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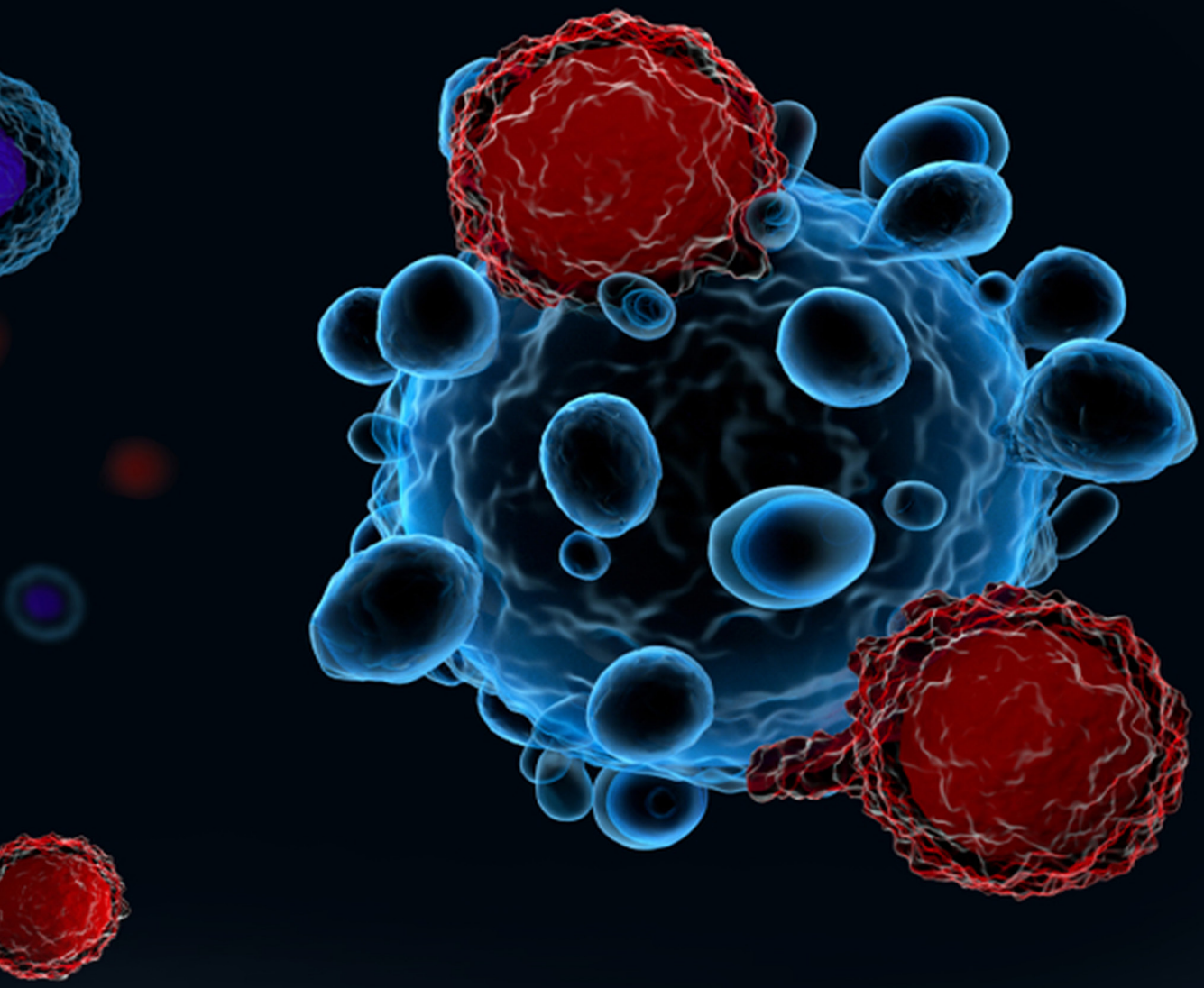
CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA TREATMENT GUIDELINES



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Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma Treatment Guidelines

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Abstract

A diagnostic and treatment guide for chronic lymphocytic leukaemia prepared by a group of Mexican experts is presented herein, in order to adapt the advances that have taken place in recent years to the reality of the Mexican population. It is a guide oriented, not only to specialists but also useful for all those health professionals involved in the management and care of patients affected by this disease.

Keywords: Chronic Lymphocytic Leukaemia; Small Lymphocytic Lymphoma; Clinical Guidelines; Diagnosis; Treatment; Mexico

Abbreviations: ISSSTE: Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado; AMHE: Agrupación Mexicana para el Estudio de la Hematología

Introduction

This guide represents the joint effort of ISSSTE (Institute for Social Security and Services for State Workers) haematologists that is aimed at bringing together the criteria for diagnosis and treatment of chronic lymphocytic leukaemia (CLL)/small lymphocytic lymphoma (SLL), by reviewing the most active concepts available in the international medical literature, that allow us a better understanding of the disease and that can be applied to the Mexican population. In recent years, CLL treatment has varied considerably from the standard treatment with chlorambucil (Clb), and the use of prednisone, continuing with the incorporation of purines (alone or in combination with alkylating agents), until the emergence of new drugs such as anti-CD20 monoclonal antibodies, bendamustine, B-cell receptor (BCR) inhibitors (idelalisib and ibrutinib) and BCL-2 antagonists such as venetoclax that redefines the treatment goal and paradigm and redefines treatment standards in clinical practice, notwithstanding their curative intent.

Currently, there are two similar studies available in the national context. The first was carried out by the Mexican Association for the Study of Haematology (AMHE), which organised and developed the first Mexican consensus on CLL, published in 2008, which addressed the clinical, diagnostic and therapeutic aspects of CLL updated in that time [1], and the second study in 2011, carried out by a group of Mexican experts led by Dr. Labardini published as an Oncology Guide dedicated exclusively to CLL [2].

