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GUIDELINES FOR DIAGNOSIS AND TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA. KROHEM B-CLL 2017

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SUMMARY – Recent developments in the diagnosis and treatment of chronic lymphocytic leukemia (B-CLL) have led to change of approach in clinical practice. New treatments have been approved based on the results of randomized multicenter trials for first line and for salvage therapy, and the results of numerous ongoing clinical trials are permanently providing new answers and further refining of therapeutic strategies. This is paralleled by substantial increase in understanding the disease genetics due to major advances in the next generation sequencing (NGS) technology. We define current position of the Croatian Cooperative Group for Hematologic Disease on diagnosis and treatment of CLL in the transition from chemo-immunotherapy paradigm into a new one that is based on new diagnostic stratification and unprecedented therapeutic results of B-cell receptor inhibitors (BRI) and Bcl-2 antagonists. This is a rapidly evolving field as a great number of ongoing clinical trials constantly accumulate and provide new knowledge. We believe that novel therapy research including genomic diagnosis is likely to offer new options that will eventually lead to time limited therapies without chemotherapy and more effective clinical care for B-CLL based on individualized precision medicine.

Key words: *Chronic lymphocytic leukemia; Practice guidelines as topic; Precision medicine; Croatia; KROHEM**

Introduction and definition

In recent years, dramatic change in therapeutic landscape led to unprecedented therapeutic results

that were translated to clinical practice, and after years of slow to modest progress in the field of B-cell chronic lymphocytic leukemia (B-CLL), the American Society of Clinical Oncology (ASCO) named transformation of CLL treatment Cancer Advance of the Year for 2015¹. New treatments were approved based on the results of randomized multicenter trials for first line and for salvage therapy, and the results of numerous ongoing clinical trials are permanently providing new

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Table 1. Steps and aims in diagnostic process that leads to definition of therapeutic goal and strategy

	STEPS AND AIMS	BASIS FOR DECISION	CRITERIA	TARGETED CATEGORIES OF CLASSIFICATION
1	Disease detection; Patient – hematologist contact	<ul style="list-style-type: none"> • Lymphocytosis (70%-80%) and/or • Lymph nodes/spleen enlargement (20%-30%) 	<ul style="list-style-type: none"> • PE and hematology lab 	
2	Diagnosis and differential diagnosis	<ul style="list-style-type: none"> • B-cell clone of typical B-CLL phenotype (in PB and/or BM and/or LN) • Other lymphoproliferative disease with lymphocytosis 	<ul style="list-style-type: none"> • Morphology + characteristic immunophenotype with quantifications • Difference in phenotype and/or morphology 	<ul style="list-style-type: none"> • CLL (73%) • SLL (7%) • MBL • B (15%): FL, MCL, MZL, PLL, HCL • T (5%): PLL, TLLO, Sezary, LGL
3	Disease extent assessment	<ul style="list-style-type: none"> • Clinical and lab (hematology) assessment 	<ul style="list-style-type: none"> • Rai, • Binet, • TTM 	<ul style="list-style-type: none"> • 0, I, II, III, IV • A, B, C • Continuous parameter of the size and distribution of tumor mass
4	Classification in prognostic groups	<ul style="list-style-type: none"> • PFs @ dg 	<ul style="list-style-type: none"> • Prognostic markers (CD38 and ZAP), FISH(4), • Prognostic indices, • Mutation status of IgHV genes 	<ul style="list-style-type: none"> • Good prognosis • Poor prognosis
5	Indications for therapy	<ul style="list-style-type: none"> • Quantitative threshold criteria • Dynamic criteria • Qualitative criteria 	<ul style="list-style-type: none"> • Anemia, thrombocytopenia, organomegaly, lymphocytosis • Trend of tumor load increase (DT), progressive cytopenias • Presence of symptoms 	<ul style="list-style-type: none"> • Early, stable, asymptomatic disease = observation only • Any other = indication for therapy
6	Immediate pre-treatment evaluation and comorbidity & general condition assessment	<ul style="list-style-type: none"> • Clinical and laboratory assessment 	<ul style="list-style-type: none"> • Updating and completing work-up • CIRS 	<ul style="list-style-type: none"> • Fit • Unfit
7	Individualization of therapy Therapy aim and strategy definition	<ul style="list-style-type: none"> • B-CLL related factors • Patient related factors 	<ul style="list-style-type: none"> • Difference in advancement and progressiveness, p53 • General condition and comorbidity 	<i>Stratification (2x2):</i> <ul style="list-style-type: none"> • High risk/Low risk • Fit/Unfit (frail)

Comment: the table shows diagnostic steps. Steps 1-4 are made in single visit. Each step is different with respect to aim, decision criteria, and the extent of work-up. Last column describes classification categories proposed. Only about 10% of patients have indication for therapy at diagnosis. Others are followed-up repeatedly until the criteria for therapy are reached (step 5). The scope of work-up is different in each step, pretreatment evaluation being most complete, aiming to provide all the necessary elements for patient stratification and definition of therapeutic goal. The overall goal of diagnostic process is to enable individualization of therapy, definition of therapeutic aim and strategy, by implementing general principles to each individual case.

answers and further refining of therapeutic strategies. This is paralleled by substantial increase in understanding the disease genetics owing to major advances in the next generation sequencing (NGS) technology^{2,3}. All these have led to change of the until now predominant paradigm based on chemo-immunotherapy (CIT) to a new one^{4,5}.

Definition. B-cell chronic lymphocytic leukemia and related disorders (monoclonal B-lymphocytosis (MBL) and small lymphocytic lymphoma (SLL)) are defined by the presence of clonal mature B-lymphocytes with typical immunophenotype in peripheral blood, bone marrow and lymphoid organs (WHO, iw-CLL)^{6,7} representing one nosologic entity. Today, it is considered that these entities are different manifestations of the same disease. MBL is most prevalent and is considered as an early stage of malignancy progressing to CLL/SLL in 1%-2% of cases *per* year. SLL accounts for less than 10% of overt malignancy, and for this reason B-CLL is most commonly used to represent both variants (CLL/SLL)^{6,8-17}.

Epidemiology. B-CLL is the most common type of leukemia in Western countries. The incidence is estimated to more than 6 *per* 100,000 people annually. The median age at diagnosis is growing globally, so that now exceeds 70 years. It should be noted that the age at treatment initiation is several years older than the age at diagnosis, depending on the duration of observation without treatment. The disease is nearly twice as common in men¹⁸⁻²². The incidence and prevalence of MBL is much higher, depends on sensitivity of the methods used, and is estimated to involve up to 12% of the population aged over 40 years^{23,24}.

Diagnostic procedure

The diagnostic process can be conditionally divided into several sections (steps, phases) with respect to different objectives to be achieved. In Table 1, the basis for decision is specified, along with the main criteria for classification and the possible categories to which the classification in this section should lead.

Setting suspicion and patient referral to hematologist

The most common finding leading to suspicion of B-CLL is absolute lymphocytosis in routine blood ex-

amination (70%-80%), and less frequently (20%-30%) the finding of organomegaly (swollen lymph nodes and/or spleen) or symptoms associated with CLL.

Diagnosis and differential diagnosis

It is mandatory to make the diagnosis of typical B-CLL on the basis of morphology and flow cytometry in peripheral blood sample and to distinguish it from other entities in the CLL syndrome. For the diagnosis of B-CLL phenotype (typical phenotype), the following is required: restriction of sIg light-chain expression of low intensity, CD5⁺, CD19⁺, CD20^{low}, CD23⁺²⁵. Basic hematologic clinical findings and blood count allow for quantification of the tumor mass in peripheral blood and lymphoid organs, which enables classification of entities that meet the diagnostic criteria for:

- (1) B-CLL (presence in the blood of more than $5 \times 10^9/L$ clonal cells), or
- (2) SLL (less than $5 \times 10^9/L$ clonal cells in the blood and clonal lymphadenopathy greater than 1.5 cm), and
- (3) MBL (less than $5 \times 10^9/L$ clonal cells in the blood and no clonal lymphadenopathy or symptoms).

So, a different size of the tumor mass between B-CLL and MBL is critical and tumor distribution is critical for distinction between B-CLL and SLL.

It is evident that for the diagnosis of disease type, a very small number of tests could be considered sufficient because if the result is positive in peripheral blood (PB), it is not necessary to analyze bone marrow (BM) or lymph nodes (LN), although these tests have their place in further diagnostic work-up. For the diagnosis of SLL, it is recommended to make lymph node biopsy to establish the diagnosis, and radiological assessment (ultrasound (US) or computed tomography (CT)) of the neck, thorax, abdomen and pelvis can be useful to distinguish between MBL and SLL.

