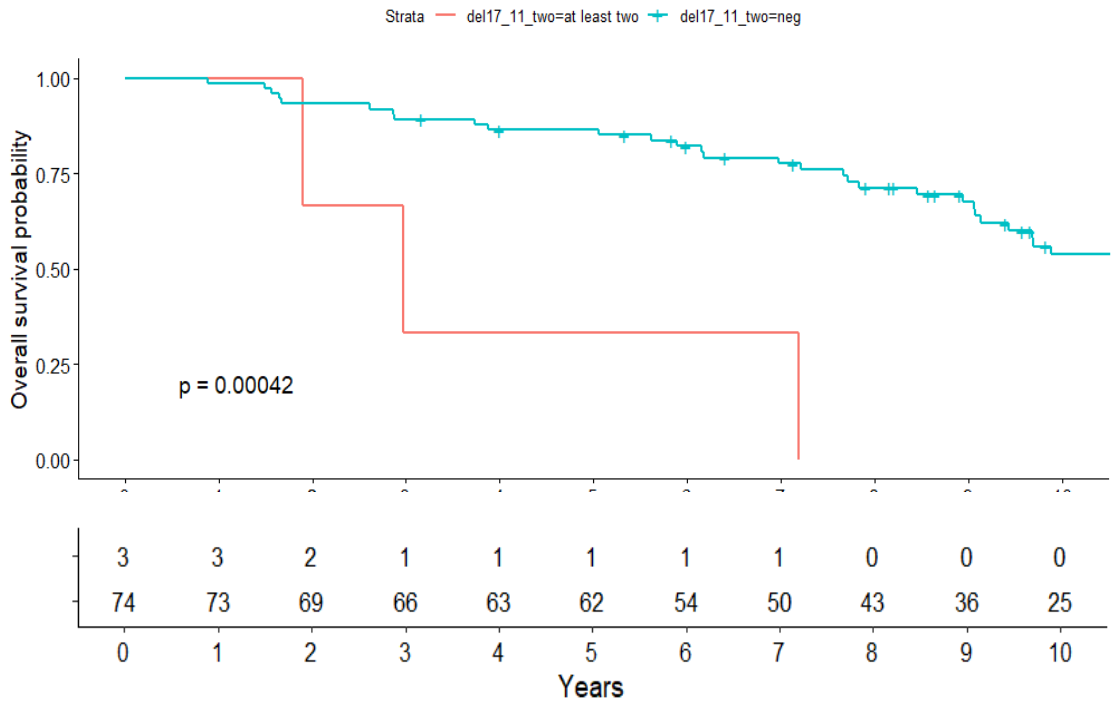

Figure 7: Kaplan-Meier analysis of overall survival in patients with and without both, del17p and dell11q mutation. The red curve represents patients with del17p and del11q mutation, while the blue curve represents patients without both mutations. Overall survival was compared between groups using the log-rank test (p=0,00042). The risk table below the plot indicates the number of patients at risk in each group at the respective time points (in years)

An isolated occurrence of either mutation (Del17p or trisomy 12 or Del11q) does not show significantly poorer survival. The corresponding survival curves are graphically shown in Figure 8-10.

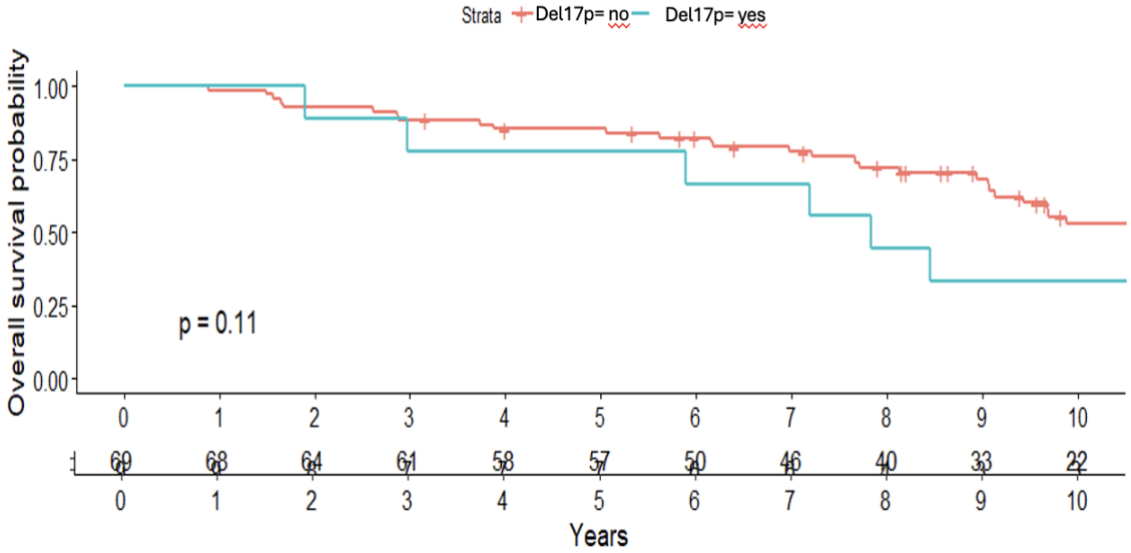

Figure 8: Kaplan-Meier analysis of overall survival in patients with and without del17p mutation. The red curve represents patients without del17p, while the blue curve represents patients with mutated del17p. Overall survival was compared between groups using the log-rank test (p=0,11). The risk table below the plot indicates the number of patients at risk in each group at the respective time points (in years)

