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# Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) updating the National Cancer Institute-Working Group (NCI-WG) 1996 guidelines

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# Guidelines for the Diagnosis and Treatment of Chronic Lymphocytic Leukemia: A Report from the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) updating the National Cancer Institute-Working Group (NCI-WG) 1996 guidelines

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From the Klinik I für Innere Medizin, Universität zu Köln, Köln, Germany; the Georgetown University Hospital, Lombardi Cancer Center, Washington, DC; the Institute of Cancer Research, London, United Kingdom; the Universita Vita-Salute San Raffaele, Milano, Italy; the Institute Pasteur, Montevideo, Uruguay; the University of Ulm, Ulm, Germany; the Pinderfields Hospital, Wakefield, United Kingdom; the Department. of Leukemia, University of Texas, MD Anderson Cancer Center, Houston, TX; the Hospital Clinic, Barcelona, Spain; the Division of Hematology/Oncology, Long Island Jewish Medical Center, New Hyde Park, NY; and the Rebecca and John Moores Cancer Center, University of California, San Diego, La Jolla, CA.

Address for correspondence:
Michael Hallek, MD
Klinik I für Innere Medizin
Universität zu Köln
Joseph-Stelzmann Str. 9
50924 Köln
Tel.: 0221/478 4400
Fax: 0221/478 5455
E mail: michael.hallek@uni-koeln.de

In 1988 and 1996, a National Cancer Institute-sponsored Working Group (NCI-WG) on chronic lymphocytic leukemia (CLL) published guidelines for the design and conduct of clinical trials for patients with CLL to facilitate comparisons between different treatments and to establish definitions that could be used in scientific studies on the biology of this disease.[1,2] The Food and Drug Administration (FDA) also adopted these guidelines in their evaluation and approval of new drugs. During the last decade, considerable progress has been made in defining new prognostic markers, diagnostic parameters and treatment options, prompting the IWCLL-sponsored Working Group to revise the 1996 criteria (Tables 1-4).

## 1. Diagnosis of CLL

The World Health Organization (WHO) classification of hematopoietic neoplasias describes CLL as leukemic, lymphocytic lymphoma, being only distinguishable from SLL (small lymphocytic lymphoma) by its leukemic appearance.[3] In the WHO classification CLL is always a disease of neoplastic B-cells, while the entity formerly described as T-CLL is now called T-cell prolymphocytic leukemia (T-PLL).[4]

It is important to verify that the patient has CLL and not some other lymphoproliferative disease that can masquerade as CLL, such as hairy cell leukemia, or leukemic manifestations of mantle cell lymphoma, marginal zone lymphoma, splenic marginal zone lymphoma with circulating villous lymphocytes or follicular lymphoma. To achieve this, it is essential to evaluate the blood count, blood smear, and the immune phenotype of the circulating lymphoid cells (see below,1.1. and 1.2.).

### 1.1. *Blood*

The diagnosis of CLL requires the presence of ≥ 5000 B-lymphocytes/μL in the peripheral blood for the duration of at least 3 months. The clonality of the circulating B-lymphocytes needs to be confirmed by flow cytometry. The leukemia cells found in the blood smear are characteristically small, mature lymphocytes with a narrow border of cytoplasm and a dense nucleus lacking discernable nucleoli and having partially aggregated chromatin. These cells may be found admixed with larger or atypical cells, cleaved cells or prolymphocytes, which may comprise up to 55% of the blood lymphocytes.[5] Finding prolymphocytes in excess of this percentage would favor a diagnosis of prolymphocytic leukemia (B-cell PLL). Gumprecht nuclear shadows, or smudge cells, found as cell debris, are other characteristic morphologic features found in CLL.

CLL or SLL might be suspected in otherwise healthy adults who have an absolute increase in the clonal B lymphocytes, but who have less than 5000 B-lymphocytes/μL in the blood. However, in the absence of lymphadenopathy or organomegaly (as defined by physical examination and CT scans), cytopenias, or disease-related symptoms, the presence of fewer than 5000 B-lymphocytes per μL blood is defined as “monoclonal B-lymphocytosis” (MBL).[6] The presence of a cytopenia caused by a typical marrow infiltrate defines the diagnosis of CLL regardless of the number of peripheral blood B-lymphocytes or of the lymph node involvement. MBL seems to progress to frank CLL at a rate of 1-2 % per year.[7]

The definition of SLL requires the presence of lymphadenopathy and the absence of cytopenias caused by a clonal marrow infiltrate. Moreover, the number of B-lymphocytes in the peripheral blood should not exceed 5000/μL. In SLL, the diagnosis should be confirmed by histopathological

evaluation of a lymph node biopsy whenever possible.

