# We are IntechOpen, the world's leading publisher of Open Access books
# Built by scientists, for scientists

## 6,900
Open access books available

## 186,000
International authors and editors

## 200M
Downloads

## 154
Countries delivered to

Our authors are among the

## TOP 1%
most cited scientists

## 12.2%
Contributors from top 500 universities

**CLARIVATE ANALYTICS**
**BOOK CITATION INDEX**
**INDEXED**

**WEB OF SCIENCE™**

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

# Interested in publishing with us?
# Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

# Prognostic Factors in Chronic Lymphoid Leukemia and Identification of New Clinically Relevant Molecular Markers

José-Ángel Hernández[1], Marcos González[2] and Jesús-María Hernández[2]
*[1]Hospital Universitario Infanta Leonor, Madrid,*
*[2]Hospital Clínico Universitario, Salamanca,*
*Spain*

## 1. Introduction

Chronic lymphocytic leukemia (CLL) is a hematological malignancy with significant clinical heterogeneity, due in part to the genetic alterations that leukemic cells present in each patient (Chiorazzi et al, 2005). CLL has a highly variable clinical course. Traditionally, it has been considered that about one-third of patients will never require treatment, as they will have prolonged survival and they will die from causes unrelated to the disease. In another third of cases, after an indolent phase disease progression occurs. In the remaining third of patients early treatment is required because of the aggressiveness of the disorder. However, due to the routine performance of blood counts in the population, the number of asymptomatic patients is increasing and, conversely, those who require initial treatment account for fewer than 15% of cases (Hernandez et al, 2010). Since the first descriptions of the disease, researchers have attempted to establish prognostic factors with which to make a risk assessment of disease progression and probability of death. The ultimate aim is to try and apply a targeted and early treatment that increases overall survival and quality of life in patients with more aggressive forms, and to determine reliably the cases who do not need further treatment. (Dighiero & Hamblin, 2008).

Historically, there have been two distinct phases in the analysis of prognostic factors in CLL. Until the late 1980s, most prognostic factors were related to clinical presentation, cellular morphology, the pattern of infiltration of the bone marrow and lymphocyte progression over a period of time. Some of these are no longer considered relevant. Since the 1990s several prognostic factors associated with the immunophenotypic profile, cytogenetic features and mutational status of the immunoglobulin heavy chain (IGVH) have been added (Moreno & Montserrat, 2008). However, nomograms and predictors that include common clinical and pathological factors are still valid.

## 2. Prognostic factors in CLL

### 2.1 Clinical characteristics

In most published series, male patients have a more aggressive clinical course and worse survival than women with CLL (Catovsky et al, 1989). More than 30 years ago, Rai and Binet

established two staging systems by which patients could be classified into low-, intermediate- and high-risk groups according to the presence or absence of certain clinical features (lymphadenopathy, visceromegalies, anemia and thrombocytopenia) (Binet et al, 1981; Rai et al, 1975). Nowadays, both clinical staging systems are widely used because of their simplicity and applicability. Different stages can be defined: early (Rai 0, Binet A), intermediate (Rai I-II, Binet B) and advanced (Rai III-IV, Binet C). These stages have a median overall survival (OS) of 10-12, 7 and 1.5-4 years, respectively (Tables 1 and 2). The Rai and Binet clinical stages have several limitations: a) they are unable to predict which patients from the initial stages will progress; b) they do not considerer tumor burden; c) they do not take into account the mechanism of cytopenias and d) they do not predict the response to therapy. At present, over 80% of cases are diagnosed in early stage A (0), since very often the diagnosis is made in the context of a routine analysis or of comorbidities that are unrelated to CLL.

| Stage | Clinical features | Overall survival (years) |
|-------|-------------------|--------------------------|
| 0 | Blood and bone marrow lymphocytosis* | 10 |
| I | Lymphocytosis and large lymph nodes | 7 |
| II | Lymphocytosis and splenomegaly and/or hepatomegaly with or without large lymph nodes | 7 |
| III | Lymphocytosis and anemia** (hemoglobin level < 11 g/dL) with or without large lymph nodes, splenomegaly or hepatomegaly | 1,5-4 |
| IV | Lymphocytosis and thrombocytopenia** (platelet count < 100 x $10^9$/L) with or without anemia, large lymph nodes, splenomegaly or hepatomegaly | 1,5-4 |

*Lymphocyte count > 5 x $10^9$/L in peripheral blood and > 30% of nucleated cells in bone marrow aspiration count.**Immune anemias or thrombocytopenias are excluded.

Table 1. Rai clinical stage system.

| Stage | Clinical features | Overall survival (years) |
|-------|-------------------|--------------------------|
| A | Lymphocytosis in peripheral blood and bone marrow* and < 3 lymphoid regions involved**. No anemia, no thrombocytopenia | 12 |
| B | Lymphocytosis in peripheral blood and bone marrow* and ≥ 3 lymphoid regions involved **, with or without splenomegaly and/or hepatomegaly. No anemia, no thrombocytopenia | 7 |
| C | Lymphocytosis * with anemia*** (hemoglobin level < 11 g/dL in male and < 10 g/dL in female) or thrombocytopenia*** (platelet count < 100 x $10^9$/L) | 2-4 |

*Lymphocyte count > 5 x $10^9$/L in peripheral blood and > 30% of nucleated cells in bone marrow aspiration count. **Each cervical, axillary and inguinal area can be unilateral or bilateral. Splenomegaly and hepatomegaly are one lymphoid region (5 areas). ***Immune anemias or thrombocytopenias are excluded.

Table 2. Binet clinical stage system.

## 2.2 Morphological features

Prolymphocytic transformation of CLL carries a worse prognosis, as well as atypical CLL ( > 15% cell morphology is not compatible with the CLL, such as the presence of cleaved nuclei lymphocytes) compared with the typical morphology of CLL. It has recently been reported that patients with more than 30% of nuclear shadows (smudge cells) in the differential count are more likely to have a mutated IGVH pattern and, therefore, a longer time to treatment and better survival (Nowakowski et al, 2007).

The pattern of bone marrow infiltration may be nodular, interstitial, mixed (the most common type) and diffuse. In practice, we have to consider the first three as non-diffuse, because there are prognostic differences between patients with diffuse vs non-diffuse pattern. Patients treated with fludarabine schedules often show a persistent nodular pattern after therapy (nodular partial response), which represents a higher quality response than a partial one. The group at the Hospital Clinic, Barcelona, showed that the presence of a diffuse pattern of bone marrow infiltration is associated with a worse prognosis (Montserrat & Rozman, 1987). However, this prognostic factor was not independently confirmed by others, when new genetic markers were included in the analysis (Geisler et al, 1996).

## 2.3 Markers of proliferation or tumor burden and markers of angiogenesis

A lymphocyte doubling time (LDT) less than 6 months or an increase in lymphocyte count > 50% in 2 months is associated with a worse prognosis because they indicate an increased activity of the disease (Viñolas et al, 1987). In any case, LDT should only be recommended to initiate the treatment of CLL or to establish the prognosis with elevated lymphocyte counts ($> 30 \times 10^9$/L). Despite their limitations, including the changes that occur during the course of the disease, LDT is a simple and inexpensive method, that is still interpreted in most clinical trials and clinical practice as an indication to start treatment.

Elevation of serum levels of lactate dehydrogenase (LDH), β2 microglobulin (B2M), thymidine kinase (TK) and soluble CD23 also indicate a high tumor burden (Hallek et al, 1996; Sarfati et al, 1996). Of these, LDH, contrary to what is observed in other lymphoproliferative disorders, is of minor relevance, although it is used in clinical practice due to the simplicity of its determination.