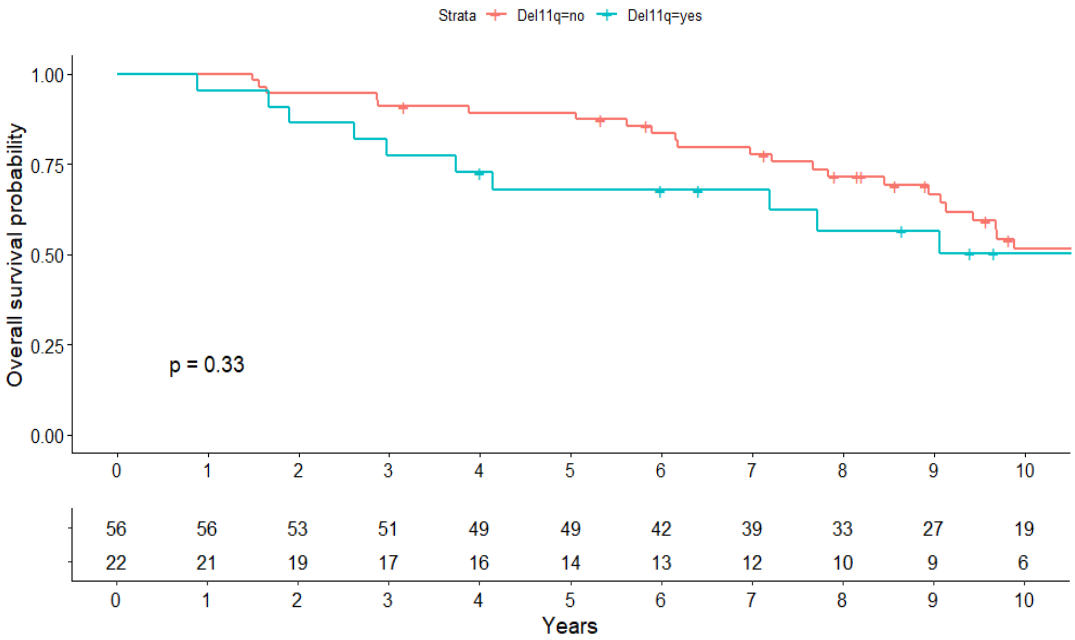

Figure 9: Kaplan-Meier analysis of overall survival in patients with and without del11p mutation. The red curve represents patients without del11p, while the blue curve represents patients with mutated del11p. Overall survival was compared between groups using the log-rank test (p=0,33). The risk table below the plot indicates the number of patients at risk in each group at the respective time points (in years)

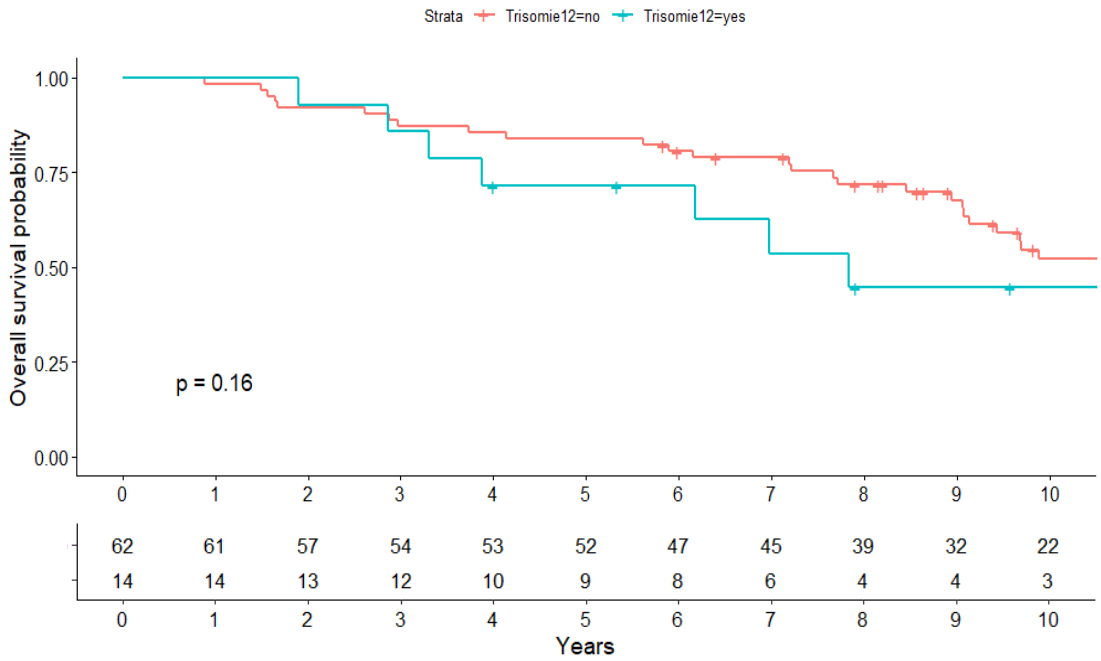

Figure 10: Kaplan-Meier analysis of overall survival in patients with and without trisomy12 mutation. The red curve represents patients without trisomy12, while the blue curve represents patients with mutated trisomy12. Overall survival was compared between groups using the log-rank test (p=0,16). The risk table below the plot indicates the number of patients at risk in each group at the respective time points (in years)

In the treatment of CLL, it is often necessary to reduce the dose, interrupt, or delay the administration of medication. This may occur, for example, due to side effects or intolerances. We investigated whether such irregularities in treatment have an impact on OS.