Other B-cell malignancies that can be accompanied by increased lymphoid cell counts in the blood should be taken in consideration in differential diagnosis. Distinction to CLL is usually made by flow cytometry assessed immune phenotype. The majority are B-cell malignancies such as follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), prolymphocytic leukemia (PLL) and

Table 2. Clinical stages and TTM

Rai stages

STAGE	DESCRIPTION	RISK
0	Lymphocytosis, in peripheral blood $>15 \times 10^9/L$ and $>40\%$ in bone marrow	Low
I	Stage 0 with enlarged lymph node (nodes)	Intermediate
II	Stage 0-I with splenomegaly, hepatomegaly or both	Intermediate
III*	Stage 0-II with hemoglobin <110 g/L	High
IV*	Stage 0-III with platelets $<100 \times 10^9/L$	High

* immune cytopenias do not fit in this stage definition

Binet stages

STAGE	DESCRIPTION	RISK
A	Hemoglobin ≥ 100 g/L and platelets $\geq 100 \times 10^9/L$ and <3 involved regions**	Low
B	Hemoglobin ≥ 100 g/L and platelets $\geq 100 \times 10^9/L$ and ≥ 3 involved regions	Intermediate
C*	Hemoglobin <100 g/L and/or platelets $<100 \times 10^9/L$ and any number of involved regions	High

* immune cytopenias do not fit in this stage definition

** the five lymphoid areas comprise uni- or bilateral cervical, axillary and inguinal lymphoid, hepatomegaly and splenomegaly

TTM-score

COMPARTMENT	REPRESENTATIVE	SIZE	RISK
TM ₁ - BM and PB	Lymphocyte count (peripheral blood)		9 Low
TM ₂ - Ly nodes	Diameter of largest palpable node	cm	9-15 Intermed
TM ₃ - spleen	Palpable spleen (below left costal margin)	cm	>15 High
TTM		TM ₁ +TM ₂ +TM ₃	Continuous variable

$|ly|$ = absolute number of lymphocytes; TTM = Total Tumor Mass score

Note: if the largest lymph node is found by imaging (US or CT), those values should be used for TM₂

Tumor Mass Distribution (TD) is calculated as quantitative parameter according to formula:

$$TD = \frac{TM_1}{TTM}$$

Doubling Time of TTM (DT) is calculated as quantitative parameter according to formula:

$$DT = \frac{M \times TTM_{beg}}{TTM_{end} - TTM_{beg}}$$

where TTM_{beg} is the size of TTM at the beginning of period M; TTM_{end} is TTM size at the end of period M; M is interval between TTM_{beg} and TTM_{end} in months.

M should be at least 3 months.

Electronic calculator available online at: <http://www.krohem.hr>

hairy cell leukemia (HCL), which all together represent less than 15% of all lymphoid appearing leukocytoses, and less than 5% express T-cell lineage marker variants.

It should be remembered that this type of disorder classification based only on the immune phenotype is not using morphological, cytogenetic, molecular, or

other characteristics of the diseases that today show significant association with prognosis.

Evaluation of the stage/extent of the disease

After the diagnosis, the next step is evaluation of the disease stage or extent. It is based on clinical and

hematologic evaluation, under the criteria shown in Table 2. Clinical stages represent a simple tool for clinical assessment of the disease extent. The basis of these systems is the assumption that the disease is gradually progressing and expanding. Therefore, the patients who have advanced disease have a higher tumor load and more extensive disease²⁶⁻²⁸.

The clinical stages according to Rai and Binet assess tumor size by simple parameters, which estimate the size of the tumor mass (without precise quantification of the affected compartments), along with the parameters for the assessment of bone marrow failure. In doing so, the greatest prognostic power contribution has the failure of bone marrow. Note that it is not a direct but an indirect sign of tumor size. Their prognostic power is relatively weak if the failure of the bone marrow is excluded.

The estimation of the size of the Total Tumor Mass (TTM) is different in that it quantitatively evaluates tumor mass in 3 major cell compartments, regardless of bone marrow failure (Table 2). Quantitative character enables unbiased monitoring of disease progression and is a very convenient tool for the assessment of therapeutic response (see later). In addition, in most patients who have both peripheral blood and lymphoid organs affected, it is possible to determine the type of distribution of the tumor mass by comparing leukemic (TM_1) and lymphoid organ ($TM_2 + TM_3$) compartments²⁹. This feature of TTM system enables to estimate the dynamics of disease progression and response to therapy and redistribution (see later)³⁰.

Prognostic factors at diagnosis and predictors of response

Analyses of prognostic factors performed in the era when therapy had little effect on clinical course identified a number of clinical or laboratory factors³¹, describing broadly the natural course of the disease. Prognostic factors that can be determined immediately at diagnosis are primarily related to the additional characterization of the tumor itself. Some of them do not change during evolution of the disease, such as the mutational status of IgVH genes that generally discriminate between more benign and more malignant disease³²⁻³⁴, and it is currently suggested to become minimal standard initial evaluation³¹. Until now, these were rarely used in routine clinical practice and

other tests showing high correlation with mutational status are used instead, such as CD38 and ZAP-70 by flow cytometry and immunohistochemistry, with the higher value found to correlate with poorer prognosis³⁵⁻⁴⁴. The usefulness of these surrogate factors is still controversial. In contrast, cytogenetic and molecular tests (FISH) to determine del (11q22-23), +12; del (13q14), del (17p13)⁴⁵ showed a strong independent prognostic effect and usually change (progress) over time. Also, finding certain mutations (including TP53, SF3B1, NOTCH1, etc.)^{46,47} can complement these findings. These tests are good predictors of response in the chemo-immunotherapy era and have proven to be useful to stratify patients into groups that react differently to certain drugs^{4,48}. Since they may change during the course of disease, testing must be performed just before each new line of therapy. All these tests that further characterize neoplastic clone cells are technically demanding and expensive, but relatively inexpensive compared to drugs, and for those validated the cost and effort are justified. Predictive factors seem to change or even lose their predictive power with novel treatments. Likewise, failure to new treatments will require discovery of new predictors.

On the other hand, a number of additional factors more related to patient's state also have a strong prognostic power, so a number of different parameters is often used, and multivariate analyses are performed to study their impact on prognosis⁴⁹.

Because of the above, we distinguish three types of factors, given the causal relationship with B-CLL. First, those that are associated with B-CLL clonal neoplastic diseases (size distribution, growth rate of tumors, mutational status, CD38, ZAP-70, FISH) may be used as predictors of response to therapy, etc.^{28,32,33,45,50-52}. Also, numerous other characteristics such as blood chemistry (serum beta₂ microglobulin, sCD23 and serum thymidine kinase⁵³⁻⁵⁵), clonogenic⁵⁶, kinetic, and computer assisted cell imaging have been shown to be related to prognosis^{57,58}; second, those that are associated with organ failure (mixed groups, they may be due to both very underlying neoplastic diseases and to consequences of associated diseases, such as anemia, thrombocytopenia, the etiology of which should be carefully evaluated); and third, those factors that are associated with the patient but not directly with the neoplasm (age, gender, performance status, comorbidity), that correlate with fitness and the ability to tolerate aggressive treatments^{40,59-62}.

On the basis of strong independent predictors, composite prognostic indices are calculated⁶³⁻⁶⁵, most recently the CLL International Prognostic Index (IPI)⁶⁶. They show very good prognostic power with respect to the length of survival in the chemo-immunotherapy (CIT) era, but much less so with new treatments. Also, one should be careful using them to stratify patients for treatment that is adapted to risk because a composite index usually consists of the factors that belong to each of the three above mentioned groups.

With new, more effective therapies, the prognostic landscape changes. Most of the important predictors in the CIT era lose their power, and new ones are yet to be identified^{4,48}.

Indications for treatment/the criteria for therapy initiation

The criteria for active disease that warrant initiation of treatment are based on iwCLL criteria, and are amended with TTM (Table 3). The decision should be based solely on the assessment of parameters that are associated with neoplastic B-CLL clone⁶. For parameters that may be unrelated to the neoplasm, it is necessary to carefully evaluate the extent to which the parameters are associated with neoplastic clone (e.g., fever, anemia, etc.). Criterion parameters can be classified into three distinct types:

1. Quantitative parameters for which threshold consensus is defined, which is considered to justify treatment initiation, such as anemia, defined by a certain level of hemoglobin, thrombocytopenia defined by platelet count, organomegaly defined by the size of spleen or lymph nodes.

2. Monitoring data to enable assessment of trends, for example, progressive cytopenia, progressive lymphadenopathy and/or splenomegaly, increased leukocyte count or TTM values (see the previous section). It should be emphasized that only measurement of dynamic parameters can directly evaluate progression of the disease, as opposed to the *a priori* estimate of a possible evolution described in the previous section (see **Prognostic features at diagnosis** section). Here, however, we should point out certain difficulties and ambiguities in quantitative measurement of the dynamic parameters, especially in the early stages of the disease. This imprecision in *a priori* definition of the

criteria for progressiveness, despite a very attractive concept, often leads to decision delay until the moment when it reaches the absolute value of the threshold that is set up as described above.