Elevated levels of TK are correlated with increased proliferation and predict the progression of CLL. In patients with early-stage CLL, high TK levels are correlated with the expression of CD38, ZAP-70, poor prognostic cytogenetic abnormalities and unmutated IGVH status. Moreover, serum TK levels have an independent value in the differentiation of CLL patients in early stages according to progression-free survival (PFS). Two limitations of the use of TK as a prognostic factor are the variation between laboratories, as well as their levels can be increased in patients with viral infections. However, its prognostic value in early-stage CLL has been fully established.

High levels of serum B2M, a protein that binds to the class I major histocompatibility complex, is one of the most important prognostic factors in some of the reported series. Moreover, their levels are correlated with the expression of CD38 and ZAP-70. Recently, the MD Anderson Cancer Center has proposed a prognostic nomogram that includes age, sex, absolute lymphocyte count, the number of lymphoid areas involved and B2M (Wierda et al,

2007). Furthermore, this group has also confirmed that low levels of B2M are independently associated with better complete responses (CR) rates, disease-free survival (DFS) and OS in patients treated with fludarabine-based schemes, with or without the addition of rituximab. The prognostic value of B2M in patients with impaired renal function is limited. Nevertheless, B2M is currently one of the most important prognostic factors for evaluating patients with advanced-stage CLL.

High levels and/or duplication of soluble CD23 also predicts a worse outcome for patients with CLL, with progression of patients in early stages and decreased survival.

Finally, the rise in the microvascular density and high levels of growth factor (VEGF) are also associated with poor prognosis (Ferrajoli et al, 2001).

## 2.4 Diagnostic imaging

Changes in abdominal computed tomography is a predictor of progression in patients with early-stage CLL, so its inclusion in the initial diagnostic tests can provide clinically relevant information (Muntañola et al, 2007). Even so, there are controversies regarding its value as a prognostic factor.

## 2.5 Immunophenotypic markers expression

### 2.5.1 Expression of CD38

CD38 is expressed in various hematopoietic cells and progenitors, thymus cells, T cells and activated B cells in later stages of differentiation. Determining the expression of CD38 is a useful tool and the results are easily analyzed to determine the prognosis of patients with CLL. However, because it is not a unique antigen of the proliferating cell in CLL it should not be analyzed independently, but along with the CD19 or CD20 and CD5, due to the aforementioned expression in other mononuclear cells. CD38 has been proposed as a prognostic marker in CLL, which has led to CD38 being proposed as a prognostic marker in CLL, indicating a more aggressive disease (Damle et al, 1999; Ghia et al, 2003). However, there is no consensus about the cutoff of positivity. Some authors suggest this to be 7%, although most choose levels above 30%. Different levels of CD38 expression can be observed over the course of the illness, during which its prognostic relevance diminishes. In fact, CD38 expression is not a perfect marker that can be a surrogates for IGVH mutational status, although it is associated with an increased incidence of organomegaly, bad prognosis cytogenetics, high B2M serum level and worse PFS and OS (Hamblin et al, 2002).

### 2.5.2 Expression of ZAP-70

The chain-associated protein zeta 70 (ZAP-70) is an intracellular tyrosine kinase Syk family/ZAP, which is associated with the zeta chain of the T cell receptor (TCR). Its expression is normally restricted to T and NK cells, which initiate the signaling pathways of T cells, resulting in the activation, differentiation and proliferation of effector cell functions in response to TCR stimulation. B cells of CLL may variably express this marker, but its positivity is one of the most powerful prognostic factors for predicting the course of the disease. The expression of ZAP-70 can be performed by various molecular techniques such as western blot, immunohistochemistry, RT-PCR, microarray expression and flow

cytometry. One of the weaknesses of its determination by flow cytometry is the lack of reproducibility of the results. Several research groups have attempted to standardize the methodology in recent years (Letestu et al, 2006). It is likely that once it has been determined, along with other clinical and biological markers, it will help clinicians to assess the prognosis in newly diagnosed CLL patients more reliably. Results published by the Barcelona group, with a 20 % cutoff of ZAP-70, determined by flow cytometry (a higher level of expression indicates a worse prognosis) demonstrated significant differences in the OS and PFS of patients with CLL (Crespo et al, 2003). Unlike the case with CD38, ZAP-70 expression seems to be better than IGVH mutational status in predicting the time to receive the first treatment. The concordance between ZAP-70 expression and IGVH mutational status is 75-90% (Rassenti et al, 2004). When the positivity of ZAP70 and CD38 expression are combined, the time to treatment is 30 months, while it is 130 months in cases where both markers are negative.

However, the expression of both CD38 and ZAP-70 has proved controversial in the scientific community regarding its prognostic value for the next reasons: a) different results may be obtained with the same samples in different laboratories (indicating lack of validity and reproducibility of the techniques used), b) there may be temporal variations in the expression of CD38, c) it is difficult to establish the correct cutoff point for the expression of CD38 (<vs> 7% <vs> 30%) and ZAP-70 (the most widely accepted value being 20%), d) a careful separation of T cells for the determination of ZAP-70 by flow cytometry techniques is mandatory, which has meant that, even recently, several experts in this area have tried to systematize the method of determination, and e) a 20-30% discrepancy in the results of ZAP-70 provided by immunophenotyping by flow cytometry and IGVH mutational status has been described.

The CD49d antigen, whose expression is associated with a worse prognosis, has acquired a special significance in recent years (Gattei et al, 2008).

## 2.6 IGVH mutational status

One of the most important genetic parameters to establish the prognosis of patients with CLL is the mutational status of VH genes. Somatic mutations of the VH gene region of the heavy chain of immunoglobulins are present in about half of all CLL cases. In 1999, two research groups reported the importance of this observation as a predictor of disease progression, with survival of 8 years in cases of patients with CLL and unmutated pattern *vs* 24 years in those with mutated status (Damle et al, 1999; Hamblin et al, 1999). Unmutated cases originate from cells in the pregerminal center and clearly have a worse prognosis than mutated CLL cells also arising from the postgerminal center. The definition of non-mutated *vs* mutated pattern resides in a cutoff point, defined arbitrarily as a homology greater than 98% (non-mutated) gene most similar to the germline (Schroeder & Dighiero, 1994).

Patients with CLL and an unmutated status have an unfavorable course and progress more rapidly, as opposed to patients exhibiting a mutated state, whose survival is much better (Figures 1, 2). Unmutated CLL patients have a greater tendency to acquire poor prognostic cytogenetic abnormalities. It has also been observed that, irrespective of mutational status, some VH regions are associated with specific clinical features and different geographical incidences (Ghia et al, 2005). This is the case for IGHV3-21 usage, whose involvement

provides a worse prognosis regardless of mutational status and, characteristically, is less prevalent in southern European countries, as confirmed by the results of an Italian group, who even showed that it is less frequent in southern than in northern Italy. This poorer clinical behavior of patients with IGHV3-21 may be explained because of the complementarity determining regions (HCDR3) are shorter and it is possible that the stimulatory influence of some unknown antigen leads to CLL progression. Other genes from the IGHV3 family, the most frequently used subgroup in CLL, are associated with prognosis. Thus, IGHV3-23 is related with a bad prognosis. On the other hand, IGHV3-72 and IGHV3-30 usages indicate good clinical outcomes, including spontaneous regression in anecdotal cases (Dal-Bo et al, 2011). Moreover, the involvement of the IGHV1-69 family, although it does not seem to have a lower survival compared to patients expressing other unmutated genes, and the IGHV4-39 usage occurs mainly in unmutated cases, while the IGHV4-34 and most cases of IGHV3 contain mutated cases. Patients with unmutated state have a poor prognosis if an autologous transplantation is performed, although the graft versus leukemia effect may counteract the therapeutic resistance of these patients if an allogeneic transplant is offered. The rearrangements of IGHV3-48 and IGHV3-53 are also associated with poor prognosis.