Definition

CLL is a lymphoproliferative disorder characterised by a clonal expansion of functionally incompetent CD5+ and CD23+ lymphocytes in the blood, bone marrow and secondary lymphoid tissues of variable chronic evolution and clinical behaviour [3]. Approximately 50% of patients will have an indolent disease course while the other 50% will have a variable aggressive clinical behaviour, requiring treatment, since they will have a shorter life expectancy than the general population. Despite the pharmacological advances that have appeared in recent years, it remains incurable, with frequent relapses during its course [4].

Epidemiology

CLL is the most frequent haematological neoplasm in the Caucasian population in western countries, with an incidence between 4 and 5 cases/100,000 inhabitants-year, which increases with age reaching 30 cases/100,000 inhabitants-year in people over the age of 70 years [5]. It has a higher incidence in males, with a male: female ratio of 2:1.5 [6]. The incidence in the Mexican population is unknown, with only three studies suggesting a lower incidence than that of Western patients (EUA/Europe), which ranges between 6.6% and 9% of leukaemia diagnosed in adults, of which approximately 50% correspond to the Mexican mestizo population and the other 50% to the Caucasian population [2]. However, a recent study published by Alvarado *et al.*, indicates that the incidence of CLL in Mexico is six times lower than in the Caucasian population, suggesting the existence of some type of genetic variability [7,8]. In the year 2017, a descriptive, retrospective study conducted in two tertiary education institutions of the metropolitan area of the Valley of Mexico [9] included 1,432 cases with a diagnosis of leukaemia between the years 2007-2014 and reported that the average age for patients with CLL was 64.8 years old [9]. In Mexico, CLL is more frequent in men than in women (60% vs. 40%) [4,7,9], similar to that reported by the European Society of Medical Oncology [5].

Pathophysiology (Genomic Mutations)

The pathogenesis of the disease is a complex process involving multiple steps of B-cell replication, some of which have already been identified, while others are still unknown. It is believed that all CLL cases are preceded by a premalignant lymphoproliferative disorder known as monoclonal B lymphocytosis. This alteration occurs in a wide range from less than 1% up to 12% in the population over 60 years of age and varies according to the method used for its detection, with a progression towards CLL/SLL or some another related lymphoproliferative disorder, at a rate of 1% per year [10]. No trigger factor for monoclonal B lymphocytosis has been detected, however multiple factors have been found, such as the response to antigenic stimulation, the microenvironment and the genetic and epigenetic mutations [11].

It has been observed that in CLL, there are defects in apoptosis that condition an increase in the survival of these cells. One mechanism involved is the overexpression of antiapoptotic molecules BCL2 and MCL1, caused by the deletion of genes such as *13q14* that codes for microRNA, inhibitors of these molecules [12]. Another mechanism involved is the mutation of the *TP 53* gene, a tumour suppressor located on the short arm of chromosome 17, involved in control points of the cell cycle, activation of DNA repair enzymes, initiation of the senescence process and activation of the apoptosis. Thus, its inactivation by deletion and/or mutation promotes the development of neoplastic cells [13].

The pathophysiological effects of CLL cells are complex and have not yet been fully understood. The accumulation of neoplastic lymphocytes in the lymph node, spleen and liver causes growth and alteration in their function and possibly in adjacent organs. In addition, the infiltration in the bone marrow and the alteration of the microenvironment cause cytopenias. CLL cells have a negative effect on the normal immune response, which causes a deterioration of the immune response to infections, a defect in the immune self-recognition and possibly a defect in

the immune surveillance for other neoplasms [14]. The mechanism of symptoms associated with CLL (symptoms B) can be explained by the dysregulation of cytokine production.

Other identified mutations associated with the pathogenesis of the disease are *NOTCH1, SF3B1, BIRC3* and *TP5* [4,7], especially when there is no *TP53* mutation [4,5]. Increased levels of CD38 and CD49 have also been associated as well as the presence of ZAP-70 [4,7,15]. In addition, the mutation of the immunoglobulin heavy chain (IGHV) has been associated with poor prognosis [16].

Clinical status

Clinically, CLL is a very heterogeneous disease; 70 to 80% of the cases are indolent (asymptomatic) and are detected by means of routine exams. Half of the patients will never have progression of their disease (with a life expectancy similar to the normal general population) and the other half will evolve quickly and will require treatment with a shorter life expectancy [1,4,16]. Some of the symptoms are of a general nature: fatigue, asthenia, fever, weight loss and nocturnal diaphoresis (symptoms B). When the disease has progressed, the manifestations are those of the anaemic, purpuric and/or infiltrative syndrome (adenopathies, splenomegaly, hepatomegaly) [1]. Up to 35% of patients develop a direct positive Coombs test in the course of their disease. Approximately 10-20% of patients develop autoimmune haemolytic anaemia and, 2-3% develop autoimmune thrombocytopenia [17,18]. The development of hypogammaglobulinemia occurs in 8% of patients, affecting three types of immunoglobulins (IgG, IgA and IgM) [19]. In less than 1% of patients, they can have pure red cell aplasia and agranulocytosis [17].

Diagnosis

The main guideline for CLL diagnosis is the presence of monoclonal lymphocytosis in peripheral blood (>5mil/mcL), sustained for at least 3 months [7,15] and that must be differentiated from monoclonal B lymphocytosis (<5mil/mcL), by sharing the characteristic phenotype: small, mature lymphocytes, with thin cytoplasmic membrane and dense nucleus without distinguishable nucleoli with partial chromatin aggregates [4,5,15,16,20].

B-cell clonality must be confirmed by peripheral blood flow cytometry where CD5 is typically expressed aberrantly on their surface and low levels (restriction) of kappa or lambda light chains but not both [5,15]. Once this essential criterion is met, it is advisable, according to international guidelines, for the patient to have the baseline tests indicated in Table 1. The criteria of the International Workshop on CLL (IWCLL) [15] indicate that the disease should be deemed active if it meets one of the following criteria (Table 2):

Table 1: Tests to establish the diagnosis of CLL [15].