3. Qualitative criteria of the occurrence of symptoms threatening organ damage and the like, considered to be the result of neoplastic disease activity.

Today, we seek to combine threshold criteria with dynamic criteria (see Table 7). Note that a number of criteria, the criteria of threshold, dynamics and qualitative changes may be used. Although it is sufficient to indicate treatment by the presence of only one of the stipulated criteria, it is important that this criterion is compelling, and the presence of multiple criteria makes decision certainly easier. It especially holds for dynamic criteria, so it is good to compare the growth trend of the tumor mass with the trends of deepening anemia and/or thrombocytopenia. The indication for treatment (according to KROHEM guidance) should be documented in patient records.

The schedule of activities and assessments

Table 4 shows the general scheme of tests that are used in pretreatment work-up, in therapy monitoring, and after therapy. Of the newly diagnosed CLL patients, about 10% require therapy immediately, so the pretreatment work-up is done immediately at diagnosis and visits 1-3 are completed without delay. Other patients (90% of all diagnosed) are observed after diagnosis at periodic visits until meeting the criteria for initiation of treatment. At that point, complete pretreatment work-up is performed. About 30% of all diagnosed patients never reach the criteria for initiation of treatment, while about 60% of all diagnosed patients reach the criteria, but in different periods, from several months to more than 10 years.

It is possible to distinguish several specific clinical questions that need to be answered in stipulated visits and require different extent of investigations. These questions are answered in the following visits: (1) diagnosis, differential diagnosis, evaluation of the extent of disease, and preferably but not mandatory, assessment of prognostic groups that require investigations (a-f). At this time, it is recommended to evaluate general health profile (j); (2) repetitive visits monitoring clinical and hematologic parameters that serve as criteria for initiation of treatment, based on simple pa-

Table 3. Criteria for active (progressive/symptomatic) disease

	CRITERION	THRESHOLD	DYNAMICS	QUALITATIVE	COMMENT
1	Hemoglobin	<100 g/L	Trend (worsening)		The level of anemia that is used in determining clinical stages according to Rai and Binet.
2	Platelets	<100x10 ⁹ /L	Trend (worsening)		The level of thrombocytopenia that is used in determining clinical stages according to Rai and Binet.
3	High tumor mass	TTM >15	TTM DT <12 mo		Below 9 is not a sufficient criterion, between 9 and 15 is a 'gray zone', above 15 is an indication present. Lymphocyte count in itself is not a sufficient criterion, except for extreme cases (TTM >15 = lymphocytes >225x10 ⁹ /L)
4	Massive splenomegaly	>6 cm below LCM or US >20 cm	(progressive)	Pain	Usually they are combined, but not necessarily. Dynamic parameters involved in TTM
5	Massive lymph nodes	>10 cm	(progressive)	Pain	Usually they are combined, but not necessarily. Dynamic parameters involved in TTM
6	Threat to organ function			Clinical judgment	For example, compressive symptoms
7	B symptoms defined as any one or more of the disease-related symptoms or signs:	Unintentional weight loss >10%/6 months; or Significant fatigue (ECOG PS 2 or worse); or Fever >38 °C for 2 or more weeks without evidence of infection; or Night sweats for more than 1 month without evidence of infection			Usually they are combined, but not necessarily. The proposed system has long been used, particularly in lymphomas, and is well validated. The presence of B-symptoms is an important and indisputable element of therapeutic indications. It suffices that one is present, but there may be several present simultaneously.
8	Autoimmune anemia or thrombocytopenia			Poorly responsive to standard therapy	Standard therapy does not imply anticancer drugs, but includes corticoids

Hypogammaglobulinemia, monoclonal or oligoclonal paraproteinemia, or absolute lymphocyte count do not by themselves constitute an indication for therapy. It is out of 8 groups of criteria theoretically possible to identify 11 individual indications based on exceeding a threshold, 3 dynamic evaluation of continuous quantitative parameters, where individual trends can be compared and thereby gain additional derived criteria, and 4 qualitative assessments. Although in principle the presence of at least one indication is sufficient, we should avoid making decisions on an isolated indication. It is clear that a larger number of indications further reinforces the decision to begin treatment. It is possible to decide that the patient needs to document the presence of at least two or more of the above indications for active (progressive/symptomatic) disease. The indication for treatment (according to KROHEM guidance) should be documented in patient records!

Table 4. General schedule of investigations before, during and after therapy

Visits→		1	2*	3	4**	5	(2'→3') (4',5')
Investigations ↓	@ dg	Monitoring to meet criteria for treatment	Pre-th work-up	Th monitoring	EOT evaluation	After therapy repeat 2'-5' before new line (3')	
a	History/PE	+	+	+	+	+	+
b	Hematology	+	+	+	+	+	+
c	TTM/stage	+	+	+	+	+	+
d	Flow cytometry	+		+		+	+
e	FISH ^{CLL(4)} / Molecular	+ ^b		+			+
f	Cyto/Histo	+ ^b		+		+	+
i	Imaging			+		+	+
j	Other ^{clin ind}	+	+	+	+	+	+
k	Research ^b	+	+	+	+	+	+

*Monitoring protocol frequency varies depending on clinical condition from several weeks to several months, or even one year if the situation is stable, without change. However, in the emergence of new circumstances, it is necessary to check-up early; **these repetitive visits depend on treatment applied. For novel agents with at present unlimited duration, these visits should enable detection of treatment failure; ^bpreferred but not required tests.

rameters (investigations a, b, c, j). The time between the visits may vary from weeks to months; (3) immediate pre-therapeutic work-up provides definite classification of the disease, definitive assessment of prognostic parameters, as well as the general state of the patient, and any associated illnesses including infection status. For this reason, this visit should be most complete, enabling stratification and providing baseline parameters for treatment; (4) these repetitive visits to monitor the course of therapy should be tailored according to the needs of respective therapy; and (5) end of therapy (EOT) visit should enable evaluation of the response achieved. After that, for CIT that is time limited, monitoring visits without therapy (2') are repeated again and in case of the need for a new line of therapy, repeat visits 3', 4' and 5' are scheduled. The situation is different for novel agents since this treatment is at present unlimited and visits 4 should enable detection of treatment failure.

Therapy individualization – defining goals and strategies, and stratifying patients

At the end of diagnostic procedure described above, there is a need to make decision on therapy, which will reflect individualization of the respective patient. Decision is based on the integration of the factors related

to the neoplasm on the one hand and the factors related to the patient on the other hand. According to new circumstances and new emerging paradigm because of the lack of curative treatment, initial therapy should maximize efficacy while minimizing overall toxicity. The overall approach is essentially based on clinical judgment and the expertise.

In general, we are comparing two risks: the risk of disease and the risk of treatment. It is clear that the risk of treatment should be reasonably lower than the risk of disease.

The risk of disease. Some treatments have been shown to be inefficient in some disease subsets. However, novel treatments are also active in those subsets, which will diminish the importance of stratification according to FISH and TP53, which was necessary until now. Nevertheless, since CIT has proved very effective in some subsets of patients and contraindicated in others, the classification according to risk should remain until head-to-head comparisons resolve pending questions.

The risk of therapy, i.e. tolerance (or acute treatment toxicity) is highly dependent on the general condition of the patient and the presence of associated diseases, which is often associated (though not exclusively) with the patient's age. However, treatments with new B-cell receptor inhibitors (BRI) have much better toxicity pro-

file that diminishes the importance of stratification according to fitness, which was necessary in the chemoimmunotherapy based paradigm. Nevertheless, for the same reason as described above, this stratification should remain for CIT. Since CIT is not indicated in patients with del(17p)/TP53 mutation, the distinction between fit and unfit becomes irrelevant.

A patient is classified as capable (fit, Go Go) when there is a low comorbidity score (e.g., CIRS-G <6). Although age does not enter in the calculation of CIRS-G, it is known that age is a very important factor and it should be taken into account. It is common to impose an age limit for stratification in therapeutic groups. Today, the limit is set at 65 years. Patients aged over 65 can be considered capable for receiving aggressive therapy if in excellent health, without substantial comorbidity.