*1.2. Immunophenotype*

CLL cells co-express the T-cell antigen CD5 and B-cell surface antigens CD19, CD20, and CD23. The levels of surface immunoglobulin, CD20, and CD79b are characteristically low compared to those found on normal B cells.[8,9] Each clone of leukemia cells is restricted to expression of either kappa or lambda immunoglobulin light chains.[8] Variations of the intensity of expression of these markers may exist and do not prevent inclusion of a patient in clinical trials for CLL.

In contrast, B-cell PLL cells do not express CD5 in half of the cases, and typically express high levels of CD20 and surface Ig.[10] Also, the leukemia cells of mantle cell lymphoma, despite also expressing B cell surface antigens and CD5, generally do not express CD23.

*1.3. Other tests performed at diagnosis*

The tests described in section 1.3. are not needed to establish the diagnosis of CLL, but may help to predict the prognosis or to assess the tumor burden. With the exception of molecular genetics (FISH), the application of these tests should not be used in routine practice to influence therapy and is not generally recommended. However, certain parameters, such as immunoglobulin mutational status, are useful for predicting the clinical course in individual cases. These tests can be recommended for patients who want a better prediction of the rate at which their disease might progress but it should be emphasized that the indication for treatment does *not* depend on any of these tests but on the clinical stage and the disease activity (see Section 4).

1.3.1 Molecular Genetics

Using interphase fluorescence-in situ hybridization (FISH), cytogenetic lesions can be identified in more than 80% of all CLL cases.[11] The most common deletions are in the long arm of chromosome 13 (del(13q14.1)). Additional, frequent chromosomal aberrations comprise deletions and/or trisomy of chromosome 12, deletions in the long arm of chromosomes 11 (del(11q)) and 6 (del(6q)), and in the short arm of chromosome 17 (del(17p)).[11] When stimulated in vitro, CLL cells can have detectable chromosomal translocations, which are of potential prognostic significance.[12] However, certain translocations can help distinguish other lymphoproliferative diseases from CLL (e.g. t(11;14), which generally is found in mantle cell lymphoma).

There is increasing evidence from prospective clinical trials that detection of certain chromosomal deletions has prognostic significance. Patients with leukemia cells that have del(17p) have an inferior prognosis and appear resistant to standard chemotherapy regimens employing alkylating drugs and/or purine analogues.[13,14] In a retrospective analysis on several chromosomal aberrations as detected by FISH, patients who had CLL cells with chromosomal aberrations del(11q) and del(17p) had an inferior outcome compared to patients who had leukemia cells with a normal karyotype or del(13q) as the sole genetic abnormality.[11] On the other hand, patients with leukemia cells having del(17p) may respond to therapy with alemtuzumab, either alone or in combination with other anti-leukemia agents.[15,16] Detection of these cytogenetic abnormalities has apparent prognostic value and may influence therapeutic decisions. For clinical trials, it is recommended that cytogenetics be performed prior to treating a patient on protocol. Additional genetic defects may be acquired during the course of the disease;[17] therefore, the repetition of FISH analyses seems justified prior to subsequent, second- and third-

line treatment.

1.3.2 Mutational status of $IgV_H$, VH3.21 usage, and expression of ZAP-70 or CD38

The leukemia cells express immunoglobulin that may or may not have incurred somatic mutations in the immunoglobulin heavy chain variable region genes ($IgV_H$ genes). The outcome of patients with leukemia cells that use an unmutated $IgV_H$ gene is inferior to those patients with leukemia cells that use a mutated $IgV_H$ gene.[18,19] In addition, the VH3.21 gene usage is an unfavorable prognostic marker independent of the $IgV_H$ mutational status.[20] Leukemia-cell expression of ZAP-70 and CD38 was found to correlate with the expression of unmutated $IgV_H$ genes and to predict a poor prognosis.[21-27] However, the association between expression of ZAP-70 or CD38 with the expression of unmutated $IgV_H$ genes is not absolute. It is uncertain whether leukemia-cell expression of unmutated $IgV_H$ genes or ZAP-70 predict the response to treatment or overall survival, once therapy is required.[14,28] Taken together, further clinical trials are needed to standardize the assessment of these parameters and to determine whether they should affect the management of patients with CLL.

1.3.3 Serum markers

Several studies have found that serum markers CD23, thymidine kinase, and $ß_2$-microglobulin may predict survival or progression-free survival.[29-35] Assays for these markers should be standardized and used in prospective clinical trials to validate their relative value to the management of patients with CLL.