Diagnostic Test	General Practice
Complete blood count and differential counting	Always
Immunophenotype of peripheral blood	Always
Pre-treatment evaluation	
Clinical history, physical examination and evaluation of functionality	Always
Complete blood count and differential counting	Always
Biopsy and bone marrow aspirate	When the clinic warrants it (cytopenias not defined)
Blood chemistry, Immunoglobulins and direct Coombs	Always
Chest radiograph	Always
Infectious disease status	Always
Additional tests before treatment	
Molecular cytogenetics (FISH) for del(13), del(11q), del(17q), add(12) in peripheral blood	Always
Conventional peripheral blood karyotype	GNI
TP53 mutation	Always
Mutational status of the variable region of the immunoglobulin heavy chain	Always
β2-microglobulin	Desirable
Chest, abdominal and pelvic CT scan	GNI
Magnetic resonance, PET-scan	GNI
Abdominal ultrasound	Possible

GNI: Generally not indicated. PET: positron emission tomography.

Table 2: Active disease criteria [3].

Active Disease Criteria	Observations
1. Evidence of progressive bone marrow failure caused by anaemia (HB <10 g/dL)/or thrombocytopenia (< 100,000 MCL)	Rule out chronic thrombocytopenia of another etiology
2. Massive or progressive symptomatic splenomegaly	Greater than 6 cm below the left costal margin
3. Bulky adenopathies	Greater than or equal to 10 cm in longitudinal diameter
4. Lymphocytosis with progressive increase equal or more than 50% in a period of two months or duplication of lymphocytes in less than 6 months	In patients with baseline <30,000, a longer period of observation should be considered and other secondary causes of lymphocytosis should be ruled out
5. Presence of autoimmune complications including anaemia and thrombocytopenia	With poor response to steroids
6. Extranodal functional or symptomatic involvement	
7. Symptoms related to the disease	Weight loss greater than 10% in the last 6 months Significant fatigue ECOG greater 2 Temperature higher than 38ºC for 2 or more weeks Profuse night time diaphoresis for a period of more than a month No evidence of infection

- Progressive medullar (infiltration) insufficiency, worsening anaemia or thrombocytopenia.

- Progressive or massive splenomegaly (>6 cm below the costal margin)

- Large-sized ganglionic conglomerates (>10 cm).

-Time of lymphocytic duplication <6 months or increase> 50% of lymphocytosis in two months.

-Anaemia and/or autoimmune thrombocytopenia that do not respond to immunosuppressive therapy.

-Weight loss (>10% in 6 months), asthenia (ECOG>2), fever> 38º (without infection for> 2 weeks) or night sweats (> 1 month).

Classification

The classification or clinical staging systems of "Rai" and "Binet" are well known, simple to apply and widely used [7] to evaluate tumour volume and predict survival. Patients are classified into three subgroups [4,7,15] according to the number of lymphadenopathies, presence of hepatomegaly and/or splenomegaly, and anaemia and/or thrombocytopenia [20].

Rai Classification

The Rai-modified classification indicates:

-Low risk patients: present lymphocytosis> 15 × 10^9/L with leukemic cells in blood and bone marrow.

-Intermediate risk patients: present lymphocytosis in blood, enlarged lymph nodes anywhere and splenomegaly and/or hepatomegaly - High risk patients: present lymphocytosis and anaemia related to the disease (Hb <11gr/dl) or thrombocytopenia (platelet count <100x10^9/L) with or without lymphadenopathy and/or organomegaly [5,15]. In these subgroups of patients, the median survival will be approximately more than 10 years, more than 8 years or more than 6.5 years respectively [5].

Binet Classification

For its part, Binet's classification is based on the number of affected lymphatic areas defined as the presence of lymph nodes larger than 1 cm in diameter or organomegaly, and whether there is anaemia and/or thrombocytopenia [15]. The stages are classified by letters that from lowest to highest risk are:

- Stage A: patients with Hb≥10 gr/dL, platelets ≥100x10^9/L and involvement of up to two lymphatic areas;

- Stage B: patients with Hb≥10 gr/dL, platelets ≥100x10^9/L and three or more affected lymphatic areas;

- Stage C: includes those with Hb <10 gr/dL and/or platelets <100x10^9/L

Survival is more than 10 years, less than 8 years and 6.5 years respectively [15].

More recently, the international prognostic index of chronic lymphocytic leukaemia (CLL-IPI) combined genetic, biochemical and clinical parameters in a prognostic model that allows patients to be allocated into four subgroups according to OS at 5 years (low, intermediate, high and very high risk) (Table 3) [21,22].

Table 3: CLIPI Classification [15].

Variable	Factor	Grade
TP53/17P	Mutated	4
IGHV	Non-Mutated	2
B2M	>3.5 mg/L	2
CLINICAL STAGE	Binet B/C or Rai II-IV	1
AGE	> 65 years	1

Differential Diagnosis

It is necessary to differentiate CLL from other chronic lymphoproliferative processes. Among these are prolymphocytic leukaemia, hairy cell leukaemia, splenic lymphoma with villous lymphocytes, follicular lymphoma, mantle cell lymphoma and lymphoplasmacytic lymphoma [5,23]. The existence of CD 5+ marker in B-lymphocytes differentiates CLL from prolymphocytic B leukaemia (although up to 30% can be weak positive) [24], hairy cell leukaemia, follicular lymphoma, splenic lymphoma with villous lymphocytes and lymphoplasmacytic lymphoma, diseases in which it is negative. Mantle cell lymphoma has lymphocytes that express CD5+, but these are habitually CD23 negative. The FMC7 can help differentiate CLL from mantle cell lymphoma as in this case they will appear as FMC7 positive. Although CLL lymphocytes express immunoglobulins on their surface, the intensity of their expression is very low, which is very useful to distinguish it from other lymphoproliferative processes where the intensity level is invariably high [5,23].