On the basis of the two above-mentioned principles, patients are today stratified into three strata with respect to antineoplastic therapy: (1) without the p53 gene deletion with good general condition (capable for aggressive, fludarabine based CIT therapy) – (LOW RISK + FIT), (2) without the p53 gene deletions with poor general condition (incapable for aggressive therapy) – (LOW RISK + UNFIT), and (3) deletion of (17p)/TP53 mutation (HIGH RISK + FIT/UNFIT). For those patients, no further division according to fitness is necessary, since the new approved treatments are well tolerated^{16,17,67,68}. In other words, these strata may in principle represent the combination of risk (high correlation with TP53 abnormality) and age (high correlation with fitness). However, for the reasons stipulated above, the chronologic age cut-off should not be rigid, so as to allow elderly patients in good health to enjoy the benefit of more aggressive treatment, and *vice versa*, to spare younger patients with comorbidity of unwarranted therapy associated risks.

The stratification is used for guidelines and reflects general principles, but for each patient, treatment plan should be individualized and set up after careful clinical evaluation, also taking into account patients' preferences.

Therapeutic procedure

Therapeutic recommendations based on evidence/clinical trials

The treatment for B-CLL consists of antineoplastic therapy and supportive measures. Antineoplastic

measures consist traditionally of chemotherapy, therapeutic antibodies, radiotherapy, stem cell transplantation methods, and recently novel agents that include B-cell receptor signal transduction inhibitors (BRI) and bcl-2 antagonists. In these guidelines, the recommendations are based on phase 3 clinical trials, in some cases on evidence from earlier phase trials, and on the approved agents and therapies in Croatia. Combination therapy is generally more efficient than monotherapy.

Choice of antineoplastic treatment options – new therapeutic paradigm

Because this disease is generally not curable, occurs in elderly population, and often progresses slowly, it is most often treated in a conservative fashion. In asymptomatic patients, treatment may be deferred until the patient becomes symptomatic as the disease progresses⁶⁹. This concept is still valid and the treatment outside clinical trials is recommended only for patients that fulfill the indications described. Since the rate of progression may vary from patient to patient, with long periods of stability and sometimes spontaneous regressions, frequent and careful observation is required to monitor the clinical course⁵. At present, about 30% live without symptoms and never need therapy. Others will progress sooner or later, and will meet the criteria for therapy introduction.

The new, very effective treatments have recently dramatically changed the therapeutic landscape and led to change in the current CIT based paradigm into a new one. In comparison to therapies available to date, the new treatments show markedly improved efficacy and considerably better tolerance. This has an impact on all aspects of patient management and choice of treatment options. This also goes for deferring therapy, but at present there are no data to support early treatment. Current and future clinical trials that include novel agents in this setting may change this current concept. For patients in whom the criteria for therapy initiation are met, the general principle in the new paradigm is that (because no curative therapy has yet been found) initial therapy should maximize efficacy with improvement of overall survival (OS), while introducing the least overall toxicity, both short term and long term. Standard chemotherapeutic agents induce not only cytopenias and sometimes fatal infections (acute treatment toxicity) but also mutational

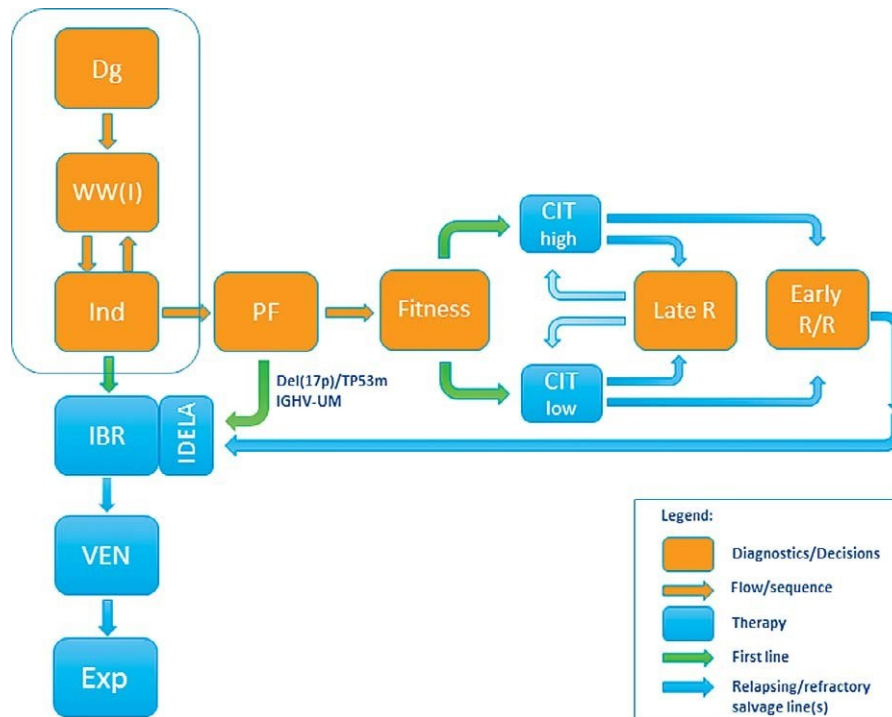


Fig. 1. Flow chart describing B-CLL diagnosis and treatment 2017 paradigm shift.

This chart shows diagnostics based decision steps (orange rectangles) and their sequence (orange arrows), currently approved therapies by EMA (in 2/2017) (blue rectangles), as well as the sequence for first-line treatment (green arrows) and salvage treatment lines (blue arrows). The minority of patients (about 10%) present with indication for treatment at diagnosis, while the majority are observed until the criteria for treatment are met. This part (framed) did not change. When the indication is present, B-CLL patients are eligible for first-line treatment. In this part, major changes have occurred because of recent approval of new options. Ibrutinib monotherapy is approved as continuous treatment of undetermined duration or until progression or unmanageable toxicity for all patient strata because of favorable efficacy/tolerance ratio in disease control. This is a new approved option, so that ibrutinib could be used to start the new path. In case of progression or toxicity, patients qualify for second-line treatment (approved option is venetoclax). If this fails, the patient is eligible for experimental treatments (combinations of novel agents with immunotherapy, allogeneic stem cell transplantation, CAR-T cell therapy, and the like). Theoretically, all of these could be done without further diagnostics and stratification, while avoiding chemotherapy. However, head-to-head comparison data between novel agents and chemo-immunotherapy (CIT) are still lacking. CIT, although associated with higher short- and long-term toxicities, has proved to be highly effective in achieving long, durable remissions and perhaps even a cure in some patient subsets. It was therefore in the CIT era essential to identify those who would respond, and a number of predicting factors have emerged in this setting. The NGS has revealed that intratumoral heterogeneity and genomic changes can be used for better CIT response prediction. Most important for CIT clinical use are two predictors, del(17p)/TP53mut and IGHV mutation status. The former can identify patient subset in which chemotherapy is ineffective and even contraindicated because of inducing adverse clonal evolution, and the latter can identify disease type, where patients with unmutated IGHV poorly respond to CIT and even if they respond, the response is short and clones that are more resistant emerge. Both predictors are considered standard minimum for stratification. If adverse features are present, the patients should be treated with ibrutinib or idelalisib in first line. Others may continue towards CIT that is tailored according to age and comorbidities. Fit patients qualify for FCR, unfit for Clb+Obi or like, and patients 'in-between' for BR. If they relapse late, the CIT may be repeated, tailored to current fitness, while early relapsed/refractory patients qualify for BRI or venetoclax salvage. At present, baseline stratification based on genetically defined risk, as well as on age and comorbidities to tailor treatment intensity is still needed for CIT, although fitness is currently not important for novel agents. The current CIT based paradigm (shown horizontally) is losing importance and the new paradigm (shown vertically) is likely to take over. However, it will require identification of new important predictors along the new path, since the majority of predictors identified for CIT lose their power in the new setting. As data accumulate, new predictors will emerge for this setting. High throughput NGS has begun to identify new predictors for targeted therapy response, as well as new predictors of failure at molecular level, as treatment proceeds. All this may eventually lead to a new upfront stratification for risk adapted precision medicine therapy in B-CLL. The ongoing trials and head-to-head comparison of novel agents and their combinations with immunotherapy *versus* CIT are under way. They will hopefully resolve current dilemmas. Novel therapy research including genomic diagnostics is likely to offer new options that will eventually lead to time limited therapies, without chemotherapy.

Dg = diagnosis; WW(I) = watch and wait (investigate); Ind = indications for treatment; PF = predictive factors; Late R = late relapse; Early R/R = early relapsing or refractory; IBR = ibrutinib; IDELA = idelalisib; VEN = venetoclax; Exp = experimental treatment; CIT = chemo-immunotherapy

damage to the genome that can manifest as more aggressive and refractory phenotypes upon relapse and can induce second malignancies. For this reason, avoiding chemotherapeutic agents upfront, when possible, is a new paradigm of sequencing therapy for CLL⁵. Major changes are explained in Figure 1.