1.3.4 Marrow Examination

Characteristically more than 30% of the nucleated cells in the aspirate are lymphoid. Although the type of marrow infiltration (diffuse versus non-diffuse) reflects the tumor burden and provides some prognostic information, recent studies of the German and Spanish study groups suggest that the prognostic value of BM biopsy may now be superseded by new prognostic markers.

A marrow aspirate and biopsy generally are *not* required for the diagnosis of CLL. However, a marrow biopsy and aspirate can help evaluate for factors that might contribute to cytopenias (anemia, thrombocytopenia) that may or may not be directly related to leukemia-cell infiltration of the marrow. Because such factors could influence the susceptibility to drug-induced cytopenias, a marrow biopsy is recommended prior to initiating therapy. It is recommended to repeat a marrow biopsy in patients with persisting cytopenia after treatment to uncover disease- versus therapy-related causes.

2. Clinical Staging

There are two widely accepted staging methods for use in both patient care and clinical trials: the Rai[36] and the Binet system.[37] The original Rai classification was modified to reduce the number of prognostic groups from five to three.[38] As such, both systems now describe three major subgroups with discrete clinical outcomes. These two staging systems are simple, inexpensive, and can be applied by physicians worldwide. Both solely rely on a physical examination and standard laboratory tests, and do not require ultrasound, computed tomography, or magnetic resonance imaging. These two systems are outlined in the following.

*2.1. Rai staging system*

The modified Rai classification defines low-risk disease as patients who have lymphocytosis with leukemia cells in the blood and/or marrow (lymphoid cells >30%) (formerly considered Rai stage 0). Patients with lymphocytosis, enlarged nodes in any site, and splenomegaly and/or hepatomegaly (lymph nodes being palpable or not) are defined as having intermediate risk disease (formerly considered Rai stage I or stage II). High risk disease includes patients with disease-related anemia (as defined by a hemoglobin (Hb) level less than 11 g/dl) (formerly stage III) or thrombocytopenia (as defined by a platelet count of less than 100 x $10^9$/L) (formerly stage IV).

*2.2. Binet staging system*

Staging is based on the number of involved areas, as defined by the presence of enlarged lymph nodes of greater than 1 cm in diameter or organomegaly, and on whether there is anemia or thrombocytopenia.

*Area of involvement considered for staging*

(1) Head and neck, including the Waldeyer ring (this counts as one area, even if more than one group of nodes is enlarged).
(2) Axillae (involvement of both axillae counts as one area).
(3) Groins, including superficial femorals (involvement of both groins counts as one area).
(4) Palpable spleen.
(5) Palpable liver (clinically enlarged).

*Stage A.* Hb ≥ 10 g/dL and platelets ≥ 100 X $10^9$/L and up to two of the above involved.

*Stage B.* Hb ≥ 10 g/dL and platelets ≥ 100 X $10^9$/L and organomegaly greater than that defined for stage A (i.e. three or more areas of nodal or organ enlargement).

*Stage C.* All patients who have Hb of less than 10 g/dL and/or a platelet count of less than 100 x $10^9$/L, irrespective of organomegaly.

3. Eligibility Criteria for Clinical Trials

The selection of CLL patients for clinical trials is similar to that for patients with other malignancies. Phase I-II clinical trials commonly, although not invariably, are intended for patients who have had prior therapy. It may be worth considering the inclusion of patients with SLL in some phase I-II trials exploring new agents in CLL. However, for SLL the response assessment should be done according to the lymphoma guidelines. The combination of new agents with standard therapy as part of phase II studies may be investigated in both untreated and previously treated patients. Phase III clinical trials are used to compare the clinical outcome employing new treatment modalities with that using current standard therapy. Other requirements for eligibility with respect to age, clinical stage, performance status, organ function, or status of disease activity should be defined for each study.

*3.1. Performance Status and Fitness*

Prior to inclusion in a trial, the performance status as defined by the Eastern Cooperative Oncology Group (ECOG) should be 0-3. Future clinical trials involving elderly patients ideally should assess the comorbidity (fitness) and/or functional activity of patients (e.g. such as that defined by "cumulative illness rating scale" (CIRS) or the "Charlson" score).[39,40]

*3.2. Organ Function Eligibility for Clinical Trials*

Most chemotherapy agents have potential toxicity for the liver, kidneys, heart, lungs, nervous

system, or other organ systems. Therefore, organ function requirements should be guided by the known or suspected toxicity of each agent based on preclinical studies or prior clinical studies. Patients enrolled on protocols evaluating agents with known or suspected toxicity for a given organ(s) should have documented the specific organ function prior to therapy.