A useful criterion for diagnostic accuracy is to use the immunophenotype and apply the Matutes Score (Table 4). By using this system, 92% of CLL cases have 4 or 5 points, 6% have 3 points and only 2% of cases have 1 or 2 [25]. Marginal zone lymphoma or lymphoplasmacytic lymphoma differs from CLL because CD43 expression is low or negative in the former [5]. Although the World Health Organization (WHO) considers CLL and small cell lymphocytic lymphoma as a single disease, lymphadenopathy and/or splenomegaly must be present in order to diagnose the latter, as well as a B-lymphocyte count in peripheral blood not more than $5x10^9$/L [5].

Table 4: Matutes Score [25].

Immunophenotype score system (Matutes Score)		
Marker	1	2
CD5	Positive	Negative
CD23	Positive	Negative
IgS	Weak (Débil)	Strong (Fuerte)
FMC7	Negative	Positive
CD22 or CD79b	Weak (Débil)	Strong (Fuerte)

Flow Cytometry

The actual degree of cytoreduction, and therefore the persistence or not of the disease, can be investigated by cell immunophenotyping using flow cytometry, the cytogenetic technique of in situ hybridization with fluorescent molecular probes (FISH) and other molecular studies [23]. The appropriate immunophenotype for flow cytometry diagnosis should have the following surface markers positive: kappa/lambda, CD19, CD20, CD5, CD23, CD10, CD200 (useful to differentiate with mantle cell lymphoma), CD 43 +/- and negative for CD10- and cyclin D1- FISH for T (11; 14), CD79b is usually weak or negative positive (Table 5). Those with atypical immunophenotype are recommended to request immunohistochemistry [16]. Table 6 shows the antigens required and recommended for the diagnosis of immunophenotype.

Table 5: Immunophenotype for the differential diagnosis of leukemic lymphoproliferative syndromes [6].

Disease	sIg	sIgM	CD5	CD10	CD20	CD11c	CD23	CD25	CD43	CD79b	CD103	FMC7	CD200
CLL	+/-	-	++	-	+/-	+/-	++	+	+	+/-	-	-	+/++
B prolymphocytic leukemia	++		+/-	-	+	+/-	+/-	+/-	+/-	+	-	+	+/-
Hairy cell leukemia	+	-	-(+)	-(+)	++	++	-	++	-	+	++	+	+/-

Mantle leukemia	+	++	+	-	+	+/-	-	+/-	+	+	-	+/-	-
Splenic B cell lymphoma	+	++	-	-	+	+	-	+/-	-	+	+/-	+	+/-
Lymphoplasmacytic lymphoma	+	+	-	-	+	-	-	+	-	+	-	+/-	+/-
Follicular lymphoma	++	+	-	++	+	+/-	+/-	+/-	-	+	-	+	+/-
Diffuse B cell lymphoma	+/-	+	-	+d	+	-	-	-	-	+	-	+	+/-

Table 6: Antigens required and recommended for the immunophenotype diagnosis [56].

	Antigen	CLL Expression	Observations
Required	CD19	Positive (>95%)	
	CD5	Positive (>20%)	
	CD23	Positive (>20%)	Negative, suggestive of mantle lymphoma
	CD20	Weak	
	Ig K, IgL	Positive and restricted	
Recommended	CD43	Positive (>20%)	
	CD79b	Weak	
	CD81	Weak	
	CD200	Positive (>20%)	Usually high antigenic intensity suggestive of CLL or tricholeukemia; Low antigenic intensity suggestive of FL, MCL.
	CD10	NEG <20%	

Flow Cytometry as Residual Disease

Minimal residual disease negative (MRD-) is the surrogate marker of the effectiveness of a treatment versus CLL. In recent years, the evaluation of MRD has been introduced in clinical studies to evaluate the intensity of the remission of the disease with a treatment [15], although this has been determined for a long time. In fact, in the first Mexican guidelines of 2008, it was already indicated that with polymerase chain reaction tests and flow cytometry, leukemic cells can be detected in a reproducible way when they represent at least 0.01% of the leukocytes [1]. Currently, this definition is still maintained so that an undetectable MRD is defined when there is less than one pathological cell CLL/10,000-100,000 leukocytes in the blood or bone marrow [15].

The MRD can be detected with multi-parametric flow cytometry techniques and/or quantitative polymerase chain reaction. The Spanish guidelines recommend the use of flow cytometry with three quadruple markings for its greater applicability and simplicity: CD5/CD19/CD20/CD38; CD5/CD19/CD81/CD22, and CD5/CD19/CD79b/CD43. Initial markers should be used, including those with aberrant expression, if they were present at the time of diagnosis. The sensitivity of these techniques lies in the detection of a tumour cell between 10,000-100,000 normal cells (4). Conversely, the ESMO 2015 guidelines indicate that the MRD detected with 4-colour flow cytometry has a high prognostic value (5). Moreover, the IWCLL guidelines for 2018 indicate that 6-colour flow cytometry presents a sensitivity reliably until the detection of less than one tumour cell among 10,000 leukocytes [15]. Likewise, in the latter, it is noted that the harmonization of these technologies has established that a typical assay based on flow cytometry comprises a central panel of six markers (CD19, CD20, CD5, CD43, CD79b and CD81) [15].

Obtaining an eradication of the MRD has been associated with better PFS and OS, regardless of other prognostic factors [4]. When MRD negative is detected in the blood it is advisable to confirm this finding in the bone marrow only in those cases of patients who have been treated with monoclonal antibodies [1], because some treatments such as these appear as negative in the blood but not in the bone marrow [1,15]. It is recommended to perform initial MRD within 2 months of reaching the maximum response of the treatment. MRD monitoring will be left at the discretion of the attending physician according to the treatment used in the patient, although it is recommended to do it every 6 months.