However, in the absence of head-to-head randomized trials to assess efficacy/tolerance ratio between novel therapies and best CIT for fit patients, in this subset CIT should not be abandoned. Also, the access and availability of new treatments will need some time, and the current cost of novel treatment may be out of reach for insurers.

Treatment options and current labels for approved antineoplastic medications

Observation. For patients who do not fulfill the criteria for starting therapy, the treatment is observation. Although it is in contrast to general oncologic tendency to treat patients with neoplasms as soon as possible, no data exist to suggest any harm in deferring therapy in those patients. Since the rate of progression may vary, frequent and careful observation is required to monitor the clinical course.

Ibrutinib. Ibrutinib is a selective irreversible inhibitor of Bruton tyrosine kinase, a signaling molecule located upstream in the B-cell receptor-signaling cascade. **Label:** IMBRUVICA as a single agent is indicated for the treatment of adult patients with previously untreated CLL; IMBRUVICA as a single agent or in combination with bendamustine and rituximab (BR) is indicated for the treatment of adult patients with CLL who have received at least one prior therapy⁷⁰. Those indications have been approved based on the randomized phase 3 studies RESONATE-2 (PCYC-1115-CA), RESONATE and HELIOS⁷¹⁻⁷³.

Idelalisib. Idelalisib is an oral inhibitor of the delta isoform of phosphatidylinositol 3-kinase, which is located in the B-cell receptor-signaling cascade. **Label:** ZYDELIG is indicated in combination with an anti-CD20 monoclonal antibody (rituximab or ofatumumab) for the treatment of adult patients with CLL who have received at least one prior therapy, or as first-line treatment in the presence of 17p deletion or *TP53* mutation in patients who are not eligible for any other therapies⁷⁴. Approval is based on randomized, double-blind, phase 3 study⁷⁵ and phase 2 study⁷⁶.

Rituximab. Rituximab is a murine anti-CD20 monoclonal antibody. **Label:** MABTHERA is indicated in combination with chemotherapy for the treatment of patients with previously untreated and relapsed/refractory CLL⁷⁷. Approval was based on randomized phase 3 studies with fludarabine and cyclophosphamide (FCR)^{78,79}, bendamustine (BR)^{80,81}, and chlorambucil^{82,83}.

Obinutuzumab. Obinutuzumab is a human anti-CD20 monoclonal antibody. **Label:** GAZYVARO in combination with chlorambucil is indicated for the treatment of adult patients with previously untreated CLL and with comorbidities making them unsuitable for full-dose fludarabine based therapy⁸⁴. Approval is based on a randomized phase 3 study⁸².

Ofatumumab. Ofatumumab is a human anti-CD20 monoclonal antibody. **Label:** ARZERRA is used in previously untreated patients who cannot be treated with fludarabine; in these patients, it is used together with chlorambucil or bendamustine (other cancer medicines); in patients whose disease has not responded to previous treatment (known as refractory disease) with fludarabine and a medicine called alemtuzumab; and in patients whose disease has come back after previous treatment (known as relapsed disease). In these patients, ARZERRA is used together with fludarabine and cyclophosphamide⁸⁵. Approval is based on a randomized phase 3 study in combination with chlorambucil COMPLEMENT-1⁸⁶.

Alemtuzumab. Alemtuzumab, a monoclonal antibody directed at CD52. The drug was withdrawn for CLL indication by the producer because of commercial reasons. However, the company can offer the drug on request for compassionate use free of charge. **Label:** (EMA EPAR 2011 authorization withdrawn). MAB-CAMPATH is used to treat patients with B-cell CLL for whom treatment combinations including fludarabine are not appropriate⁸⁷. Approval was based on randomized clinical trials and showed activity in *TP53* mutation⁸⁸⁻⁹⁰.

Venetoclax. Venetoclax is a highly selective inhibitor of *Bcl2*. **Label:** VENCLYXTO monotherapy is indicated for the treatment of CLL in the presence of 17p deletion or *TP53* mutation in adult patients who are unsuitable for or have failed a B-cell receptor pathway inhibitor. Venclyxto monotherapy is indicated for the treatment of CLL in the absence of 17p deletion or *TP53* mutation in adult patients who have failed

both chemo-immunotherapy and B-cell receptor pathway inhibitor⁹¹. Approval is based on a phase 1 escalation study⁹² and phase 2 study⁹³.

Oral alkylating agents with or without corticosteroids. Chlorambucil was used for the treatment of CLL for more than 60 years. **Label:** LEUKERAN is indicated for the treatment of CLL⁹⁴. Although the role of chlorambucil has considerably diminished, regulators paradoxically still accept drugs for registration on the basis of phase 3 trials, which use chlorambucil in very low doses as comparator^{89,95,96}, while claiming at the same time that the therapeutic success of such therapies is extremely modest. Thus, low doses are still considered standard therapy, although it was shown that medium and especially high doses had significantly greater effectiveness⁹⁷⁻¹⁰⁰. A meta-analysis of six trials of immediate *versus* deferred therapy with chlorambucil showed no difference in OS at 10 years⁶⁹.

Purine analogs. Fludarabine is a purine analog, one of a group of chemotherapy drugs known as anti-metabolites. They stop cells making and repairing DNA. Cancer cells need to make and repair DNA in order to grow and multiply. **Label:** Fludarabine (gen) is used in the treatment of B-cell CLL in patients with sufficient healthy blood cell production. First treatment for CLL with this medicine should only be started in patients with advanced disease having disease-related symptoms or evidence of disease progression¹⁰¹. Approval is based on a phase 3 randomized study⁹⁵. This drug is also used in combination therapies (see below).

Bendamustine. Bendamustine is a cytotoxic agent with bifunctional properties of an alkylator and a purine analog. **Label:** bendamustine (gen) is used as monotherapy or in combinations with other drugs for treatment of CLL in cases when combination chemotherapy containing fludarabine is not appropriate¹⁰². Approval is based on a randomized phase 3 trial for monotherapy¹⁰³ and for combinations on phase 2 trials in previously treated⁸⁰ and untreated patients⁸¹.

Combination therapy

Fludarabine based combinations include FCR, FCOfa, FR, and FC. Fludarabine plus cyclophosphamide plus rituximab has proved very effective in those patients who can tolerate the treatment. For this reason, in the last 10 years, FCR has become the gold standard of CIT for fit patients⁷⁸. Long-term results have confirmed overall efficacy and a subset of long-

term responders defined by genomic risk groups has emerged¹⁰⁴⁻¹⁰⁶. Although no head-to-head comparisons have yet been completed in line with the new paradigm, the indication is narrowed to a subset of fit patients with hypermutated IVGH in whom the likelihood of very long remission may outweigh the concerns of chemotherapy toxicity.

Bendamustine combinations are used in those patients in whom fludarabine cannot be tolerated. In head-to-head comparison to FCR, BR combination was found inferior¹⁰⁷.

Combination therapy without anti-CD20 monoclonal antibodies. For FC, CVP and CHOP, a meta-analysis of ten trials compared combination chemotherapy (before the availability of rituximab) with chlorambucil alone and showed no difference in OS at 5 years⁶⁹.

Combination with novel agents. It is likely that combinations of BRI and Bcl2 inhibitors with anti-CD20 antibodies will be the basis in the era of the new emerging paradigm. At present, only ibrutinib + bendamustine + rituximab and idelalisib + anti-CD20 monoclonal antibodies are approved.

Bone marrow and peripheral stem cell transplantations. Although this modality is considered the only option for cure, it is still under clinical evaluation, especially in the context of novel agents and new emerging paradigm^{108,109}.

The overall therapeutic effect is a consequence of total therapeutic interventions, including antineoplastic and **supportive measures** that are particularly important.

Criteria for evaluation of response to therapy

Table 5 shows the criteria for assessment of therapeutic effect. The criteria generally used the same grounds, based on the estimation of the tumor mass parameters in different compartments on the one hand, and the parameters for the assessment of myelopoiesis on the other hand. The criteria are somewhat different in the current NCI/iwCLL criteria^{6,13} and the criteria described below (IGCI, EORTC)^{97-99,110,111}.