IGVH mutational status and cytogenetic abnormalities identified by FISH have a major impact on the survival of patients with CLL, but while cytogenetic changes during the course of the disease are relatively common, IGVH mutational status remains constant over time. One of the limitations of its use is the high cost of testing, due to its laboriousness and the expertise required.
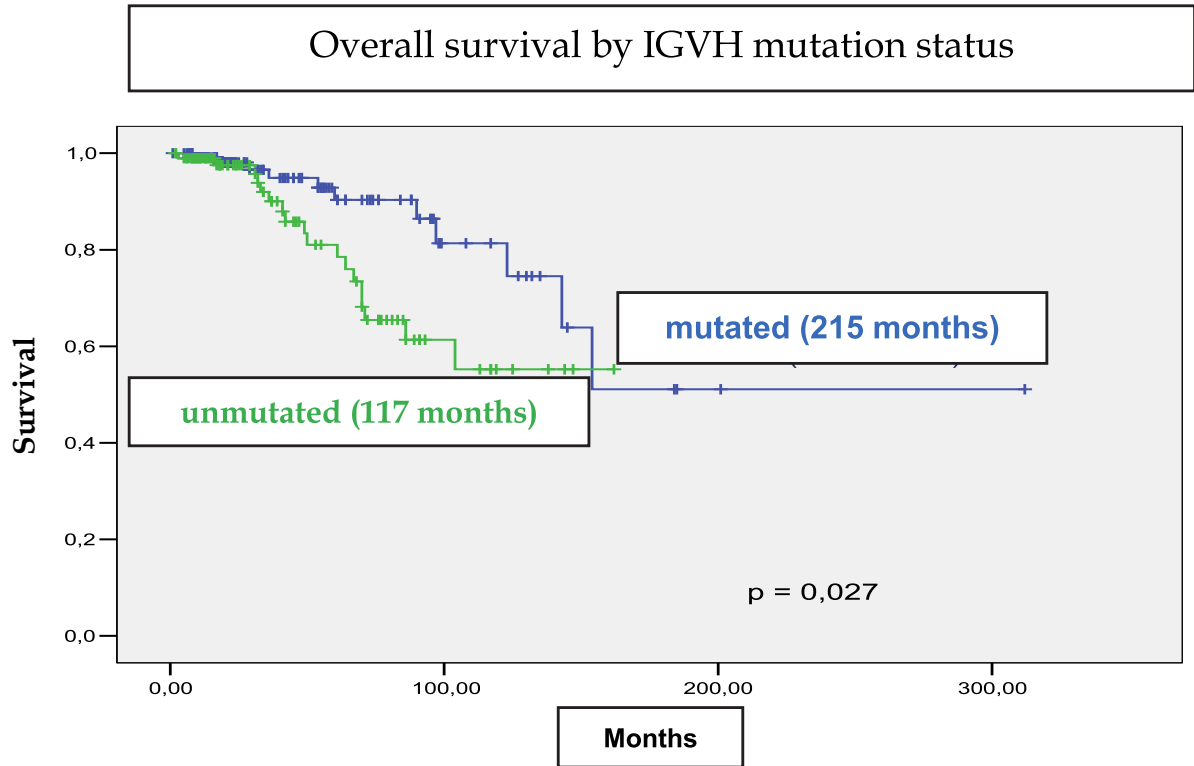


Fig. 1. Overall survival of the Salamanca University series of 226 patients with CLL by IGVH mutation status.
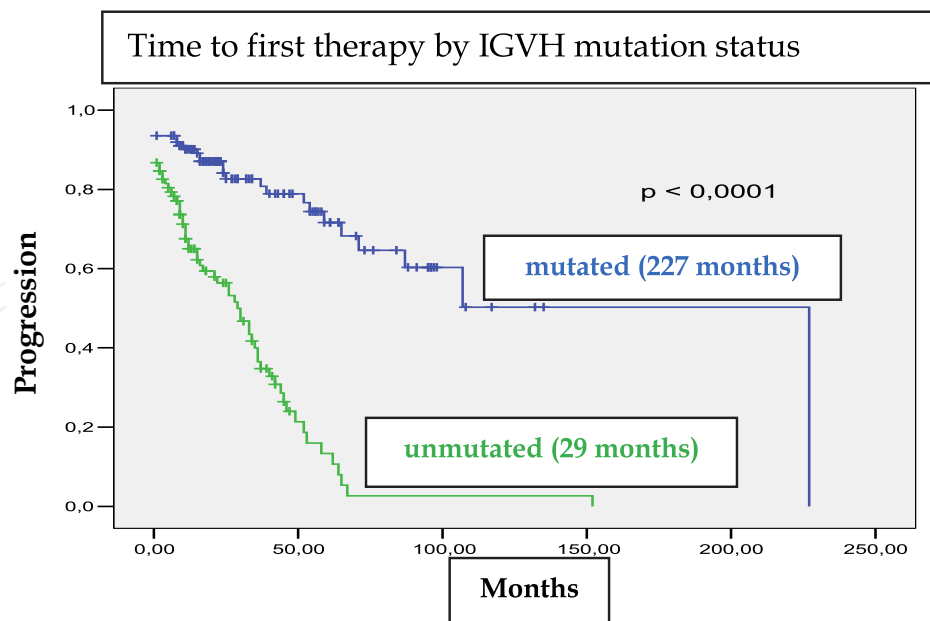
Fig. 2. Time to first therapy of the Salamanca University series of 226 patients with CLL by IGVH mutation status.

## 2.7 Cytogenetics and fluorescence in situ hybridization (FISH)

In 2000, the University of Ulm Group published their results from 325 patients with CLL concerning the relationship of various cytogenetic abnormalities, determined by FISH, with survival (Dohner et al, 2000). Using a panel of eight FISH probes, they analyzed the losses in 6q, 11q, 13q and 17p, the trisomies of 3q26, 8q24 and 12q13 and 14q32 translocations. They found that 82% of patients had chromosomal abnormalities, some of which were of prognostic relevance. In order of frequency, loss of 13q14 was the most frequent (present in 55% of cases), followed by loss of 11q22-23 (18%), trisomy of chromosome 12 (16%), loss of 17p13 (7%) and loss of 6q21 (6%). Only 57 patients (18%) had no abnormalities according to FISH, while 67 and 26 patients had two or more cytogenetic abnormalities, respectively. Median survival of patients with 17p-, 11q-, trisomy 12, normal cytogenetics and 13q- as a single alteration were 32, 79, 114, 111 and 133 months, respectively. In addition, patients with 17p- had the shortest interval before first treatment (9 months), whereas this period was longest in those with 13q- (92 months). In the Cox regression of overall survival time, patients with 17p deletion had a hazard ratio eight times that of other patients, whereas for those with 11q loss the hazard ratio was somewhat less than 3. These results have been reproduced in several series (Figure 3) (Tables 3, 4).

It is of note that some of the cytogenetic changes are related to characteristics of the disease: patients with 11q- tend to be younger and have marked lymphadenopathy, while those with 17p- are resistant to standard treatments, including that with fludarabine. 17p and 11q deletions were independently associated with other prognostic factors, such as IGVH mutation state, and the patients with these deletions had an adverse clinical outcome with progression of CLL and decreased survival (Krober et al, 2006). The British Group recently reported that patients with a more than 20% loss of function of TP53, a gene located on 17p, have a worse prognosis than those with a lower percentage loss (Gonzalez et al, 2011).