Immunohistochemistry

As for immunohistochemistry, all cases should be considered, if possible, and those with atypical immunophenotype (CD 23+/- O CD 23-, CD 20++, IGS++). The marker to be investigated will be cyclin D1, which must be negative. In case of positivity, consider another diagnosis as mantle lymphoma [16].

Complications

The most important complications before or after treatment are:

Infections

Patients with CLL are at an increased risk of infections, either related to the disease or with the immunosuppressive effect of the treatment [4,15,16]. These infections are usually more frequent in the initial treatment stages and in patients that have to take corticosteroids as part of their treatment [4].

In general, vaccination is recommended before treatment starts, and vaccines with live attenuated virus are contraindicated [15]. Antiviral prophylaxis and antibiotic therapy can be used in patients with recurrent or high-risk infections [5]. However, there are studies that indicate that in patients under the age of 65 years with fludarabine, with an adequate functional status and without a previous history of infections, the systematic use of antiviral or antimicrobial prophylaxis is not recommended, due to the low risk of infections [26]. Infections related to the herpes virus are more frequent in patients treated with alemtuzumab or idelalisib, while those treated with antiCD20 have more infections with hepatitis B virus [15]. It is advisable to carry out a serological study against hepatitis B and C viruses before the start of treatment with immunochemotherapy [4,15]. Progressive multifocal leukoencephalopathy has been observed in some patients treated with antiCD20 [15]. The Spanish guidelines do not recommend conducting global prophylaxis for fungal infections. It is only recommended in patients undergoing steroid treatment, especially if it is part of a combined treatment with chemotherapy or alemtuzumab [4], or those with a history of fungal infections, mainly aspergillosis [27].

Cytopenias

Cytopenias may be caused by CLL activity at the bone marrow level in which case the proper management of this problem will be indicated, or by autoimmune phenomena (secondary neutropenia, selective erythropoietic aplasia) due to the production of autoantibodies directed against the progenitor cells or as a paraneoplastic problem associated with CLL, hence, it should be established if they are presented with a stable CLL which will require treatment as any independent autoimmune process, or if it is the disease activity, in which case treatment becomes critical [15,18]. The majority of patients with autoimmune cytopenias (autoimmune thrombocytopenia, autoimmune haemolytic anaemia) respond to corticosteroids [5,15]. In case of non-response, other immunosuppressants such as rituximab [5,15] can be administered in monotherapy or combined with cyclophosphamide and dexamethasone, bendamustine, or alemtuzumab [4], and/or by performing splenectomy [15].

Tumour Lysis Syndrome

The appearance of the tumour lysis syndrome is rare, as it is very rare in patients treated with purine analogues, but cases of this syndrome have been described in patients treated with lenalidomide, venetoclax or antiCD20 type II [15]. Follow-up is recommended in patients with large tumour burden (lymphocytosis greater than 25x109 L), and impaired renal function [28,29].

Second Neoplasms

Approximately 15% to 18% more frequent in patients with CLL than in the general population, even in patients before treatment. The most frequent cases are skin and prostate cancer, although they can occur at other levels. The mechanism by which they occur most often is not well understood, but it seems to be associated with the immune defects of the CLL [28].

Occasionally, CLL can transform into a diffuse large B cell lymphoma (95%) or Hodgkin's lymphoma (5%) (Richter syndrome), which usually occurs between 2 to 6 years after diagnosis, usually in a very aggressive clinical manner.

Others

The other associated autoimmune problems that have been described are glomerulonephritis and other renal alterations [30], angioedema and pemphigus. This section considers the non-haematological toxicities that some drugs present and that must be considered. Among these, autoimmune colitis and pneumonitis have been described, related to idelalisib; arrhythmias, arterial hypertension, diarrhoea and haemorrhages (digestive, central nervous system or others) with ibrutinib [15] in addition to transient lymphocytosis that appears in the first weeks of the treatment with this drug, and that does not mean failure or progression [16].

Treatment

CLL is an indolent disease, so not all patients require treatment. The criteria for active disease have already been discussed previously (Table 2). As CLL is a disease that occurs mainly in elderly patients, it is necessary to classify them according to functional scales, because not all of them will be candidates for treatment (Table 7). Conversely, and due to the clinical and molecular heterogeneity of CLL, it is recommended that patients be classified into risk groups according to molecular prognostic factors, in centres where there is availability (Table 8). Currently, a wide variety of therapeutic options are available for CLL treatment, which have been used in different clinical trials, demonstrating its effectiveness. The therapeutic options, combined treatments and different population groups used in the clinical studies are shown in (Table 9). The most common treatment schemes can be seen in Table 10.

Table 7: Classification of patients according to functionality criteria [54].

Functionality criteria	Observations
Fit	No comorbidity
Not fit	Presence of comorbidities grade 0-2 WHO
Fragile	With severe comorbidities WHO 3-4

Table 8: Risk classification of patients according to functional and molecular criteria [55].

Functionality and molecular criteria	
Fit and low risk	Low-risk molecular prognostic factors (mutated IgVH, 13q) Young patients, no comorbidity
Fit and high risk	Prognostic factors of high molecular risk (non-mutated igVH, mutation of TP53, del17p, del11q, complex karyotype
Fragile	Includes elderly patients with poor functional status and multiple comorbidities
Not fit for treatment	Not candidates to any therapies, except palliative care

Table 9: Therapeutic options used in the different CLL clinical trials [40].