To monitor the dynamics of the disease (both progression and response to therapy), TTM score (described in clinical stage) is very convenient because it is the only clinical system that is based on a continuous, quantitative parameter that is easy to apply and

Table 5. Definition of response to treatment (NCI updated guidelines, Blood 2008)

	PARAMETER	CR ¹	PR ¹	PD ¹
NCI / IWCLL	Group A			
	Lymphadenopathy ²	There is none >1.5 cm	Decrease $\geq 50\%$	Increase $\geq 50\%$
	Hepatomegaly	No	Decrease $\geq 50\%$	Increase $\geq 50\%$
	Splenomegaly	No	Decrease $\geq 50\%$	Increase $\geq 50\%$
	Lymphocytosis in blood	$<4 \times 10^9/L$	Decrease $\geq 50\%$ from baseline	Increase $\geq 50\%$ from baseline
	Bone marrow ³	Normocellular, <30% lymphocytes, No B-lymphoid nodules. Hypocellular marrow defines CRi.	50% reduction of marrow infiltration or B lymphoid nodules.	
	Group B			
	Platelets	$>100 \times 10^9/L$	$>100 \times 10^9/L$ or increase $\geq 50\%$ from baseline	Reduction by $\geq 50\%$ from baseline as a result of CLL
	Hemoglobin	>110 g/L	>110 g/L or increase $\geq 50\%$ from baseline	Reduction by >2 g/dL from baseline as a result of CLL
	Neutrophils ³	$>1.5 \times 10^9/L$	$>1.5 \times 10^9/L$ or $>50\%$ improvement from baseline	
TTM* (EORTC/IGCI/KROHEM)	TTM	TTM <2 (lymphocytosis $<4 \times 10^9/L$, no lymphadenopathy, no organomegaly)	TTM decrease $\geq 50\%$ and TTM <9	TTM increase $\geq 25\%$
	BM function (Plt, Hb, Neutro): same as NCI/IWCLL (group B)			

Group A criteria define tumor mass, group B criteria define hematopoietic system (or bone marrow) function.

¹ CR (complete remission): all criteria must be present, and patients must be without general symptoms associated with CLL; PR (partial remission): at least two criteria in group A plus one in group B must be present; SD (stable disease) is the absence of progressive disease (PD) if at least PR is not reached; PD (progressive disease): at least one criterion from group A or B must be present.

² The sum of the products of multiple lymph nodes (as evaluated by CT in clinical trials or by physical examination in general practice).

³ These parameters are irrelevant for certain types of responses.

validated in thousands of patients in various international clinical trials. To estimate the doubling time (DT), it is more reliable than just the number of lymphocytes because it can compensate for changes in the distribution of the tumor mass occurrence after the administration of corticosteroids or TKIs, when there may be an increase in the number of leukocytes while reducing the nodes or spleen. For this reason, the NCI/iwCLL criteria have recently been amended, but are still suboptimal for monitoring disease response^{23,112}, while the TTM scoring system is much better for measuring redistribution of clonal cells among compartments^{30,113}.

When assessing the response to therapy, complete remission (CR) is assessed equally in the TTM and

NCI based criteria, but TTM shows the advantage in assessing partial remission (PR) by comparing total tumor mass before and after treatment, so it is possible to set a minimum threshold for minimal remission (MR), e.g., reduction by $>25\%$, partial remission (PR) $>50\%$, and very good PR $>75\%$ and more. Likewise, the TTM based criteria are more accurate and without bias on estimating stable disease (SD) and progressive disease (PD). The continuous quantitative character of TTM size allows for comparison of trends between the criterion group A (TTM) and group B (function of residual normal hematopoiesis). It is possible to evaluate the beneficial antineoplastic effect of therapy independently of the toxic effect on hematopoiesis.

Minimal residual disease (MRD) is an important end point, and should reflect no measurable disease in the body. However, MRD testing should be standardized and the sample source well defined. By doing this, we should avoid reports of MRD negative patients with persistent significant organomegaly. Those cases in fact have 'clean' blood but sometimes may not fulfill common criteria for partial remission, and the term MRD without specification is misleading.

Antineoplastic therapy – current treatment options

There are several treatment options. Therapeutic recommendations summary for **first-line treatment** and for **salvage treatment** in major therapeutic stratification groups are shown in respective tables.

Management of patients with no accepted criteria for therapy

If the patient is not showing any signs of active/progressive/symptomatic disease, the antineoplastic therapy is not recommended, but the patient is monitored and reviewed without therapy. Thus 'Watch and Wait' should be transformed to 'Watch and Investigate' (W&I). This view is based on the evidence collected in randomized trials during the 1980s, when it was shown that chlorambucil based treatment did not contribute to longer survival, moreover, despite the relative ease of controlling symptoms and achieving clinical remission, overall survival was marginally worse^{97,110,114}. Until now, there are no data indicating that harm is due to deferring therapy in asymptomatic, stable disease. However, trials that are under way, which include novel agents and/or combinations, may change this concept, but it will take time since those trials require prolonged follow up.

While for these patients antineoplastic medication is not recommended, standard care should include infection prophylaxis such as annual flu and pneumococcal every 5 years, and in case of infection early treatment.

Initial treatment (first-line treatment)

The **first-line treatment** relates to previously untreated patients. All patients in standard care must

have clinical indication for treatment initiation (i.e. must fulfill the criteria stipulated above). Recommendation depends on the risk associated with B-CLL (High or Low) and patient general condition (Fit or Unfit) (Table 6, Fig. 1). Each stratum will be discussed separately.

Initial treatment for patients with no del(17p)/TP53 mutation that are fit (LOW RISK + FIT)

As a rule, the patients in this stratum (about one-third of first-line treatment) are younger than 65. Therapeutic goal is to be set high, to achieve complete, durable remission, prolong survival, and perhaps even offer a possible cure.

FCR (fludarabine, cyclophosphamide, rituximab) is recommended as a standard initial therapy for previously untreated fit patients outside clinical trials⁷⁸. According to the DCLLSG CLL8 protocol, 6 cycles at intervals of 28 days if the patients tolerate the treatment well, and after EOT, no further treatment is anticipated, only follow up visits. Long-term follow up identified a subset of patients in whom long and durable response was achieved^{105,106,115}. Those patients had mutated IGHV, no 11q and were aged <65. Other patients may respond, but they tend to relapse soon. For this reason, for fit patients aged <65, mutated IGHV and no 11q (nor del(17p)/TP mutation) who fulfill the criteria for treatment, FCR is the treatment of choice. In others, according to the new paradigm, it is suggested to avoid chemotherapy, especially FC.

In patients who are unsuitable for fludarabine therapy, bendamustine + rituximab (BR) can be tried. It seems to be both less toxic and less effective.

Ibrutinib is also approved for this indication. According to the new paradigm, BRI may replace CIT but at present, there are no data of randomized trials to support it. Head-to-head comparison of FCR or BR with ibrutinib is under way and is likely to resolve this question.

Initial treatment for patients with no del(17p)/TP53 abnormality that are unfit (LOW RISK+UNFIT)

The majority of patients (more than 60% of treated in first line) belong to this group. As a rule, the patients are older than 65, with comorbidity, and therefore not capable to tolerate aggressive CIT therapeutic approach; thus, it is necessary to modify therapeutic goal

Table 6. First-line treatment of CLL (KROHEM v1 2017)

Stage	% ^a	Molecular cytogenetics	% ^b	General condition	% ^b	First-line treatment ^c
						Standard
Asymptomatic; Binet: A-B ; Rai 0-II; TTM<9 (15)	33	Irrelevant		Irrelevant		Nothing (W&I)
Binet C, Rai III-IV; TTM>15; or symptomatic disease (indication for treatment met)	67	No del(17p) / TP53 mut	93	Fit	32	FCR (1) ^d B + R ^e Ibrutinib (1)
				Unfit	61	Chl + Obi (1) Chl + R Chl + Ofa B + R Ibrutinib (1)
		del(17p)/ TP53 mut	7	Irrelevant	7	Ibrutinib Idelalisib + R HDMP+R A ^f

Clinical trials are highly recommended for all subsets and we strongly believe that they improve the level of care.

^a Projected percentages are based on compiled data from Western countries and Croatia.

^b Percentages of patients with distinct general condition and molecular genetics groups refer to treated patients. Fit patients are less than 65 years of age and with CIRS score less than 6. Younger patients with CIRS score of 6 and more and patients aged 65 years or more (regardless of CIRS score) qualify as unfit.

^c Standard treatments are in order of preference, all are 2A or less according to NCCN consensus, treatments with higher grade are marked (1).

^d In patients with hypermutated IGHV and no 11q.

^e For less fit patients.

^f Alemtuzumab is withdrawn from market, but can be obtained free of charge from producer upon request.

FCR (fludarabine, cyclophosphamide and rituximab); B = bendamustine; Chl = chlorambucil; R = rituximab; Obi = obinutuzumab; Ofa = ofatumumab; A = alemtuzumab; HDMP (high dose methylprednisolone).

and choose remission or stabilization of disease with a well-preserved quality of life.

We recommend chlorambucil plus one of the anti-CD20 antibodies as a standard^{82,83,86,116}. Best results have been published with chlorambucil + obinutuzumab.

Also, BR is an option of chemo-immunotherapy for patients with appropriate fitness.