On the other hand, although it has customarily been considered that patients with del (13q) have a better prognosis, those with a high number of losses and/or the size of the deletion is greater have a worse prognosis in terms of time to receive the first treatment and survival, as shown recently by four independent groups (Figures 4, 5) (Dal Bo et al, 2011). Our genomic expression profile (GEP) studies have shown that those cases with a high number of losses of 13q have a higher expression of genes related to proliferation and reduced expression of apoptosis-related genes (Hernandez et al, 2009).

The 14q32/IGH translocation is present in 5-7% of CLL cases. Patients with IGH rearrangements can be classified in the intermediate prognosis group, as occurs with 6q deletion CLL patients (Cavazzini et al, 2008).

Clonal evolution may be observed during the course of CLL with the acquisition of new cytogenetic abnormalities (Stilgenbauer et al, 2007). These cytogenetic aberrations occur in 20-45% of patients and are associated with the presence of unmutated state and/or ZAP-70 expression. Therefore, FISH analysis should be done to diagnose CLL, before starting treatment and during relapse.

Finally, it has recently been shown that the presence of chromosomal translocations is associated with poor prognosis in CLL patients and that the length of telomeres is a prognostic factor related to mutation status (Sellmann et al, 2011).
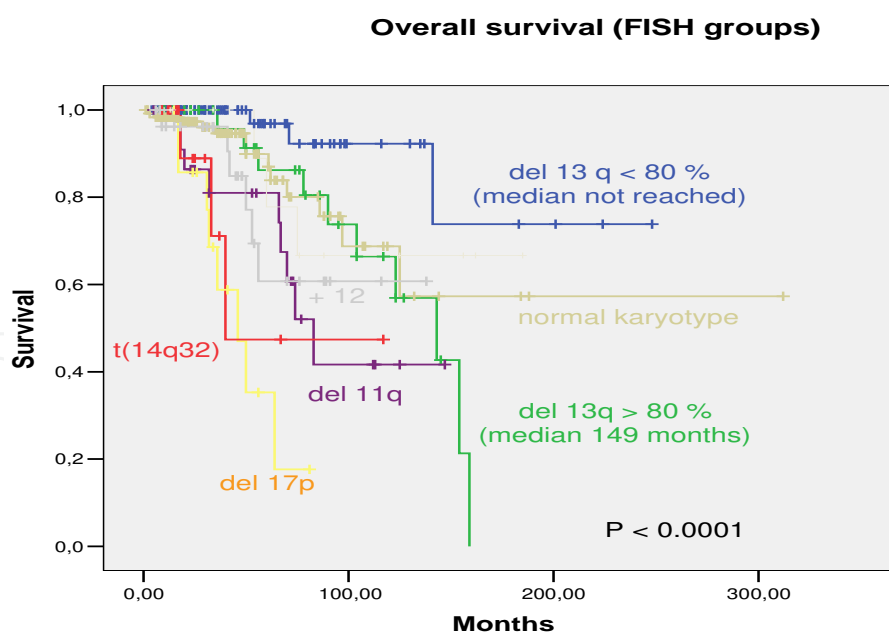


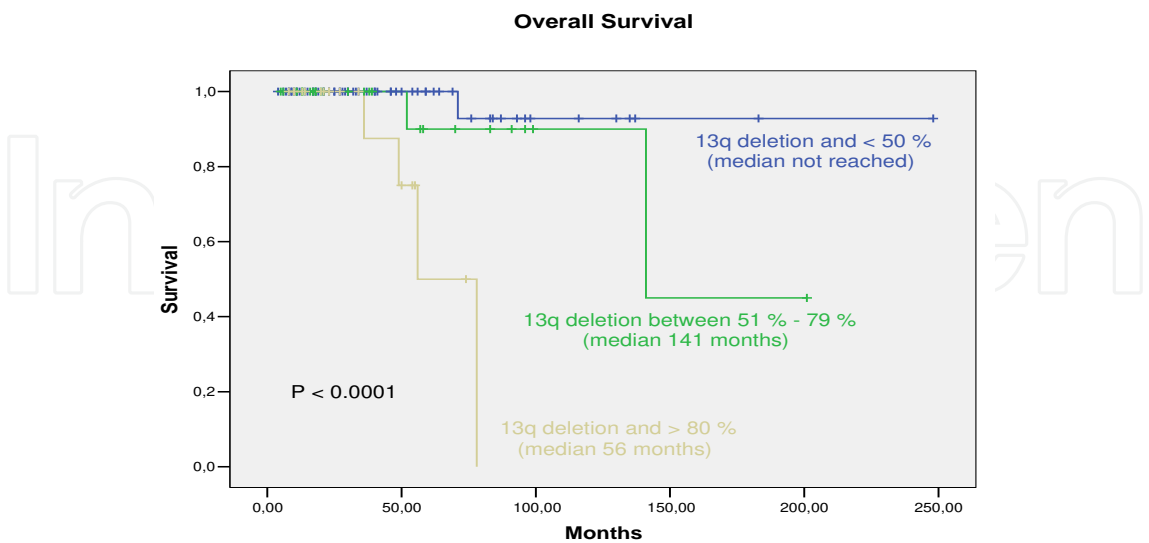Fig. 3. Overall survival of the Salamanca University series of 350 patients with CLL by FISH group.

Fig. 4. Overall survival of the Salamanca University series of 109 patients with 13q deletion as unique alteration at diagnosis. A high number of losses in 13q is associated with a worse prognosis.
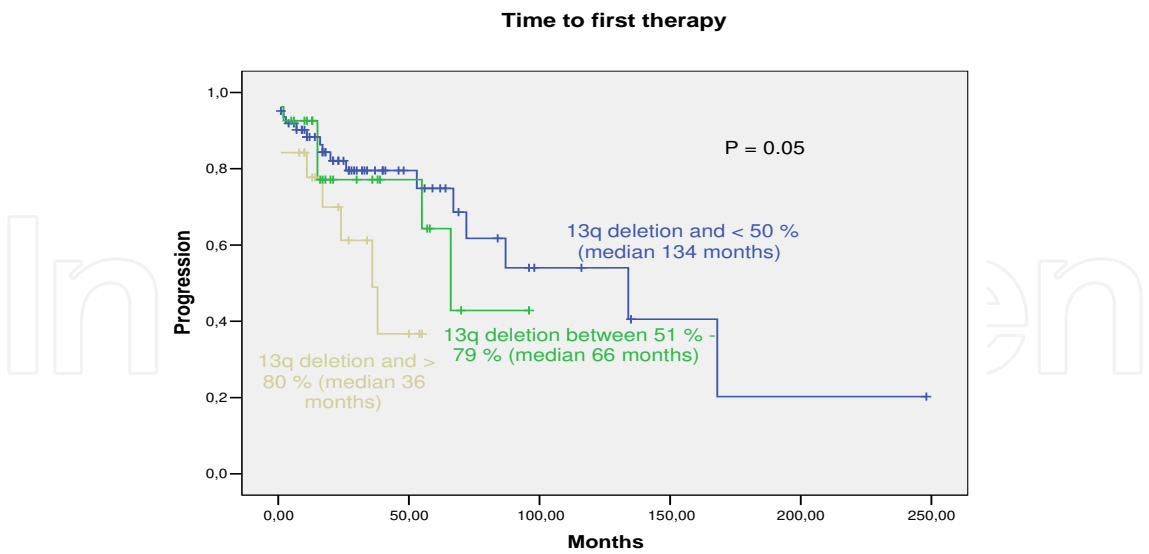