*3.3. Infectious Disease Status*

The status of specific infectious diseases as outlined in section 3.5 should be documented. Patients with active infections requiring systemic antibiotics, antifungal or antiviral drugs should have their infection resolved prior to initiating therapy in a clinical trial.

*3.4. Second Malignancies*

Patients with a second malignancy, other than basal cell carcinoma of the skin or in situ carcinoma of the cervix or the breast, generally are not considered candidates for entry into clinical trials unless the tumor was successfully treated with curative intent at least 2 years prior to trial entry.

*3.5. Required Pretreatment Evaluation*

Parameters considered necessary for a complete pretreatment evaluation may differ depending on whether or not the patient is treated in a clinical protocol. Therefore, a clear distinction is made in sections 3.5 and 5 between recommendations for general practice and the requirements for clinical trials (Tables 1, 2, and 3). If not indicated otherwise, recommendations are identical for clinical trials and general practice. In general, studies for defining these parameters should be performed within 2 weeks of clinical trial enrollment (except for marrow aspirate and biopsy and computed tomography (CT) scans (see sections 3.5.1 and 3.5.2).

3.5.1. Essential pretreatment tests (see Table 1)

3.5.1.1. Physical examination: The bidimensional diameters of the largest palpable lymph nodes in each of the following sites should be recorded: cervical, axillary, supraclavicular, inguinal, and femoral. The size of the liver and spleen, as assessed by palpation, should also be recorded.

3.5.1.2. Assessment of performance status (ECOG score).

3.5.1.3. A complete blood cell count (CBC; white blood cell count, hemoglobin and hematocrit, platelet count) and differential count, including both percent and absolute number of lymphocytes, and reticulocyte count should be performed. Reporting the proportion of prolymphocytes is desirable when these are present.

3.5.1.4. Marrow biopsy: Prior to initiating treatment in a clinical trial with potentially myelosuppressive agents, patients should undergo a unilateral marrow aspirate and biopsy. Repeat marrow biopsies may be compared with the pre-treatment marrow specimen.

3.5.1.5. Serum chemistry (e.g., creatinine, bilirubin, LDH, transaminases, alkaline phosphatase).

3.5.1.6. Serum immunoglobulin levels.

3.5.1.7. Direct antiglobulin test (DAT).

3.5.1.8. Chest radiograph.

3.5.1.9. Human immunodeficiency virus (HIV)

Patients who are infected with HIV should be given special consideration because of the potential

risks for immune suppression with most anti-leukemia therapies and the potential for compounded myelotoxicity of treatment with anti-retroviral therapy.

3.5.1.10. Cytomegalovirus (CMV)

Therapies associated with the potential for reactivation of infection with CMV, such as alemtuzumab or allogeneic stem cell transplantation, should include plans for monitoring for active CMV disease and/or for providing anti-CMV therapy.[41] These should cover screening or early diagnosis of CMV reactivation and its subsequent management. However, a positive CMV serology does not represent a contraindication for alemtuzumab treatment or allogeneic stem cell transplantation. As a general recommendation for patients treated with alemtuzumab, close monitoring and/or therapy for active CMV disease should be considered for patients found to have evidence for increased levels of CMV in the blood by the polymerase chain reaction (PCR), even in the absence of clinical symptoms. Also, evaluation and therapy for CMV is recommended for any patient with clinical symptoms of active CMV infection.

3.5.1.11. Hepatitis B and Hepatitis C

Before initiating treatment, the evaluation for infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) is recommended, since reactivation of HBV and HCV infections may occur under therapy with immunosuppressive or myelosuppressive drugs. Chronic HBV carriers as defined by positive surface antigen (HBsAg) undergoing chemotherapy should receive prophylactic therapy with nucleoside analogs such as lamivudine to prevent HBV reactivation.[42,43]

3.5.2 Additional pretreatment tests (Table 1)

The following tests may be performed in clinical trials or in the presence of specific clinical problems.

3.5.2.1. The assessment of molecular cytogenetics (FISH) prior to therapy is recommended.

3.5.2.2. Computed tomography (CT) scans: CT scans generally are *not* required for the initial evaluation, or follow up. Moreover, the staging of CLL does not use CT scans but relies on physical examination and blood counts. A recent study has found that patients in Rai stage 0 but with detectable abdominal disease by CT scans may have a more aggressive disease.[44] Therefore, clinical studies evaluating the use of CT scans in CLL are strongly encouraged. Moreover, enlarged lymph nodes if detected only by CT scan do not change the clinical Binet or Rai stage.