We investigated whether any patients treated for chronic lymphocytic leukemia (CLL) had undergone a dose reduction at any time. Those who did experience a dose reduction did not have a worse overall survival rate than those who did not (p=0,12). The Kaplan–Meier curve is shown in Figure 11.

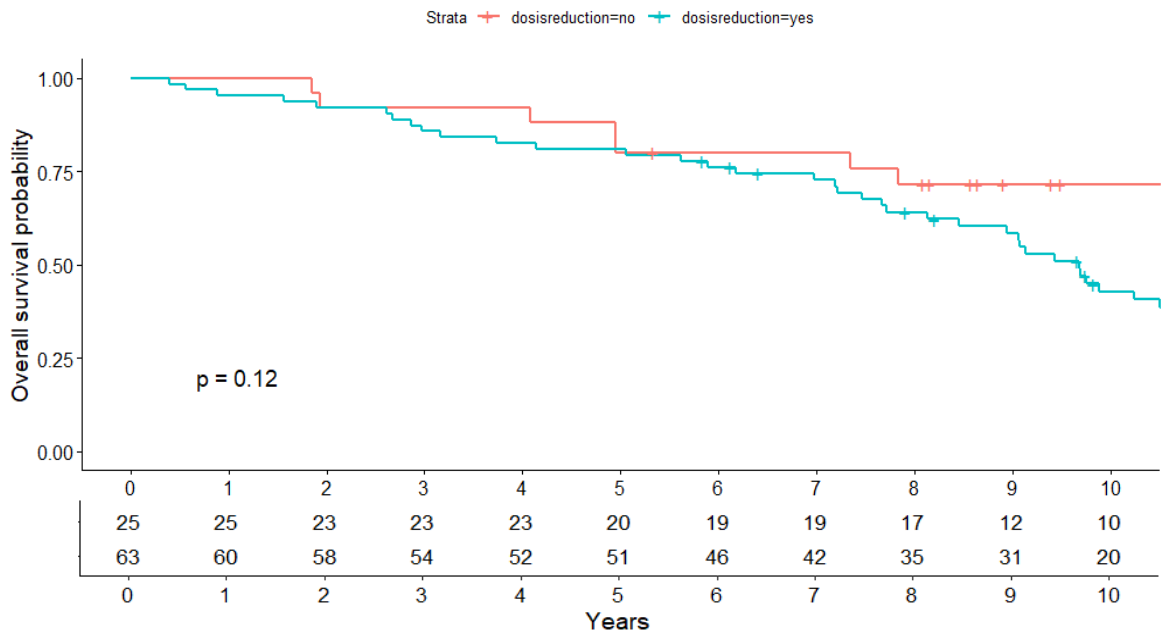

Figure 11: Kaplan-Meier analysis of overall survival in patients who underwent dosisreduction and who never underwent dosisreduction during CLL therapy. The red curve represents patients without any dosisreduction, while the blue curve represents patients who underwent dosisreduction. Overall survival was compared between groups using the log-rank test (p=0,12). The risk table below the plot indicates the number of patients at risk in each group at the respective time points (in years)

3.2.2 Time to therapy

The time from diagnosis to first administration of therapy usually depends on the activity of the disease according to the Rai and Binet classification.
We investigated the effect of parameters on the time to therapy and came to the following observation:

Table 8 Comparison of lymph node regions and Richter transformation (RT) between groups

	Group 1 (n)	Group 2 (n)	P value
LN regions	1-2 (67)	3-5 (56)	P= 0,0033
RT	Yes (24)	No (100)	P= 0,00043

Table 8 shows that the number of lymph node regions affected significantly influences time to therapy (p = 0,0033). Patients with three to five affected lymph node regions, which is corresponding to a Binet B stage experienced significantly

shorter times to therapy than patients with less than three affected lymph node regions. Figure 12 illustrates the associated Kaplan–Meier curve.