Ibrutinib is also approved for this indication because of excellent results in this patient subset. According to the new paradigm, BRI may replace CIT but at present, there are no direct data to support it. Head-to-head comparison of ibrutinib + obinutuzumab with chlorambucil + obinotuzumab is under way and is likely to resolve this question.

Initial treatment for fit/unfit patients with del(17p) /TP53 mutation (HIGH RISK + FIT/UNFIT)

In this stratum, we expect less than 7% of all patients treated in first line. CIT is contraindicated in

this subset, since the TP53 mutated clone is not responding and CIT may enhance unfavorable clonal selection and is therefore harmful. We recommend for both fit and unfit the induction with ibrutinib or idelalisib plus rituximab. Ibrutinib appears superior to idelalisib in all settings as first choice BRI¹¹⁷⁻¹¹⁹. In selected cases, this therapy can be followed by elective AlloSCT. HDMP plus rituximab^{120,121} or alemtuzumab¹²² should be used if BCI therapy is unavailable.

Treatment for relapsed/refractory CLL

This relates to previously treated patients. Again, they should fulfill the criteria for retreatment, essentially the same as described above. For this reason, time to progression (measured as PFS) is distinct from time to next treatment (TTNT). The situation in this setting is much more complex, since in addition to four major therapeutic strata, special attention should be paid to previous treatment(s) (type of treatment, num-

ber of treatment lines, the period that has elapsed from previous treatment, etc.). In principle, with the exception of very late relapses, the patients require more therapy to achieve less response.

Since the vast majority (>90%) of all CLL first-line treated patients have no TP53 abnormality, their treatment allocation was essentially dependent on their general condition (Fit or Unfit). Thus, the fit patients receive more aggressive treatment aiming at MRD negativity (hopefully eradication of the disease), while the unfit patients receive less aggressive treatment that is less likely to achieve MRD negativity and consequently the therapeutic aim is less ambitious.

The relapse is, therefore, primarily linked to the first-line therapeutic stratum. It is an indicator of respective therapy failure. In principle, the longer the period to relapse, the more effective first-line treatment was.

General principles of therapeutic strategies in relapsing/refractory patients are shown in Table 7 and Figure 1. The relapsing patient in early relapse is considered refractory and requires change of therapy. If the treatment results in remission of long duration, it is reasonable to try the same treatment that has proved effective again in the relapse.

Table 7. Treatment of relapsed/refractory CLL (KROHEM v1 2017)

Relapse	% ^a	Molecular cytogenetics	%	General condition	%	Salvage treatment		Extended / Maintenance
						Standard ^{b,c}		
Early (<2 years) Refractory disease (<1 year)	30	No del(17p) / TP53mut	22	Fit	7	Ibrutinib (1) Idelalisib + R (1) Venetoclax (1) ^c	FCR ^d B+R ^d F + A ^e	→AlloSCT →antiCD20 ^g
				Unfit	15	HDMP + R Ofa	B + R Chl + antiCD20 ^d	→antiCD20 ^g
		Del(17p) / TP53mut	8	Fit & Unfit	8	Ibrutinib (1) Idelalisib + R (1) Venetoclax (1) ^f HDMP + R A ^e ± R		→AlloSCT (fit) →antiCD20 ^g
Late (>2 years)	70		70	Fit & Unfit		Repeat first line (or choose from above)		

The guidelines for salvage treatment are more complex than in first-line treatment. It should take into consideration additional criteria depending on the type of treatment in first line, and on the observed duration of response. Clinical trials are highly recommended for all subsets and we strongly believe that they improve the level of care.

^a Projected percentages of early and late relapses are based on Dubrava University Hospital data for 2015 and 2016. Percentages of unfit patients and patients with del(17p) tend to increase. Fit patients = less than 65 years of age and with CIRS score less than 6. Younger patients with CIRS score of 6 and more and patients aged 65 years or more qualify as unfit;

^b Standard treatments are in order of preference, but for each individual patient should be based on integration of clinical data and patient preference. All treatments are 2A according to NCCN consensus, treatments with higher grade or lower grade are marked;

^c In patients who are unsuitable for or have failed a B-cell receptor pathway inhibitor and chemo-immunotherapy;

^d If not in 1st line;

^e Alemtuzumab is withdrawn from market, but can be obtained free of charge from producer upon request;

^f In patients who are unsuitable for or have failed a B-cell receptor pathway inhibitor;

^g Ofatumumab is found to significantly prolong PFS in responsive patients in second or third response to chemo-immunotherapy, approved by FDA.

FCR (fludarabine, cyclophosphamide and R); B = bendamustine; Chl = chlorambucil; R = rituximab; Obi = obinutuzumab; Ofa = ofatumumab; A = alemtuzumab; Allo SCT = allogeneic stem cell transplantation; HDMP (high dose methylprednisolone); antiCD20 (ofatumumab or obinutuzumab or rituximab).

For this reason, relapses will be described as a function of first-line stratification therapeutic failures. However, in all patients in relapse, TP53 status should be checked (before each new line of therapy) to assess whether the risk grade has changed in comparison to front-line stratification. Most of the patients currently relapsing early were in first line treated by CIT adjusted to fitness status. Occasionally, some patients in first relapse were in first line treated by chemotherapy (chlorambucil, fludarabine alone or in combination with cyclophosphamide).

Patients relapsing from the LOW RISK + FIT stratum

Patients relapsing early are considered refractory and should be treated with ibrutinib, or idelalisib plus rituximab.

If the anti BCR drugs cannot be provided, the current options include BR, HDMP+R, FA, other chemo-immunotherapy, ofatumumab and alloSCT.

Patients relapsing late who have not acquired TP53 abnormality remain fit enough for fludarabine-based treatment, and those with a clinical indication for treatment may receive FCR, provided that they have mutated IGHV, no TP53 or 11q^{108,123}. If the patient at the time of relapse changes to unfit stratum, the relapse treatment described in respective section applies.

Patients relapsing from the LOW RISK + UNFIT stratum

Over 50% of all treated patients belong to this group. In this group, less aggressive treatment was applied in front line because these patients are not likely to tolerate FCR. The response obtained is less likely MRD negative and relapses are expected in a wide range from early (less than 24 months) or late (more than 24 months).

In case of early relapse, patient is considered refractory to given treatment and ibrutinib or idelalisib + rituximab is recommended. Less benefit can be expected from BR or chlorambucil + antiCD20 or HDMP + R. In selected responsive cases, ofatumumab maintenance is applied.

In case of late relapse, the patients relapsing after chlorambucil can be retreated with chlorambucil + anti-CD20 monoclonal antibody¹¹⁶. This therapy may be repeated several times (in function of achieved du-

ration) until the duration of remission is shortened to 2 years, after which it is justified to go to the second-line therapy (ibrutinib or idelalisib+R).

Patients relapsing from the HIGH RISK + FIT/UNFIT stratum

These patients are at a particularly high risk. If the patients did not receive BRI treatment, those drugs are recommended. If the patients relapse on BRI drugs, venetoclax or alternative BRI is indicated. If those are not available, HDMP + R or alemtuzumab ± R can be tried.

If the fit patients were not previously allo transplanted, reinduction should be considered with different combinations (including ofatumumab) and if successful, proceed to transplantation.

In selected responsive cases, ofatumumab maintenance may be considered¹²⁴.

Consolidation/Maintenance therapy

It has been observed that MRD negative remission is associated with prolonged progression free survival both in previously untreated¹²⁵ and relapsed¹²⁶ patients. This has led to studies of additional treatment in patients with residual disease post therapy. In line with the new paradigm, although chlorambucil maintenance can prolong survival¹²⁷, maintenance treatment should be chemotherapy free. Ofatumumab in selective responsive patients was shown to prolong PFS but not OS¹²⁸, and is approved for this indication by FDA. Other anti-CD20 antibodies, although approved in some other B-cell malignancies¹²⁹, are not yet approved in B-CLL by regulators. Lenalidomide was also shown to improve PFS, but is not yet approved by regulators^{130,131}.

As described earlier, BRI therapies are given for a prolonged period to maintain the response as distinct from chemo-immunotherapy. Early (months) period of treatment is characterized by marked tumor mass redistribution, so that monitoring should be adjusted accordingly (see section on response criteria).

The role of allogeneic transplantation

The indication for allogeneic SCT is currently changing in line with excellent results of BRI and venetoclax. Current indications include poor respond-

ers to BRI and Bcl-2 antagonist, and appearance of Richter syndrome¹⁰⁹.

The role of radiotherapy

Radiotherapy can provide effective palliation in cases with symptomatic bulky lymphadenopathy and should be offered to patients in which chemo-immunotherapy has been ineffective or is contraindicated. Low doses of external beam radiotherapy (2x2 Gy) can be highly effective in this situation and a higher dose (30 Gy in 2-3 Gy fractions) may be required in patients with transformed aggressive disease or those known to have a *TP53* abnormality¹³².