CLL Clinical Trials						
Trial	**Agents**	**Population**	**OR rate**	**PFS**	**OS**	**Author**
CLL08	FCR VS FC	Young and fit	90% VS 80%	51.8% VS 32.8%	3 years 87% VS 83%	Halleck et al.
CLL10	FCR VS BR	Young and fit, without del17p	95% VS 96%	55.2% VS 41,7%	3 years 91% VS 92%	Eichhorst
CLL11	Chlorambucil VS RClb VS R obinutuzumab	Fragile	31.4% VS 61.7% VS 77.3%	11.1% VS 16.3% VS 26.7%	Not reached HR for death: Obinituzumab- CLB VS Clb without benefit for other comparisons	Goede
RESONATE 2	Ibrutinib VS Clb	< 65 years	86% VS 35%	Not reached VS 18.9%	2 years 98% VS 85%	burger
Other trials - phase III						
MURANO	Venetoclax-Rituximab VS BR	CLL in relapse and fit	92% VS 72%	2 years 84.9% VS 36.3%	Not reached HR for progression or death 0.48 in favour of VR	Seymour
HELIOS	Ibrutinib-BR VS placebo-BR	<prior 1st line treatment and fit Exclusion 17p-	86% VS 68%	79% VS 24%	Not reached HR 0.62 in favour of bendamustine	Chanan-Khan
IDELALISIB-R	Idelalisib Rituximab vs placebo-Rituximab	CLL relapse Fragile	81% vs 13%	24 weeks 93 vs 46%	12 months 92% vs 80%	Furma

OR: Overall Response; PFS: Progression; Free Survival; OS: Overall Survival

Table 10: Usual treatment schemes.

Scheme	Drug			Duration
FCR	**Cyclophosphamide**	**Fludarabine**	**Rituximab**	
	250 mg m^2 iv 10 to 30 min days 1-3	25 mg m^2 iv en 20 to 30 min days 1-3	375 mg m^2 iv day 1 (up to 500 mg m^2)	Cycles every 28 days, up to 6 cycles
BR	BENDAMUSTINE	RITUXIMAB		
	70 mg m2 iv days 1 and 2	375 mg m2 iv day 1 (up to 500 mg m2)		Cycles every 28 days, up to 6 cycles
RC	RITUXIMAB	CHLORAMBUCIL		
	375 mg m^2 iv day 1 (up to 500 mg m^2)	0.3 mg/kg/day days 1-5		Cycles every 28 days, up to 6 cycles
IBRUTINIB	IBRUTINIB 420 mg day p.o. (3 capsules 140 mg)			Until disease progression and/or unacceptable toxicity
IDELALISIB	IDELALISIB 150mg p.o. every 12 hours			Until disease progression and/or unacceptable toxicity

VR	VENETOCLAX	RITUXIMAB		
	Week 1= 20 mg Week 2= 50 mg/day Week 3= 100 mg/ day Week 4= 200 mg/ day Week 5= 400 mg/ day	RITUXIMAB 375 mg m2 IV Day 1 (up to 500 mg m2) From week 5 every 28 days iv		Until disease progression and/or unacceptable toxicity
OBINUTUZMAB	OBINUTUZUMAB Cycle 1 Day 1,8,15= 100 mg Cycle 2 to 6 Day 1=100mg			Cycles every 28 days, up to 6 cycles

Second Line Treatment

Refractory Disease or Relapse

Relapse is defined as evidence of disease progression in a patient who had previously met criteria for partial or complete remission for a period of 6 months or longer [15]. Refractory disease is one that does not respond to treatment or progresses within 6 months from the last dose of treatment [15]. The treatment of relapses or refractory disease should be initiated only in symptomatic patients [5,15]. The ESMO2015 guidelines indicate that in case of relapse or progression within 24-36 months after chemotherapy, and if the existence of a TP53 mutation has been ruled out, the same first line treatment should be repeated [5]. If relapse appears in less time or there is a lack of response to the first line treatment, the therapeutic scheme must be changed [5] and second line treatment must be started [15].

The recommended options in patients with refractory disease or relapse, irrespective of age and comorbidities of the patient, are Ibrutinib, Idelalisib/Rituximab, Idelalisib and Venetoclax (the latter particularly in patients who are refractory to ibrutinib or idelalisib treatment) [5,16]. Adding Idelalisib or ibrutinib to treatment with Bendamustine/Rituximab (BR) has been shown to improve progression-free survival (PFS) in these patients, so it can be considered an alternative in refractory disease or relapse [16]. It has been proven that the main reason for discontinuation of treatment with kinase inhibitors (ibrutinib, idelalisib) is due to its toxicity, mainly atrial fibrillation with ibrutinib and pneumonitis with idelalisib, being the reason for abandonment in 51% of patients [31].

Bendamustine

Bendamustine is a drug that was developed in the former German Democratic Republic more than 50 years ago, having been used in patients with CLL and in low-grade non-Hodgkin's lymphoma [32]. Currently, it is one of the main first line treatments for CLL as well as in relapsed patients [32]. In routine clinical practice, bendamustine is mainly used in combination with rituximab based on data from phase II studies [32]. In one of them, performed in patients with refractory disease or in relapse by the German group [33], an overall response to treatment rated at 60% was observed in patients sensitive to fludarabine compared to 45% in the group of patients refractory to it, with a median event-free survival of nearly months. The authors considered that this combination was effective in all patients except those with *del17p* or in those who were refractory to FCR (Fludarabine + cyclophosphamide + rituximab) [33].

Subsequently, studies have been published indicating that the FCR combination continues to be the standard treatment for those with advanced CLL, such as the one published by Eichhorst et al. [34] which included patients without previous treatment and without *del17p* and compared this combination with patients treated with bendamustine and rituximab (BR) [34]. The study observed a median PFS with the BR combination of 41.7 months while the FCR was 55.2 months, however, it was noted that the BR combination was less toxic for patients [34].