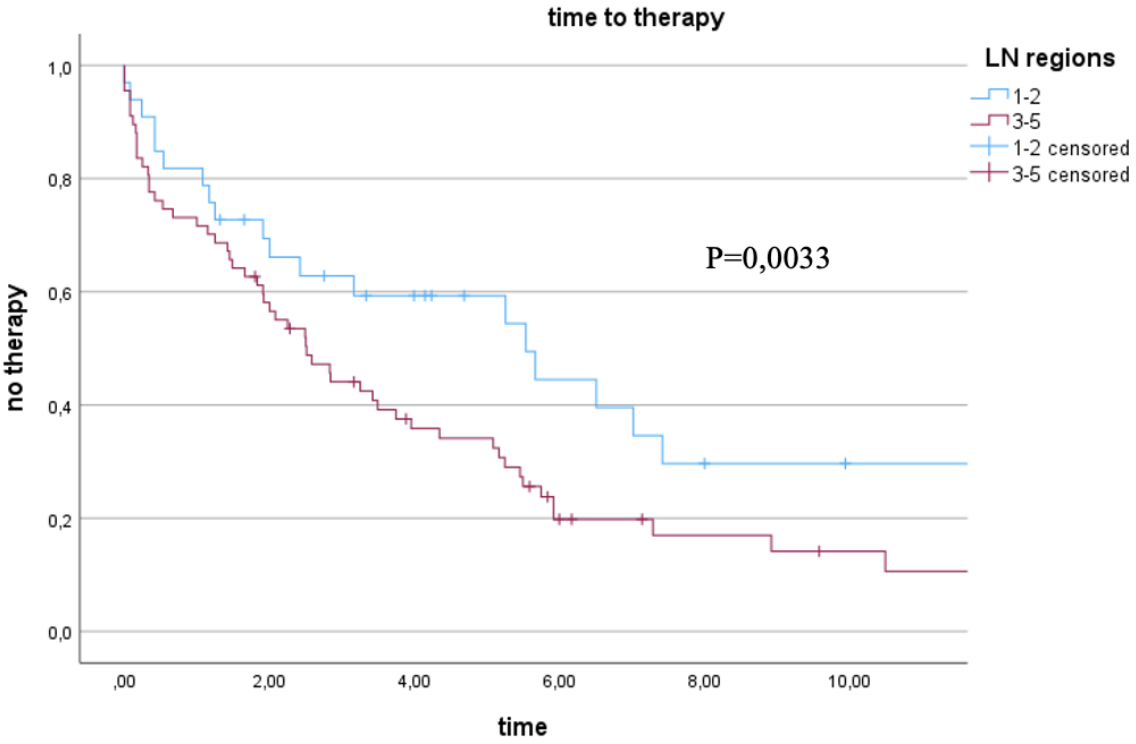

Figure 12: Kaplan-Meier analysis of the time to therapy in patients with 1-2 and patients with more than 2 affected lymph node regions. The blue curve represents patients with 1-2 affected lymph node regions, while the red curve represents patients with more than two affected lymph node regions. Time to therapy was compared between groups using the log-rank test (p=0,0033).

Patients who developed a Richter's transformation at any point, which means both before and after therapy initiation, showed a significantly shorter time to therapy than those who never developed one (p = 0,00043). It is important to note that patients who received their first therapy before developing RT are also included. The corresponding Kaplan–Meier curve is shown in Figure 13.

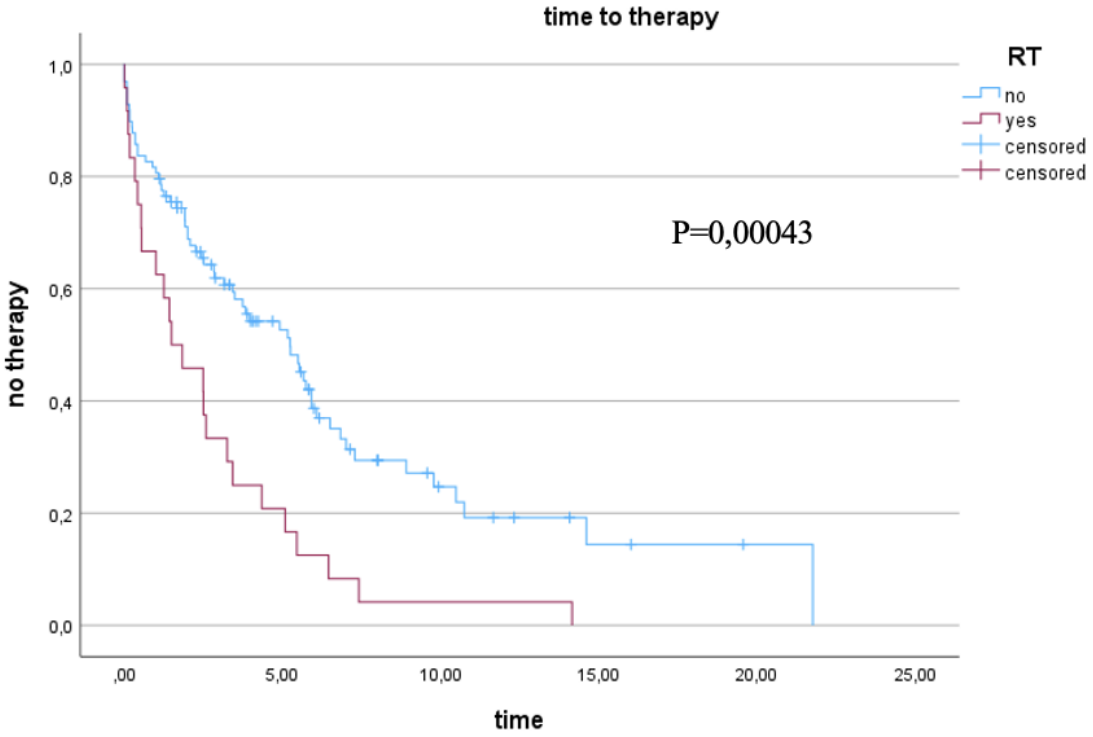

Figure 13: Kaplan-Meier analysis of the time to therapy in patients with and without Richter transformation. The red curve represents patients with Richter transformation, while the blue curve represents patients without Richter transformation. Overall surivival was compared between groups using the log-rank test (p=0,00043).