Treatment of SLL

The biological similarities between SLL and CLL are so close that a similar response to treatment could be expected. This is supported by the MDACC single centre retrospective study¹³³. Indications for and choices of treatment are the same as for CLL. Rare patients in whom SLL is diagnosed following biopsy of an enlarged lymph node in the absence of detectable disease at any other site may be offered local radiotherapy with curative intent.

Risk of other diseases

Infections

The risk of infections is related to progressive defect both in antibody- and cell-mediated immunity. In addition, therapy may worsen immune impairment, particularly fludarabine and anti-CD20 antibodies, but also B-cell receptor inhibitors. For this reason, infections represent a frequent cause of morbidity and mortality in CLL. Infections are typically bacterial and should be treated early. For those who have repeated infection and require repeated antibiotics, immunoglobulin replacement therapy should be considered. Prophylactic vaccination is advised, but live vaccine should be avoided.

Autoimmune complications

Autoimmune complications are common in CLL, occurring in 10%-20% of patients¹³⁴. They almost exclusively target blood cells, most commonly red blood cells. Hemolytic anemia (AIHA) is predominant and

immune thrombocytopenia (ITP) is 4-5 times less common. A bone marrow aspirate is usually required to confirm the diagnosis of autoimmune cytopenia.

AIHA or ITP should be treated before deciding whether therapy for CLL is needed. First-line therapy is prednisolone. Second-line therapies for patients intolerant of or refractory to steroids include cyclosporine, intravenous immunoglobulin (ITP), thrombopoietin mimetic agents (ITP), CVP, low-dose cyclophosphamide, rituximab, alemtuzumab and splenectomy¹³⁵. CLL treatment may be initiated to control recurrent or refractory AIHA/ITP. Rituximab-containing regimens are recommended in patients who do not have a *TP53* abnormality. If AIHA/ITP develops during CLL treatment, the same regimen should be used again in this patient with extreme caution and only if no effective alternative is available. Autoimmune neutropenia usually responds to GCSF.

Prolymphocytic transformation

B-cell prolymphocytic transformation is diagnosed in progressive organomegaly and lymphocytosis with increase of prolymphocytes >55% and is treated as aggressive CLL. It occurs rarely in <1% of cases.

Richter syndrome

Richter syndrome is CLL transformation to aggressive lymphoma, usually DLBCL or Hodgkin like. LN biopsy is mandatory for diagnosis, PET may be helpful. It occurs in 2%-7% of patients. While novel agents are under investigation, CIT is still the recommended approach. Depending on the histologic subtype of lymphomatous transformation, patients who are suitable for intensive therapy should receive regimens currently employed for either primary diffuse large B cell lymphoma (Richter syndrome) or Hodgkin's lymphoma. Younger patients who achieve good response are candidates for allogeneic stem cell transplantation.

Check point inhibitor pembrolizumab showed activity in RS, but not in CLL¹³⁶⁻¹³⁸.

Myelodysplastic syndrome and acute leukemia

Although MDS and AML are uncommon in CLL, the rate of therapy related to fludarabine based CIT is about 5% and much higher after autologous stem cell

Table 8. Supportive therapy in patients with B-CLL

	PROBLEM	RECOMMENDATIONS
1	Vaccination	<ul style="list-style-type: none"> • Annual vaccination against influenza. Care must be taken of the fact that the recovery of B-cell system after anti CD20 antibody therapy lasts for about 9 months, so that the response to vaccination in this period is inadequate. • Pneumococcal vaccine every 5 years • Avoid all live vaccines, including Zoster
2	Anti-infective prophylaxis	<ul style="list-style-type: none"> • For patients receiving purine analogs and/or alemtuzumab, and in period after that the following prophylaxis is recommended: <ul style="list-style-type: none"> ✓ Herpes viruses (acyclovir) ✓ PCP (sulfamethoxazole/trimethoprim) • Special attention in patients receiving alemtuzumab should be paid to the problem of CMV reactivation. Although there is no common position in the literature, most reports recommend that prophylactic ganciclovir is prescribed if viremia present. The viral load levels must be monitored every few weeks. • For patients receiving anti CD20 antibody and positive for HBV, HCV consult GE/infectologist for antiviral prophylaxis.
3	Respiratory infections requiring IV antibiotics and hospitalization	<ul style="list-style-type: none"> • Apply appropriate antibiotic therapy. • Determine serum IgG, and if the value is less than 5 g/L: <ul style="list-style-type: none"> ✓ Apply monthly IVIG 0.3-0.5 g/kg ✓ Adjust the dose so that the value is maintained above 5 g/L
4	Immunoglobulin replacement therapy	<ul style="list-style-type: none"> • Should be considered as a means of reducing the incidence of bacterial infections in patients with a low serum IgG level who have experienced previous major or recurrent minor bacterial infections despite optimal antibacterial prophylaxis. • The goal should be to reduce the incidence of infection and the immunoglobulin dose should be adjusted accordingly. • Patients should be reviewed regularly to evaluate the effectiveness of immunoglobulin replacement therapy and whether there is a continuing need for treatment. • Patients who develop serious and/or recurrent infections despite antimicrobial prophylaxis and immunoglobulin replacement should be managed in conjunction with a microbiologist, infectious disease specialist, and/or immunologist.
5	Blood transfusion	<ul style="list-style-type: none"> • The use of irradiated blood products should be considered in the following situations: indefinitely in patients treated with a purine analog, following bendamustine until more evidence emerges about the risk of transfusion-associated graft <i>versus</i> host disease, following alemtuzumab and for 3 months post conditioning with chemotherapy or immunotherapy (6 months after total body irradiation) for patients undergoing autologous transplantation.
6	Tumor lysis	<ul style="list-style-type: none"> • Venetoclax can cause severe tumor lysis syndrome. Special precautions and ramp-up therapy should be followed strictly.

transplantation. The response to therapy is poor. Whether novel agents that do not induce genotoxic stress to stem cells reduce the incidence of this serious complication is currently under evaluation.

Supportive therapy

Supportive therapy plays an important role in management and is carried out in accordance with the generally accepted good clinical practice^{16,17}.

Table 8 shows the basic characteristics of supportive therapy in B-CLL. This covers the area of vaccination, anti-infective prophylaxis, respiratory recurrent infections requiring IV antibiotics and hospitalization, immunoglobulin replacement therapy, blood transfusions, and tumor lysis.

Extended version and updates can be found on KROHEM website: <http://www.krohem.hr/Documents/AMENDMENT%20KROHEM%20CLL%20GUIDELINES%20v1.%202016%20%20ENGL.pdf>

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Sažetak

SMJERNICE ZA DIJAGNOSTIKU I LIJEČENJE KRONIČNE LIMFOCITNE LEUKEMIJE
– KROHEM B-CLL 2017.

B. Jakšić, V. Pejša, S. Ostojić-Kolonić, I. Kardum-Skelin, S. Bašić-Kinda, B. Coha, V. Gverić-Krečak, R. Vrhovac,
O. Jakšić, I. Aurer, J. Sinčić-Petričević, A. Načinović-Duletić i D. Nemet, za Radnu skupinu KROHEM CLL

Nedavni događaji u dijagnostici i liječenju kronične limfocitne leukemije (B-KLL) doveli su do promjene pristupa u kliničkoj praksi. Nova liječenja su odobrena na temelju rezultata randomiziranih multicentričnih pokusa za prvu liniju terapije i za liječenje relapsa/refraktorne bolesti, a rezultati brojnih kliničkih pokusa u tijeku trajno doprinose daljnjem unaprjeđenju terapijskih strategija. Uz to prisutan je bitan porast razumijevanja genskih promjena bolesti zbog velikog napretka tehnologije nove generacije sekvencioniranja. Definiramo trenutni stav Hrvatske suradne skupine za hematološke bolesti o dijagnostici i liječenju B-KLL u sadašnjoj tranziciji iz kemo-imunoterapijske paradigme u novu koja se temelji na novoj dijagnostičkoj slojevitosti i izvrsnim terapijskim rezultatima inhibitora B-staničnih receptora (BRI) i Bcl-2 antagonista. To se područje brzo razvija kako velik broj kliničkih ispitivanja koja su u tijeku neprestance doprinosi i pruža nova znanja. Vjerujemo da će istraživanje novih terapija uz genomsku dijagnostiku pružiti nove mogućnosti koje će na kraju dovesti do vremenski ograničenog liječenja bez kemoterapije i do učinkovitije kliničke skrbi B-KLL na temelju individualizirane i precizne medicine.

Ključne riječi: *Kronična limfocitna leukemija; Praktične smjernice; Precizna medicina; Hrvatska; KROHEM*