Ibrutinib

Ibrutinib is an irreversible oral inhibitor of Bruton's tyrosine kinase (BTK) that is approved for CLL treatment in many countries including the United States of America and in Europe. It is also approved in some countries for mantle cell lymphoma and for Waldenstrom's macroglobulinemia [35]. Its effectiveness has been proven through its clinical development. The RESONATE-2 study was conducted in patients ≥65 years with CLL who had not received previous treatment, and without *del17p* to compare the efficacy of ibrutinib against chlorambucil, demonstrating its superiority as a first line treatment, prolonging PFS, with a risk of progression or death 84% lower than those treated with chlorambucil. OS (overall survival) was also superior with ibrutinib [36]. Its effectiveness in monotherapy was evaluated by comparing it with intravenous ofatumumab in patients who had received previous treatments and could not be treated with purine analogues. In the RESONATE study, patients with *del17p* and *del11q* were included. The results indicated a greater PFS and OS with ibrutinib rather than with ofatumumab [37]. The efficacy of ibrutinib in combination with other drugs was evaluated in the HELIOS study, in which it was combined with BR in

previously treated patients. In this study, patients with *del17p* were not included. The results also showed that this combination prolongs the PFS when compared with the BR treatment [38].

Idelalisib

It is an inhibitor of phosphoinositide 3-kinase (PI3K). The use of idelalisib is recommended in combination with rituximab or with ofatumumab in patients in whom there is no other first-line therapeutic alternative [39]. Efficacy has been assessed in combination with rituximab or with ofatumumab. A phase III study compared the efficacy of the combination of idelalisib + rituximab versus rituximab + placebo and observed a prolonged PFS with the combination, as well as OS and the response rate [40]. Another study analysed the idelalisib + ofatumumab combination in previously treated patients, including those with *del17p* or mutated TP53, showing that PFS in patients treated with the combination was significantly higher than that observed in patients treated only with ofatumumab [41]. Regarding the use of idelalisib, prophylactic management should be considered due to the high rate of infections and associated death demonstrated in phase III studies [41].

Venetoclax

Venetoclax is a potent and selective inhibitor of the antiapoptotic protein BCL-2 (B-cell lymphoma) with activity in patients with CLL, including patients who have been previously treated and patients with del17p. [42,43]. Two studies conducted in patients with refractory or relapsing disease, including patients with *del17p*, showed that treatment with venetoclax monotherapy produced an objective response in approximately 80% of patients [44,45]. A phase II study, in which 36 patients were administered venetoclax after treatment with idelalisib, observed an objective response rate of 67% and an estimated PFS median at 12 months of 79% [43]. It has also shown efficacy in patients refractory to ibrutinib in a study that has recently published data from an intermediate analysis [46]. At the time of the analysis, 65% of patients had presented an overall response (CI 95%: 53-74).

Recently, data from the MURANO study were published in phase III [47] that evaluated the efficacy of venetoclax in combination with rituximab in patients with refractory CLL or in relapse, compared with patients treated with the BR combination. The study conducted in 389 patients followed for 2 years showed a PFS of 84.9% in patients treated with the venetoclax-rituximab combination versus 36.3% in those treated with BR combination (HR: 0.17, CI 95%: 0.11-0.25, p<0.001), maintaining this therapeutic benefit in all the subgroups analysed, including those with *del17p*. In the subgroup of patients with *del17p*, PFS was 81.5% compared to 27.8% respectively (HR: 0.13, CI 95%: 0.05-0.29), while in those who did not present it, PFS was 85.9% versus 41.0% (HR: 0.19, CI 95%: 0.12-0.32). The results of this study were confirmed by an independent evaluation committee [47].

Hence, the use of venetoclax combined with rituximab is recommended in patients with refractory CLL or in relapse.

Obinutuzumab

Obinutuzumab is a humanized anti-CD20 monoclonal antibody type II of subclass IgG1. Its use is approved in Europe by the EMA and in the United States of America by the FDA in combination with chlorambucil for the treatment of adult patients with chronic lymphocytic leukaemia (CLL), not previously treated and with comorbidities that cannot be treated with fludarabine [48,49]. In clinical studies, obinutuzumab has been shown to be effective and well tolerated in these patients, both when administered as monotherapy and in combination with chemotherapy [48,49]. Phase IIIb of the GREEN study conducted in 971 patients with previously untreated or recurrent CLL, in which 1000mg obinutuzumab was administered alone or in combination with chemotherapy (bendamustine, fludarabine/cyclophosphamide, or chlorambucil), proved that the treatment is safe and well tolerated. In this study, it was observed that patients submitted to first line treatment presented overall response rates at 3 months after treatment higher than 80% for all obinituzumab combinations with chemotherapy [49].

Transplantation of hematopoietic progenitors

Autologous transplantation (auto-TPH) has been shown to prolong PFS but not OS [4]. Although it is a treatment that must be carefully evaluated and individualized, it is considered to be indicated in high-risk patients (with *del17p*/*mutTP53*) who have achieved clinical response after first line treatment with BTK inhibitors (ibrutinib, idelalisib) or in relapse [4]. In patients younger than 65 years, auto-TPH should be considered after rescue treatment with another BTK or venetoclax inhibitor [4].

Response Criteria

The response assessment must be performed at least two months after the therapy is completed, and includes a physical examination and blood and bone marrow tests. It is suggested to classify the parameters to be evaluated in two groups, lymphoid tumour burden and constitutional symptoms (Group A) and response of the hematopoietic system (Group B). In case of maintenance therapy, the response assessment must be performed at least two months after the maximum response has been reached, which is defined as the treatment phase in which no further improvement is observed for at least 2 months of treatment [15]. The types of response are defined according to the criteria of Table 11. Patients who do not achieve FR (Full Response) or PR (Partial Response) and who do not have criteria for DP (Disease Progression) should be considered as stable disease [15].

Table 11: Criteria to assess response to treatment [15].