Studies have shown that an unmutated IgHV status significantly shortens the time to therapy.(55, 56) However, he presence or absence of somatic hypermutation had no effect on time-to-therapy in our cohort(p = 0,19).

3.3 Clinical, molecularbiological and genetic associations

CLL is a highly heterogeneous disease that presents itself very differently both clinically and molecularly.(57)We attempted to find associations between clinical, molecular biological, and genetic factors that could be useful for CLL risk stratification.

3.3.1 Associations between clinical factors

A comparison of clinical factors revealed a significant association between Richter's transformation and the number of lymph node regions affected, which is demonstrated in table 9. Patients with RT were more likely to have three to five affected lymph node regions than those who had never undergone RT (p = 0,038). Figure 14 illustrates these proportionalities.

Table 9 association between RT and lymph node regions

	LN1-2	LN3-5	Total	
CLL with Richter	3	17	20	P=0,038
CLL without Richter	35	51	86	
Total	38	68	106	

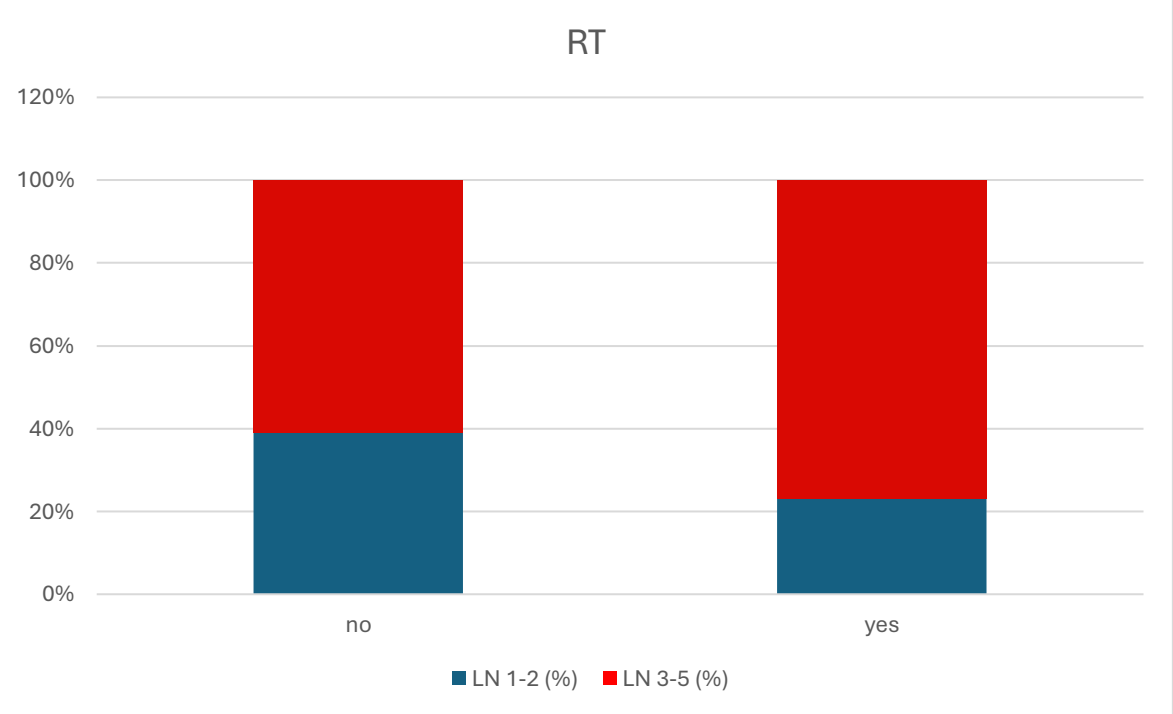

Figure 14: association between lymph node regions and RT

We assessed the various comorbidities of patients to determine whether an increased number of comorbidities increases the need for a reduced dose. The cohort was divided into two groups: patients with 0–2 comorbidities, and patients

with three or more comorbidities. There was no significantly increased frequency of dose reduction in patients with at least three comorbidities. (p > 0,99 as shown in table 10). The numbers are shown in Table 10.

Table 10 : association between dosisreduction and comorbidity

comorbidities	0,1,2	>2	Total	
dosisreduction	35	28	63	P= >0,99
no dosisreduction	14	12	26	
Total	49	40	89	

3.3.2 Associations between clinical and molecularbiological factors

Since unmutated IgHV is known to have a poorer survival in CLL patients, we tested if those patients are also more likely to develop RT. Our results showed that there was no significant association between the presence of somatic hypermutations and Richter transformation in our cohort, (p = > 0,99) as shown in table 11.