Fig. 5. Time to first therapy of the Salamanca University series of 109 patients with 13q deletion as unique alteration at diagnosis. A high number of losses in 13q is associated with a worse prognosis.

| Study | 13q⁻ | 13q⁻as unique alteration | 11q⁻ | +12 | 17p⁻ | Mutated status | Unmutated status |
|---|---|---|---|---|---|---|---|
| Ulm University | 55 | 36 | 18 | 16 | 7 | 56 | 44 |
| CLL1[1] | 59 | 40 | 10 | 13 | 4 | 41 | 59 |
| CLL4[2] | 53 | 34 | 21 | 11 | 3 | 69 | 31 |
| CLL3[3] | 52 | 27 | 22 | 12 | 3 | 68 | 32 |
| CLL2H[4] | 48 | 14 | 32 | 18 | 27 | 81 | 19 |
| Salamanca University[5] | 46 | 36 | 9 | 12 | 4 | 56 | 44 |

*Results indicate percentages. 1. Patients in Binet A clinical stage without classical indication of therapy. 2. Patients aged < 65 years and Binet B and C clinical stages, included in randomized clinical trial (fludarabine *vs* fludarabine + cyclophosphamide). 3. Patients aged < 60 years and Binet B and C clinical stages, included in a clinical trial of autologous transplantation. 4. Patients refractory to fludarabine included in a clinical trial of subcutaneous alemtuzumab. 5. t(14q32) in 6 % of patients.

Table 3. Incidence of genomic aberrations and IGVH mutational status in several series of the CLL German Study Group and our series from the Salamanca University*

| Variable | Hazard ratio  (95% CI*) |
|---|---|
| Deletion 17p | 7.03 (3.23-15.3) |
| IGVH1-69 usage | 2.60 (1.04-6.44) |
| Deletion 11q | 2.52 (1.29-4.90) |
| Normal cytogenetics | 0.47 (0.23-0.97) |
| Deletion 13q as unique aberration | 0.34 (0.18-0.64) |
| Mutated IGVH status | 0.43 (0.20-0.91) |

*CI: confidence interval.

Table 4. Cox regression analysis of overall survival in the series of CLL patients of the CLL patients of the Salamanca University

## 2.8 MicroRNAs

MicroRNAs (miRNA) are a class of RNAs that modulate the expression of post-transcriptional genes. miRNAs are small, non-coding RNAs involved in cancer genesis, apoptosis and the cell metabolism. These molecules show a role in CLL pathogenesis and prognosis (Calin et al, 2005). So, miR-29 has been described as a tumor-suppressing molecule that targets oncogenes like TCL1. This oncogene is overexpressed in CLL and is associated with an unmutated IGVH status, a high level of ZAP-70 expression and high-risk cytogenetics. In CLL patients, TCL1 protein expression is inversely correlated with miR-29 and miR-81 expression. Recently, it has been confirmed that the expression of miR-29 and miR-223 are correlated with poor prognosis. These two miRNAs, ZAP-70 and lipoprotein lipase (LPL) are the four variables comprising a new progressive prognostic score from 0 to 4 with the median TFS decreased from 312 months in the very good prognosis group to 12 months in the poor prognosis group. Also, high and low levels of expression of miR-21 and miR-181b, respectively, have been reported as risk prognostic factors, and miR-15a and miR-

16 expression is related to IGVH mutation status. Finally, a correlation between 17p deletion, TP53 and miR-34a has been reported. It seems likely that miRNA research will be increasingly influential in determining the prognosis of CLL, and some of these molecules may prove to be surrogate markers in this disorder (Ward et al, 2011).

## 2.9 Lipoproteinlipase (LPL) and ADAM 29 gene expression

Recently, several studies have demonstrated the importance of LPL expression, such as that of the principal RNA prognostic marker. A comparative study of RNA-based markers in CLL revealed LPL to be a powerful predictor of clinical outcome. In the initial report the LPL/ADAM29 expression ratio was described as a strong prognosis indicator in CLL, that enabled a better assessment than ZAP-70 in advances stages of CLL (Oppezzo et al, 2005).

## 2.10 Genomic expression profiles

Another area of development is the analysis of gene expression by RNA microarrays, which has led to improved diagnosis and classification of the neoplasms of leukemia patients. Although little information is currently available, the discovery through the analysis of microarray gene expression of a group of genes associated with survival of patients reflects the potential of this technology to detect new markers that may be prognostically relevant. In 2001, it was demonstrated the association between gene expression and mutational pattern and the existence of a homogeneous phenotype related to memory B cells in patients with CLL (Klein et al, 2001; Rosenwald et al, 2001). Subsequently, it was observed that the study of gene expression profiles showed a common molecular signature in patients with CLL, what contributed to the identification of markers of progression or it was different in mutated and unmutated CLL. In addition, genes that are significantly more highly expressed are located in the corresponding aberrant chromosomal regions, indicating the existence of a genetic effect of dose, which may have a pathogenic role in CLL. Significant differences in gene expression according to sex were also found, which suggests that differences in molecular signatures relating to IGVH mutational status may be related to the sex of the patient (Haslinger et al, 2004). Several genes have been implicated in the pathogenesis and prognosis of CLL. Other research lines related to the DNA methylation and the phosphorylation of receptor and adaptor proteins are providing an increasing amount of information about this disease and could have a prognostic role (Prieto-Sanchez et al, 2006). Even so, more work remains to be done in this field (Codony et al, 2009).

## 2.11 Treatment response and prognosis

The quality, depth and length of the response in the CLL treatment are of great prognostic importance. The achievement of a complete or a nodular partial response predicts better DFS and OS. In addition, some therapy schedules, mainly those based on immunochemotherapy (i.e., FCR combination) might overcome the dismal prognosis of patients with CLL and del (11q). On the other hand, negative minimal residual disease (MRD) improves the outcome of CLL patients, in terms of PFS, TTT and OS. Nevertheless, MRD assessment is not recommended in clinical practice, although it might be of great value in the coming years (Cramer & Hallek, 2011).

### 2.12 Comorbidities and prognosis

Performance status, physical fitness and comorbidities are important features in the selection of therapy and thereby in the prognosis of patients with CLL (Zenz et al, 2010). In this context, patients with CLL are divided in three groups: a) Fit or 'Go go' patients, for whom a standard treatment can be administered with the aim of achieving the best response, such as FCR; b) 'Slow go' patients, who should be treated with modified therapies in order to control the disease; c) Unfit or 'No go' patients, who should receive palliative care.

### 2.13 Other prognostic factors

### 2.13.1 Bcl2 and other immunophenotypic markers

Patients who are CD71+ and Bcl2+ have a shorter PFS and OS than those who are CD71- and Bcl2. In a recent paper, the independent prognostic value of bcl-2 was confirmed within ZAP-70 negative patients (Del Poeta et al, 2010). Other immunophenotypic markers, such as soluble CD20, have been investigated as potential prognostic factors in CLL.

### 2.13.2 CD26; CD44

CD26 antigen is strongly upregulated in activated B cells. CD26-positive patients show a shorter time to treatment and this positivity is correlated with ZAP-70 expression or IGVH mutational status (Cro et al, 2009). On the other hand, high levels of soluble CD44 predict the risk of illness progression in patients with early-stage CLL.

### 2.13.3 Circulating endothelial cells (CECs)

In patients with CLL, as occurs in other malignancies, CECs are increased and are correlated with an aggressive clinical outcome. In a recent report, the gene expression profile in patients with higher levels of CECs indicated increased cell survival and proliferation, diminished cell adhesion to the extracellular matrix, and enhanced proangiogenic function. CECs might be considered a biological marker for new targeted antiangiogenic therapies (Rigolin et al, 2010).