Group	Parameter	FR *	PR **	DP
GROUP A	Lymph node	None ≥ 1.5cm	Decrease of≥ 50% of the baseline	Increase of ≥ 50% of baseline or response
	Spleen / liver	Spleen <13cm, normal liver	Decrease of≥ 50% of the baseline	Increase of ≥ 50% of baseline or response
	Constitutional symptoms	None	Any	Any
	Lymphocytes in peripheral blood	<4x10(9)/L	Decrease of ≥ 50% of the baseline	Increase of ≥ 50% of baseline
GROUP B	Platelets	>100, 000/mcL	or 50% over the baseline	Decrease of more than 50% of the secondary baseline to CLL
	Haemoglobin	>11 g/dL	or 50% over the baseline	Decrease of >2g/dL from the secondary baseline to CLL
	Bone marrow	Normal cells, without CLL cells^	Presence of CLL cells or B lymph nodes, or not performed	Increase of >50% of CLL cells in successive biopsies

* Must meet all criteria, plus neutrophil count> 1.5 x 10 (9)

^ In morphological evaluation of bone marrow aspirate

** To define a partial response, improvement must be achieved in at least two parameters of Group A and one parameter of Group B.

FR: Full response; PR: Partial Response; DP: Disease Progression

In order to assess the response to treatment, a complete physical examination of the patient should be performed as well as a complete blood test [5,15]. In order to define the response to treatment, the tumour burden, the constitutional symptoms and the haematopoietic system should be assessed [15]. Maximum response is defined as the treatment phase in which no significant improvement occurs during at least 2 months of treatment [15]. To determine if complete remission has occurred, a bone marrow biopsy will be performed [5]. Criteria for defining complete remission include: Neutrophils ≥1.5x10^9/L, Lymphocytes less than 4.0x10^9/L, Platelets> 100x10^9/L, Haemoglobin ≥11.0 gr/dL without transfusions, bone marrow aspirate <30% lymphocytes, no detection of hepatomegaly or splenomegaly and lymph nodes [2,15]. The determination of MRD with 4-colour cytometry has a strong prognostic value, and it has been observed that patients with MRD- have a longer response duration as well as longer survival [5]. To consider a partial response, patients will present a 50% reduction in the lymph nodes compared to the start of treatment, as well as in hepatomegaly or splenomegaly, Haemoglobin ≥11.0 gr/dL or 50% improvement over the initial response without transfusions, Neutrophils ≥1.5x10^9/L or a 50% improvement, 50% reduction of lymphocytes, Platelets> 100x10^9/L or 50% improvement in platelet count [2,15].

The progression of the disease will be characterised by lymphadenopathy at 50% or more against the initially observed status, 50% or more increase of splenomegaly or appearance thereof, in case of no previous symptoms, as well as hepatomegaly, increase of lymphocytes at 50% or more with at least 5,000 B lymphocytes/μL, evolution to a more aggressive histology and the appearance of cytopenias directly related to the disease [2,15].

Prognosis

Despite advances in CLL treatment, it remains an incurable disease [5]. CLL prognostic factors are related to the patient's and disease characteristics (Table 12) [4,23,50-52].

Table 12: Prognostic factors in patients with CLL [50-52].

Prognostic factors	Favourable prognosis (>10 years)	Favourable prognosis (>10 years)
CLASSIC		
Clinical Stages	A,0	B,C;I,II,III,IV
Leukocyte Count	<50.000/MM3	>50.000/MM3
Bone Marrow Infiltration	Non-Diffuse	Diffuse
Prolymphocytes in Peripheral Blood	<10%	>10%
Lymphocyte Duplication Time	>12 Months	<12 Months
New		
B2M Rate	Normal	Elevated
Cytogenetics	DEL (13q) Isolated	DEL (11q).DEL (17P), Complete Karyotype

Flow Cytometry	CD38 < 30% ZAP-70 <20% CD49 <30%	CD38 >30% ZAP-70 >20% CD49d >30%
Igvh Mutations	YES	NO
Genetic Mutations	MYD88,	TP53,NOTCH1,SF3B1,BIRC3,ATM

IGHV heavy chain mutational status: Patients with CLL can be divided into two groups based on the mutagenic state of the immunoglobulin heavy chain, influenced by the origin of the B cells: pregerminal B cells (naive cells) carry a sequence of germline and post-germline DNA (memory). Mutated IGHV genes are defined with> 2% non-homology in the nucleotide sequence compared to the germline DNA. The mutational state of the IGHV heavy chain remains constant in the course of the disease, the status with non-mutated IGHV have worse prognosis than in patients with the mutated form and predicts a lower PFS and OS.

Genomic Aberrations

The interphase fluorescence in situ hybridization (FISH) technique is the current standard for the detection of chromosomal abnormalities in CLL and is the most sensitive method compared to conventional cytogenetic analysis. CLL is characteriSed by having at least one chromosomal abnormality by FISH in 80% of patients. The most common aberrations are partial deletions of chromosomes *6q, 11q, 13q* or *17p*. Chromosomal gains (e.g., trisomy 12) are less frequent.

Genomic Mutations

High-throughput (next-generation) sequencing has improved our understanding of the genetic characteristics of CLL, with the identification of recurrent mutations that carry new prognostic information.

Flow Cytometry

in terms of the prognostic parameters of this technique (CD38, CD49d, ZAP-70), CD 49d is the greatest predictor of OS and treatment-free survival, associated with advanced clinical status, high levels of DHL and B2M. The positivity of ZAP-70 and CD38 is correlated with the non-mutated status of IGHV and could represent a marker thereof.

Beta 2 Microglobulin

The elevation of this marker is a strong predictor of the indeterminate prognosis of PFS, OS and response to treatment in patients in the first line treatment.

Minimal Residual Disease

The evaluation of minimal residual disease (MRD) is an important objective in the treatment of CLL. Its collection is predictive of a prolonged PFS and OS, it could be considered a surrogate variable of PFS in the context of treatment with chemo immunotherapy. The evaluation of MRD by flow cytometry or molecular techniques in the age of the new BTK or Bcl-2 inhibitors identifies the most cost-effective treatment sequence and their duration. A therapeutic approach guided by the level of MRD could also determine which patients could benefit from a consolidation treatment or an early termination thereof [50-56].

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