Table 11 association between RT and SHM

	No SHM	SHM	Total	
CLL with Richter	2	1	3	P=>0,99
CLL without Richter	12	9	21	
Total	14	10	24	

Table 12 shows our additional comparison of the presence of somatic hypermutation (SHM) with the presence of therapy initiation, which demonstrates no significant difference, (p = 0,61)

Table 12: association between therapy and SHM

	no SHM	SHM	Total	
CLL never needed Therapy	2	3	5	P=0,61
CLL needed Therapy	10	6	16	
Total	12	9	21	

3.3.3 Associations between clinical and genetic factors

We wanted to find out whether there were associations between genetic factors and clinical markers.

The result was a positive correlation between the presence of a Del17p mutation and the occurrence of a Richter transformation.

Patients who underwent a Richter transformation were significantly more likely to have a dell 17p mutation. (p= 0,0001 as shown in table 13). The correlation is graphically shown in figure 15.

Table 13: association between RT and del17p

	Del 17 p	no Del 17p	Total	
CLL without Richter	2	60	62	P=0,0001
CLL with Richter	7	9	16	
Total	9	69	78	

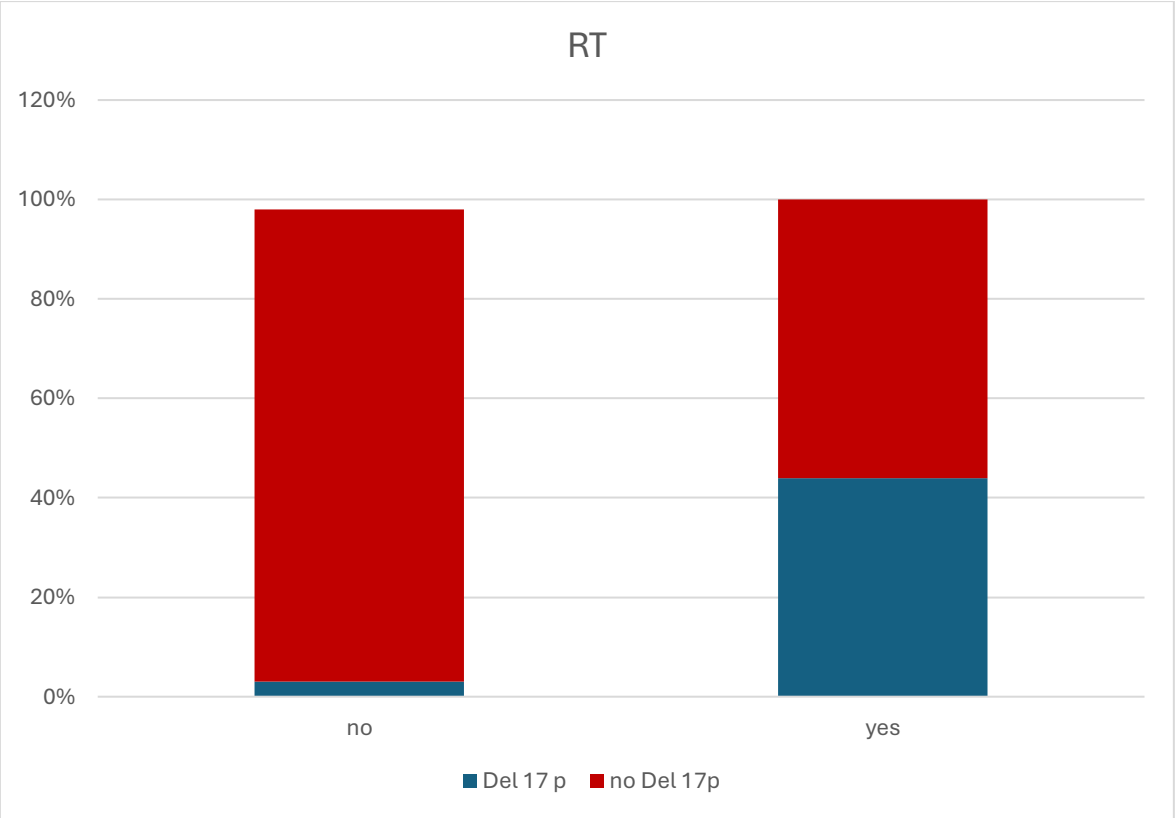

Figure 15: association between RT and del17p

Since there was a positive association between an increased number of lymph node regions and RT, as well as between a del17p mutation and RT, we additionally investigated whether an increased number of lymph node regions is also more frequently associated with a del17p mutation. However, this did not prove to be significant (p=0,79), as demonstrated in table 14.

Table 14: association between LN regions and del17p

	Del17p	No Del17p	Total	
CLL LN<=2	7	21	28	P=0,79
CLL LN>2	15	35	50	
Total	22	56	78	

3.4 Therapy

Over the past decade, a lot has changed in the treatment of CLL. Newer targeted therapies have largely replaced conventional immunochemotherapies, partly due to their more favorable side effect profile.(58) Since our cohort included patients receiving many different treatment regimens, including both conventional and newer therapies, we compared the side effect profiles of both treatment approaches.

We investigated unwanted side effects, in the form of neutropenia and infections in patients receiving therapy for CLL, comparing newer targeted therapies with conventional chemotherapy. Targeted Therapy include Ibrutinib and Venetoclax. Conventional chemotherapy includes Rituximab -Bendamustin (RB), (Rituximab)-Fludarabine-Cyclophosphamid (R-FC). The results showed that there were no significant differences in the occurrence of adverse effects (p= 0,69), as shown in table 15 and figure 16.