### 2.13.4 CLLU1 expression

High CLLU1 expression levels are associated with shorter OS in patients younger than 70 years of age. CLLU1 expression analysis adds prognostic information in risk prediction in CLL patients with the exception of those who have an unmutated IGVH status (Josefsson et al, 2007).

### 2.13.5 Interleukin (IL) 6, IL-8 and IL-10

IL-6 is a strong predictor of shorter survival in CLL patients with advanced disease. Furthermore, high levels of IL-8 are associated with shorter OS (Wierda et al, 2003). Finally, IL-10 levels are elevated in patients with CLL and are correlated with adverse clinical and biological characteristics of the disease and with shorter survival. The role of several IL inhibitors in the treatment of patients with CLL is currently being explored (Fayad et al, 2001) .

### 2.13.6 Matrix metalloproteinase-9 (MMP9)

MMP9 is involved in migration and tissue invasion in patients with CLL. The combined macromolecular cell surface complex formed by CD38, CD49d, CD44 and MMP9 is associated with a dismal prognosis, and, recently, it has been suggested as a novel therapeutic target (Buggins et al, 2011).

### 2.13.7 PEG10 expression

The overexpression of the paternally expressed gene 10 (PEG10) is observed in high-risk CLL patients, defined by high levels of LPL mRNA expression. Recently, PEG10 has been proposed as a new marker in CLL by Austrian and German researchers (Kainz et al, 2007).

### 2.13.8 Telomerase activity and telomere length

Several reports have illustrated the role of telomerase activity and telomere length in cancer prognosis. In CLL, short telomeres and high telomerase activity are associated with poor prognosis (Sellmann et al, 2011). Telomerase inhibitors are being investigated as novel targeted therapies in CLL.

### 2.13.9 TOSO/FCMR expression

Recently, the overexpression of the new gene TOSO (or FC mu receptor [FCMR, FAIM3/TOSO]) has been shown to be associated with the Binet clinical stage, IGVH mutation status, age and time to treatment in CLL. Furthermore, a high level of expression of TOSO is an independent predictor of shorter SLT in CLL (Hancer et al, 2011). However, no correlation has been found between the expression of TOSO and ZAP-70 or CD38. On the other hand, overexpression of FCMR seems to promote chromosomal abnormalities.

### 2.13.10 Tumor necrosis factor (TNF) alpha

MD Anderson Clinical Cancer investigators reported several years ago a correlation between elevated TNF-alpha levels and advanced clinical stage patients, high B2M levels and lower hemoglobin and platelet counts (Ferrajoli et al, 2002).

### 2.13.11 Rel A DNA binding

Researchers from Cardiff University recently demonstrated the importance of the NF-kappa B subunit Rel A in CLL (Hewamana et al, 2009). Rel A DNA binding appears to be strongly associated with advanced Binet stage, time to first therapy and survival. In addition, it seems to have the unique capacity to predict the duration of response to therapy.

## 3. Conclusions

A wide variety of prognostic factors have been studied in CLL, but clinical staging according to Binet or Rai systems, LTD and B2M are the main clinical and biological prognostic markers. Cytogenetics, using FISH (especially using the del (17p) probe), and expression of CD38 and ZAP-70 as surrogate markers for the IgVH mutational status are used routinely worldwide, although CD38 and ZAP-70 are not mandatory in clinical

practice. New markers such as LPL, miR29c and TCL7 predict OS in CLL. On the other hand, the evaluation of patient physical fitness and the assessment of the response to therapy are critical elements.

Recently, sequencing the CLL genome and advances in computing and robotics have produced a revolution in general genetics and CLL (Puente et al, 2011). The combination of these methodologies has led to the development of microarray technology, enabling thousands of genes to be analyzed simultaneously. In CLL patients, the gene expression profile indicates that significantly differentiated genes are located in regions with chromosomal aberrations. However, the evidence linking specific genetic alterations using FISH or mutational status to the results of arrays is still not very consistent.

## 4. References

Binet, JL.; Auquier, A.; Dighiero, G., et al. (1981). A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. *Cancer*, Vol.48, No.4, pp. 198-206, ISSN 0008-543X.

Buggins, AG.; Levi, A.; Gohil, S., et al. (2011). Evidence for a macromolecular complex in poor prognosis CLL that contains CD38, CD49d, CD44 and MMP-9. *Br J Haematol*, Vol.154, No.2, pp. 216-222, ISSN 1365-2141.

Calin, GA.; Ferracin, M.; Cimmino, A., et al. (2005). A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med*, Vol.353, No.17, pp. 1793-1801. ISSN 1533-4406.

Catovsky, D.; Fooks, J. & Richards, S. (1989). Prognostic factors in chronic lymphocytic leukaemia: the importance of age, sex and response to treatment in survival. A report from the MRC CLL 1 trial. MRC Working Party on Leukaemia in Adults. *Br J Haematol*, Vol.72, No.2, pp. 141-149. ISSN 0007-1048.

Cavazzini, F.; Hernández, JA.; Gozzetti, A., et al. (2008). Chromosome 14q32 translocations involving the immunoglobulin heavy chain locus in chronic lymphocytic leukemia identify a disease subset with poor prognosis. *Br J Haematol*, Vol.142, No.4, pp. 529-537. ISSN 1365-2141.

Chiorazzi, N.; Rai, KR. & Ferrarini, M. (2005). Chronic lymphocytic leukemia. *N Engl J Med*, Vol.352, No.8, pp. 804-815. ISSN 1533-4406.

Codony, C.; Crespo, M.; Abrisqueta, P., et al. (2009). Gene expression profiling in chronic lymphocytic leukaemia. *Best Pract Res Clin Haematol*, Vol.22, No.2, pp. 211-22.ISSN 1532-1924.

Cramer, P. & Hallek, M. (2011). Prognostic factors in chronic lymphocytic leukemia-what do we need to know?. *Nat Rev Clin Oncol*, Vol.8, No.1, pp. 38-47. ISSN 1759-4782.

Crespo, M.; Bosch, F.; Villamor, N., et al. (2003). ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med*, Vol.348, No.18, pp. 1764-1775. ISSN 1533-4406.

Cro, L.; Morabito, F.; Zucal, N., et al. (2009). CD26 expression in mature B-cell neoplasia: its possible role as a new prognostic marker in B-CLL. *Hematol Oncol*, Vol.27, No.3, pp. 140-7. ISSN 1099-1069.

Dal Bo, M.; Rossi, FM.; Rossi, D., et al. (2011a). 13q14 deletion size and number of deleted cells both influence prognosis in chronic lymphocytic leukemia. *Genes, Chromosomes Cancer*, Vol.50, No.8, pp. 633-643. ISSN 1098-2264.

Dal-Bo, M; Del Giudice, I; Bomben, R., et al. (2011b). B-cell receptor, clinical course and prognosis in chronic lymphocytic leukaemia: the growing saga of the IGHV3 subgroup gene usage. *Br J Haematol*, Vol.153, No.1, pp. 3-14. ISSN 1365-2141.

Damle, RN.; Wasil, T.; Fais, F., et al. (1999). Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*, Vol.94, No.6, pp. 1840-1847. ISSN 0006-4971.

Del Poeta, G.; Del Principe, MI.; Maurillo, L., et al. (2010). Spontaneous apoptosis and proliferation detected by BCL-2 and CD71 proteins are important progression indicators within ZAP-70 negative chronic lymphocytic leukemia. *Leuk Lymphoma*, Vol.51, No.1, pp. 95-106. ISSN 1029-2403.