Table 15: adverse effects of chemoimmunotherapy compared to targeted therapy

	no adverse effects	adverse effects	Total	
CLL - immuno chemo therapy	25	33	58	
CLL - targeted therapy	17	28	45	P = 0,69
Tot	42	61	103	

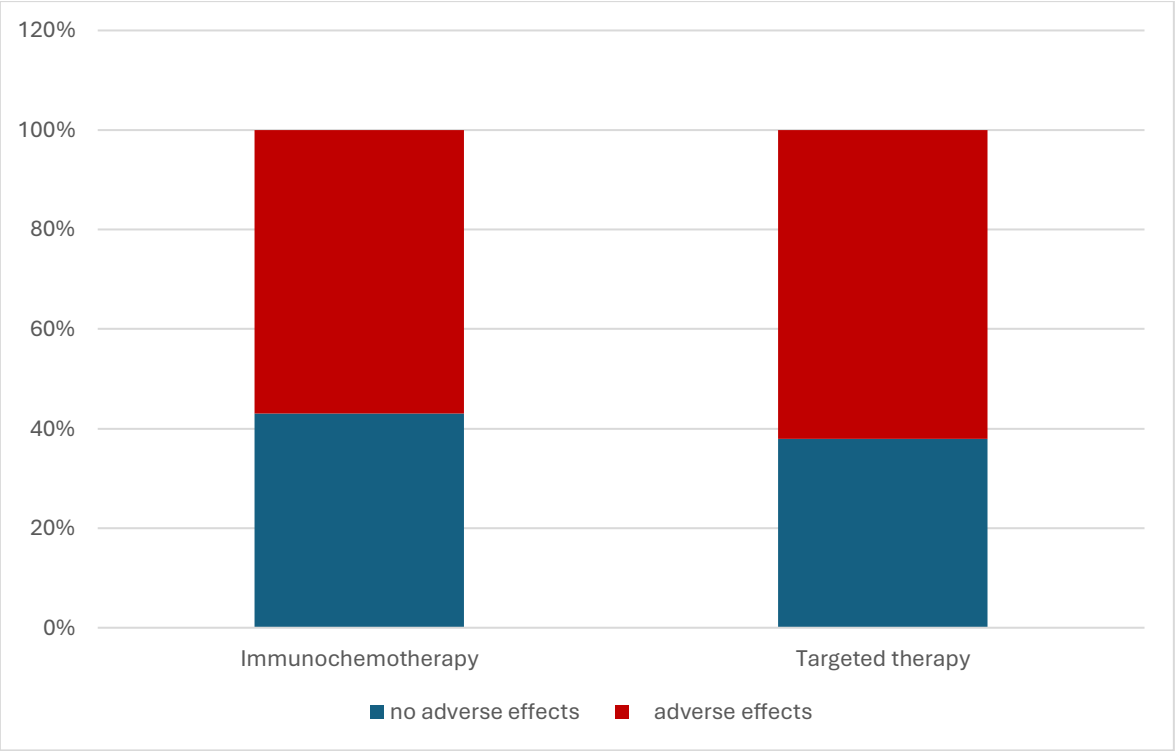

Figure 16: adverse effects of chemoimmunotherapy compared to targeted therapy

We also evaluated the cardinal side effects of ibrutinib therapy in form of de novo atrial fibrillation, since several studies have detected a higher incidence of atrial fibrillation in patients treated with Ibrutinib.(59)We came to the result that 10,4% (n=4) of patients treated with ibrutinib in our cohort suffered from de novo atrial fibrillation as shown in table 16.

Table 16: de novo atrial fibrillation during therapy with ibrutinib

	Patients treated with ibrutinib	De novo atrial fibrillation	No de Novo atrial fibrillation
n	39	4	35

4. Discussion

Although CLL is a highly variable disease in terms of prognosis and survival, average survival tends to be favorable compared to other non-Hodgkin lymphomas. Many cytogenetic parameters play a role, not only in the development and progression of CLL, but also in terms of outcome. Over the last decade, our growing understanding of CLL biology has been used to develop therapeutic options aimed at improving CLL prognosis.

In 2007, Wierda attempted to create prognostic models for the outcome of CLL and concluded, among other things, that the number of affected lymph node areas in previously untreated patients has a significant influences overall survival (OS).(60) However, analyzing our cohort revealed no significant influence of the number of lymph node areas on OS. This may be because more advanced disease responds better to modern therapies. However, it should be noted that all of our patients had a positive lymph node status at the time of diagnosis, so no conclusions can be drawn about the prognostic significance of lymph node status per se. Nevertheless, it is a little surprising, because more affected lymph node regions were associated with the development of Richter transformation, which has a significantly poorer prognosis.(61) However, additional studies with more cases are necessary to examine this question. Furthermore, if you look at the time to therapy, it decreases with the number of affected regions. This has already been described in the literature and indicates that several affected regions are also associated with more advanced disease, meaning that the indications for therapy are therefore reached more quickly.(62)

The 24 patients in our cohort who underwent RT during the course of CLL had a significantly worse overall survival. This is in line with previous studies.(63, 64) Furthermore, we can describe the time to therapy (TTT) in patients with RT as significantly shorter than in patients who never developed RT. A pooled analysis by Al-Sawaf in 2021 already showed that patients who developed RT at a later

stage, had a median TTT of 12,4 months, whereas the TTT of patients without RT at any stage was 90,2 months.(63)

Certain genomic aberrations have both positive and negative effects on overall survival. In our cohort, we focused on those with negative effects.(22) We investigated the outcome of patients with del11q, del17p and trisomy12 in our cohort in terms of overall survival. When the mutations occurred individually, no significant deterioration in overall survival was found. In the case of del 17p mutation this is probably due to the small number of patients with this mutation in our cohort. However, other studies with a higher number of cases have demonstrated that a 17p deletion results in significantly shorter overall survival,(22) including in patients treated with ibrutinib.(65) Furthermore, del17p mutations are a risk factor for RT.(66) Our cohort also revealed a significant association between the occurrence of del17p and RT. However, there was no association exposed between a higher number of lymph node regions and the occurrence of del17p. Whether there is a direct association between these two markers has not yet been investigated, but considering that both are risk factors for RT, a causal relationship may exist. However, further studies are need to further elaborate this association. In 2000, Döhner showed that del11q was associated with the second worst overall survival among chromosomal abnormalities after del17p.(22) In 2019, Thomas J. Kipps examined the impact of dell1q on survival in patients treated with ibrutinib, revealing that his cohort experienced a significantly better outcome than previous studies had demonstrated, suggesting that the prognostic statement of del11q no longer has the same relevance and significance.(67) The prognosis for trisomy 12 mutations was classified as intermediate by Döhner. However, this depends on the parallel occurrence of other mutations such as trisomy 18 and the presence of a Notch1 mutation.(22, 68) Our cohort revealed no significant difference in overall survival between patients with and without trisomy 12.
While del17p and dell1q mutations alone did not significantly worsen survival in our cohort, the combined presence of both mutations was associated with worse overall survival despite the small number of cases. The synergistic effect of both mutations at the same time was already suggested by Jennifer Goy.(69)

The immunoglobulin heavy chain variable gene (IgHV) counts as one of the most important factors in determining the outcome of CLL. Studies have shown the importance of this gene's mutational status on survival. Several studies demonstrated the more malignant course of IgHV unmutated CLL (U-CLL) compared to IgHV mutated CLL (M-CLL) in terms of Overall survival (OS).(19, 70, 71) It has been also been found a shorter time to therapy in patients with unmutated IgHV.(55, 62)
Our study supports the finding of reduced overall survival in patients with unmutated IgHV, however, the significance of our results is limited by the small number of patients for whom IgHV status was determined. This probably also explains why we did neither find a shorter time to therapy in patients with unmutated IgHV nor had they received therapy more frequently.

In our cohort, the event of a dose reduction had no significant impact on overall survival. This is consistent with the results of various studies investigating the impact of reduced doses of individual substances on overall survival, especially with Ibrutinib, but also with Fludarabine.(72) (73) Therefore, a dose reduction appears reasonable, particularly in the event of side effects and in patients with risk factors, as it does not appear to significantly affect mortality.
In 2014, Goede examined the influence of comorbidities on CLL therapy. He exposed that patients with two or more comorbidities did not suffer from adverse effects more frequently but had more often undergone dose reductions of study drugs and treatment discontinuations.(74) In contrast, comorbidities did not seem to influence the necessity of a dose reduction in our cohort. However, our study has limitations in this respect. We only evaluated the quantity and not the severity of the comorbidities Furthermore, we did not record when the comorbidities occurred or how long they existed. We also only compared two groups of patients: those with 0–2 comorbidities and those with more than 2. Consequently, these results are only of limited informative value.

When we compared conventional chemo immunotherapies with new, targeted therapies in terms of adverse effects, there was no significant difference. It should be noted, however, that we only considered neutropenia and infections. A larger

2022 study showed a significantly higher number of adverse effects in patients undergoing F-CR therapy compared to patients receiving ibrutinib-rituximab (IR), the same applied specifically to infections and neutropenia. One possible reasons for the different results in our cohort could be the lower number of cases. Our comparison is also different because we included ibrutinib and venetoclax in the targeted therapies, and venetoclax carries a high risk of neutropenia.(75) The same study also suggested that there is a significantly higher risk of atrial fibrillation and other cardiac adverse effects during therapy with IR.(76) A few other studies described more frequent appearance of atrial fibrillation when receiving therapy with ibrutinib.(77, 78) We also investigated this and found an occurrence of de novo atrial fibrillation in patients receiving treatment with ibrutinib of 10,2%.

4.2 Limitations

Our retrospective study was a one center study. Findings from other health care facilities were not available to us, meaning that some of our documentation was incomplete or not fully comprehensible. The number of cases was comparatively low, in particular the number of patients in whom certain diagnostics were performed, especially IgHV. Our cohort was also very heterogeneous in terms of age and comorbidities and their treatment regimens.

4.3 Conclusion

Our findings suggest that specific clinical and genetic parameters—particularly the number of involved lymph node regions and del17p status—have a substantial impact on disease progression, Richter transformation, and overall survival in CLL. The integration of these factors may facilitate the development of refined risk stratification models for CLL management.

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