Dighiero, G. & Hamblin, TJ. (2008). Chronic lymphocytic leukaemia. *Lancet*, Vol.371, No.9617, pp. 1017-1029. ISSN 1474-547X.

Dohner, H.; Stilgenbauer, S.; Benner, A., et al. (2000). Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*, Vol.343, No.26, pp. 1910-1916. ISSN 0028-4793.

Fayad, L; Keating, MJ.; Reuben, JM., et al. (2001). Interleukin-6 and interleukin-10 levels in chronic lymphocytic leukemia: correlation with phenotypic characteristics and outcome. *Blood*, Vol.97, No.1, pp. 256-263. ISSN 0006-4971.

Ferrajoli, A; Keating, MJ; Manshouri, T., et al. (2002). The clinical significance of tumor necrosis factor-alpha plasma level in patients having chronic lymphocytic leukemia. *Blood*, Vol.100, No.4, pp. 1215-1219. ISSN 0006-4971.

Ferrajoli, A.; Manshouri, T.; Estrov, Z., et al. (2001). High levels of vascular endothelial growth factor receptor-2 correlate with shortened survival in chronic lymphocytic leukemia. *Clin Cancer Res*, Vol.7, No.4, pp. 795-799. ISSN 1078-0432.

Gattei, V.; Bulian, P.; Del Principe, MI., et al. (2008). Relevance of CD49d protein expression as overall survival and progressive disease prognosticator in chronic lymphocytic leukemia. *Blood*, Vol.111, No.2, pp. 865-873. ISSN 0006-4971.

Geisler, CH.; Hou-Jensen, K.; Jensen, OM., et al. (1996). The bone-marrow infiltration pattern in B-cell chronic lymphocytic leukemia is not an important prognostic factor. Danish CLL Study Group. *Eur J Haematol*, Vol.57, No. 4, pp. 292-300. ISSN: 0902-4441.

Ghia, P.; Guida, G.; Stella, S., et al. (2003). The pattern of CD38 expression defines a distinct subset of chronic lymphocytic leukemia (CLL) patients at risk of disease progression. *Blood*, Vol.101, No.4, pp. 1262-1269. ISSN 0006-4971.

Ghia, P.; Stamatopoulos, K.; Belessi, C., et al. (2005). Geographic patterns and pathogenetic implications of IGHV gene usage in chronic lymphocytic leukemia: the lesson of the IGHV3-21 gene. *Blood*, Vol.105, No. 4, pp. 1678-1685. ISSN 0006-4971.

Gonzalez, D,; Martínez, P., Wade, R., et al. (2011). Mutational status of the TP53 gene as a predictor of response and survival in patients with chronic lymphocytic leukemia: results from the LRF CLL4 trial. *J Clin Oncol*, Vol.29, No.16, pp. 2223-2229. ISSN 1527-7755.

Haferlach, T.; Kohlmann, A.; Schnittger, S., et al. (2005) Global approach to the diagnosis of leukemia using gene expression profiling. *Blood*, Vol.106, No.4, pp. 1189-1198. ISSN 0006-4971.

Hallek, M.; Wanders, L.; Ostwald, M., et al. (1996). Serum beta(2)-microglobulin and serum thymidine kinase are independent predictors of progression-free survival in

chronic lymphocytic leukemia and immunocytoma. *Leuk Lymphoma*, Vol.22, No.5-6, pp. 439-447. ISSN 1042-8194.

Hamblin, TJ.; Davis, Z.; Gardiner, A., et al. (1999). Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*, Vol.94, No.6, pp. 1848-1854. ISSN 0006-4971.

Hamblin, TJ.; Orchard, JA.; Ibbotson, RE, et al. (2002). CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. *Blood*, Vol. 99, No.3, pp. 1023-1029. ISSN 0006-4971.

Hancer, VS.; Diz-Kucukkaya, R. & Aktan M. (2011). Overexpression of Fc mu receptor (FCMR, TOSO) gene in chronic lymphocytic leukemia patients. *Med Oncol*. 2011 Jan 25. [Epub ahead of print]. ISSN 1559-131X.

Haslinger, C.; Schweifer, N.; Stilgenbauer, S., et al. (2004). Microarray gene expression profiling of B-cell chronic lymphocytic leukemia subgroups defined by genomic aberrations and VH mutation status. *J Clin Oncol*, Vol.22, No.19, pp. 3937-3949. ISSN 0732-183X.

Hernández, JA.; González, M. & Hernández, JM. (2010). Chronic lymphoid leukemia. *Med Clin (Barc)*, Vol.135, No.4, pp. 172-178. ISSN 0025-7753.

Hernández, JA.; Rodríguez, AE.; González, M., et al. (2009). A high number of losses in 13q14 chromosome is associated with a worse outcome and biological differences in patients with B chronic lymphoid leukemia. *Haematologica*, Vol.94, No.3, pp. 364-371. ISSN 1592-8721.

Hernandez, JM.; Mecucci, C.; Criel, A., et al. (1995). Cytogenetic analysis of B cell chronic lymphoid leukemias classified according to morphologic and immunophenotypic (FAB) criteria. *Leukemia*, Vol.9, No.12, pp. 2140-2146. ISSN 0887-6924.

Hewamana, S.; Lin, TT.; Rowntree, C., et al. (2009). Rel a is an independent biomarker of clinical outcome in chronic lymphocytic leukemia. *J Clin Oncol*, Vol.27, No.5, pp. 763-769. ISSN 1527-7755.

Josefsson, P.; Geisler, CH.; Leffers, H., et al. (2007). CLLU1 expression analysis adds prognostic information to risk prediction in chronic lymphocytic leukemia. *Blood*, Vol.109, No.11, pp. 4973-4979. ISSN 0006-4971.

Kainz, B.; Shehata, M.; Bilban, M., et al. (2007). Overexpression of the paternally expressed gene 10 (PEG10) from the imprinted locus on chromosome 7q21 in high-risk B-cell chronic lymphocytic leukemia. *Int J Cancer*, Vol.121, No.9, pp. 1984-1993. ISSN 0020-7136.

Klein, U.; Tu, Y.; Stolovitzky, GA., et al. (2001). Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. *J Exp Med* , Vol.194, No.11, pp. 1625-1638. ISSN 0022-1007.

Krober, A.; Bloehdorn, J.; Hafner, S., et al. (2006). Additional genetic high-risk features such as 11q deletion, 17p deletion, and V3-21 usage characterize discordance of ZAP-70 and VH mutation status in chronic lymphocytic leukemia. *J Clin Oncol*, Vol.24, No.6, pp. 969-975. ISSN 1527-7755.

Lai, R.; O'Brien, S.; Maushouri, T., et al. (2002). Prognostic value of plasma interleukin-6 levels in patients with chronic lymphocytic leukemia. *Cancer*, Vol.95, No.5, pp. 1071-1075. ISSN 0008-543X.

Letestu, R.; Rawstron, A.; Ghia, P.; et al. (2006). Evaluation of ZAP-70 expression by flow cytometry in chronic lymphocytic leukemia: A multicentric international harmonization process. *Cytometry B Clin Cytom*, Vol.70, No.4, pp. 309-314. ISSN 1552-4949.

Molica, S.; Vitelli, G.; Levato, D., et al. (2003). Increased serum levels of matrix metalloproteinase-9 predict clinical outcome of patients with early B-cell chronic lymphocytic leukaemia. *Eur J Haematol*, Vol.70, No.6, pp. 373-378. ISSN 0902-4441.

Montserrat, E. New prognostic markers in CLL. (2006). *Hematology Am Soc Hematol Educ Program*, pp. 279-284. ISSN 1520-4391.

Montserrat, E. & Rozman, C. (1987). Bone marrow biopsy in chronic lymphocytic leukemia: a review of its prognostic importance. *Blood Cells*, Vol.12, No.2, pp. 315-326. ISSN 0340-4684.

Moreno, C. & Montserrat, E. (2008). New prognostic markers in chronic lymphocytic leukemia. *Blood Rev*, Vol.22, No.4, pp. 211-219. ISSN 0268-960X.

Muntañola, A.; Bosch, F.; Arguis, P., et al. (2007). Abdominal computed tomography predicts progression in patients with Rai stage 0 chronic lymphocytic leukemia. *J Clin Oncol*, Vol.25, No.12, pp. 1576-1580. ISSN 1527-7755.

Nowakowski, GS.; Hoyer, JD.; Shanafelt, TD., et al. (2007). Using smudge cells on routine blood smears to predict clinical outcome in chronic lymphocytic leukemia: a universally available prognostic test. *Mayo Clin Proc*, Vol.82, No.4, pp. 449-453. ISSN 0025-6196.

Oppezzo, P.; Vasconcelos, Y.; Settegrana, C., et al. (2005). The LPL/ADAM29 expression ratio is a novel prognosis indicator in chronic lymphocytic leukemia. *Blood*, Vol.106, No.2, pp. 650-7. ISSN 0006-4971.

Orfao, A.; Gonzalez, M.; San Miguel, JF., et al. (1989). B-cell chronic lymphocytic leukaemia: prognostic value of the immunophenotype and the clinico-haematological features. *Am J Hematol*, Vol.31, No.1, pp. 26-31. ISSN 0361-8609.

Prieto-Sanchez, RM.; Hernandez, JA.; Garcia, JL., et al. (2006). Overexpression of the *VAV* proto-oncogene product is associated with b-cell chronic lymphocytic leukaemia displaying loss on 13q. *Br J Haematol,* Vol.133, No.6, pp. 642-645. ISSN 0007-1048.

Puente, XS.; Pinyol, M.; Quesada, V., et al. (2011). Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukemia. *Nature*, Vol.475, No.7354, pp. 101-105. ISSN 1476-4687.

Rai, KR.; Sawitsky, A.; Cronkite, EP., et al. (1975). Clinical staging of chronic lymphocytic leukemia. *Blood*, Vol.46, No.2, pp. 219-234. ISSN 0006-4971.

Rassenti, LZ.; Huynh, L.; Toy, TL., et al. (2004). ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. *N Engl J Med*, Vol.351, No.9, pp. 893-901. ISSN 1533-4406.

Rigolin, GM.; Maffei, R.; Rizzotto, L., et al. (2010). Circulating endothelial cells in patients with chronic lymphocytic leukemia:clinical-prognostic and biologic significance. *Cancer,* Vol.116, No.8, pp. 1926-1937. ISSN 0008-543X.

Rosenwald, A.; Alizadeh, AA.; Widhopf, G., et al. (2001). Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. *J Exp Med,* Vol.194: No.11, pp. 1639-1647. ISSN 0022-1007.

Sarfati, M.; Chevret, S.; Chastang, C., et al. (1996). Prognostic importance of serum soluble CD23 level in chronic lymphocytic leukemia. *Blood*, Vol.88, No.11, pp. 4259-4264. ISSN 0006-4971.

Schroeder, HW. Jr. & Dighiero, G. (1994). The pathogenesis of chronic lymphocytic leukemia: analysis of the antibody repertoire. *Immunol Today*, Vol.15, No.6, pp. 288-294. ISSN 0167-5699.

Sellmann, L.; de Beer, D.; Bartels, M., et al. (2011). Telomeres and prognosis in patients with chronic lymphocytic leukaemia. *Int J Hematol*, Vol.93, No.1, pp. 74-82. ISSN 1865-3774.

Stilgenbauer, S.; Sander, S.; Bullinger, L., et al. (2007). Clonal evolution in chronic lymphocytic leukemia: acquisition of high-risk genomic aberrations associated with unmutated VH, resistance to therapy, and short survival. *Haematologica*, Vol.92, No.9, pp. 1242-1245. ISSN 1592-8721.

Viñolas, N.; Reverter, JC.; Urbano-Ispizua, A., et al. (1987). Lymphocyte doubling time in chronic lymphocytic leukemia: an update of its prognostic significance. *Blood Cells*, Vol.12, No.2, pp. 457-470. ISSN 0340-4684.

Ward, BP.; Tsongalis, GJ. & Kaur,P. (2011). MicroRNAs in chronic lymphocytic leukemia. *Exp Mol Pathol*, Vol. 9, No.2, pp. 173-178. ISSN 1096-0945.

Wierda, WG.; Johnson, MM.; Do, KA., et al. (2003). Plasma interleukin 8 level predicts for survival in chronic lymphocytic leukaemia. *Br J Haematol*, Vol.120, No.3, pp. 452-456. ISSN 0007-1048.

Wierda, WG.; O'Brien, S.; Wang, X., et al. (2007). Prognostic nomogram and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. *Blood*, Vol.109, No.11, pp. 4679-4685. ISSN 0006-4971.

Yi, S.; Yu, Z.; Zhou, K., et al. (2011). TOSO is overexpressed and correlated with disease progression in Chinese patients with chronic lymphocytic leukemia. *Leuk Lymphoma*, Vol.52, No.1, pp. 72-78. ISSN 1029-2403.

Zenz, T.; Fröling, S.; Mertens, D., et al. (2010). Moving from prognostic to predictive factors in chronic lymphocytic leukaemia (CLL). *Best Pract Res Clin Haematol*, Vol.23, No.1, pp. 71-84.ISSN 1532-1924.
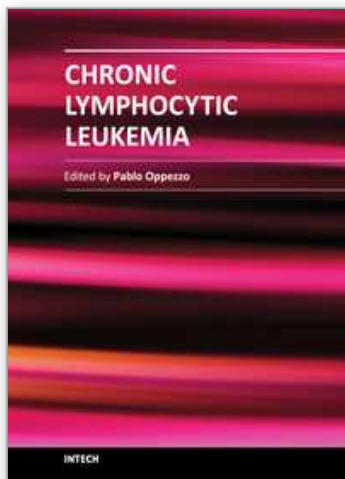
**Chronic Lymphocytic Leukemia**

Edited by Dr. Pablo Oppezzo

B-cell chronic lymphocytic leukemia (CLL) is considered a single disease with extremely variable course, and survival rates ranging from months to decades. It is clear that clinical heterogeneity reflects biologic diversity with at least two major subtypes in terms of cellular proliferation, clinical aggressiveness and prognosis. As CLL progresses, abnormal hematopoiesis results in pancitopenia and decreased immunoglobulin production, followed by nonspecific symptoms such as fatigue or malaise. A cure is usually not possible, and delayed treatment (until symptoms develop) is aimed at lengthening life and decreasing symptoms. Researchers are playing a lead role in investigating CLL's cause and the role of genetics in the pathogenesis of this disorder. Research programs are dedicated towards understanding the basic mechanisms underlying CLL with the hope of improving treatment options.

**How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

José-Ángel Hernández, Marcos González and Jesús-María Hernández (2012). Prognostic Factors in Chronic Lymphoid Leukemia and Identification of New Clinically Relevant Molecular Markers, Chronic Lymphocytic Leukemia, Dr. Pablo Oppezzo (Ed.), ISBN: 978-953-307-881-6, InTech, Available from: http://www.intechopen.com/books/chronic-lymphocytic-leukemia/prognostic-factors-in-chronic-lymphoid-leukemia-and-identification-of-new-clinically-relevant-molecu

# INTECH
open science | open minds