In *clinical trials* where the treatment intent is to maximize complete remissions chest, abdominal and pelvic CT scans are recommended to evaluate the response to therapy. CT scans should be performed prior to the start of therapy and at the first restaging following therapy if previously abnormal.

3.5.2.3. Other imaging methods: Except in some patients with Richter's transformation, positron emission tomography (PET) scans do *not* provide information that is useful in the management of CLL. Similarly, nuclear magnetic resonance imaging and other imaging techniques are generally *not* useful in the management of CLL.

3.5.2.4. Abdominal ultrasound: In some countries, the use of abdominal ultrasound is popular to assess the extent of lymphadenopathy and organomegaly in CLL. While it may be used in the clinical management of individual patients, this methodology is strongly investigator-dependent and should therefore not be used for the response evaluation in clinical trials.

3.5.2.5. A lymph node biopsy is generally *not* required, unless such tissue is necessary for companion scientific studies or in rare cases with difficult diagnosis. A lymph node biopsy is

requested to establish the diagnosis of a transformation into an aggressive lymphoma (Richter's syndrome).

## 4. Indications for Treatment

### 4.1. *Primary Treatment Decisions (Table 2)*

Criteria for initiating treatment may vary depending on whether or not the patient is treated in a clinical trial. In general practice, newly diagnosed patients with asymptomatic early-stage disease (Rai 0, Binet A), should be monitored without therapy unless they have evidence of disease progression. Studies from both the French Cooperative Group on CLL,[45] the Cancer and Leukemia Group B (CALGB),[46] the Spanish Group Pethema,[47] and the Medical Research Council (MRC)[48] in the UK in patients with early-stage disease confirm that the use of alkylating agents in patients with such early-stage disease does not prolong surviva1. This result was confirmed by a meta-analysis.[49] In one study, treated patients with early-stage disease had an increased frequency of fatal epithelial cancers compared to untreated patients.[45] Therefore, the potential benefit, if any, of an early-intervention therapy with anti-leukemia drugs, alone or in combination with monoclonal antibodies, requires further study.

Whereas patients at intermediate (stage I and II) and high risk (stage III and IV) according to the modified Rai classification or at Binet stage B or C usually benefit from the initiation of treatment, some of these patients (in particular Rai intermediate risk or Binet stage B) can be monitored without therapy until they have evidence for progressive or symptomatic disease.

Active disease should be clearly documented for protocol therapy. At least one of the following criteria should be met:

(1) Evidence of progressive marrow failure as manifested by the development of, or worsening of, anemia and/or thrombocytopenia

(2) Massive (i.e., > 6 cm below the left costal margin) or progressive or symptomatic splenomegaly

(3) Massive nodes (i.e., >10 cm in longest diameter) or progressive or symptomatic lymphadenopathy

(4) Progressive lymphocytosis with an increase of >50% over a 2-month period, or lymphocyte doubling time (LDT) of less than 6 months. LDT can be obtained by linear regression extrapolation of absolute lymphocyte counts (ALC) obtained at intervals of two weeks over an observation period of 2-3 months; patients with initial blood lymphocyte counts of less than 30.000/µl may require a longer observation period to determine the LDT. Also, factors contributing to lymphocytosis or lymphadenopathy other than CLL (e.g., infections) should be excluded.

(5) Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroids or other standard therapy (see 10.2).

(6) A minimum of any one of the following disease-related symptoms must be present:
   (a) Unintentional weight loss ≥ 10% within the previous 6 months.
   (b) Significant fatigue (i.e., ECOG PS 2 or worse; cannot work or unable to perform usual activities).
   (c) Fevers of greater than 100.5° F or 38.0° C for 2 or more weeks without other evidence of infection.
   (d) Night sweats for more than 1 month without evidence of infection.

Hypogammaglobulinemia or monoclonal or oligoclonal paraproteinemia does not by itself constitute a basis for initiating therapy. However it is recommended to assess the change of these protein abnormalities, if patients are treated.

Patients with CLL may present with a markedly elevated leukocyte count; however, the symptoms associated with leukocyte aggregates that develop in patients with acute leukemia rarely occur in patients with CLL. Therefore, the absolute lymphocyte count should not be used as the sole indicator for treatment

4.2. *Second-Line Treatment Decisions*

In general, second-line treatment decisions follow the same indications as those used for initiation of first-line treatment. Patients who have resistant disease, a short time to progression after the first treatment, and/or leukemia cells with del(17p) often do not respond to standard chemotherapy and have a relatively short survival. Therefore, such patients should be offered investigative clinical protocols including allogeneic hematopoietic stem cell transplantation.[50-54]

5. Definition of Response, Relapse and Refractory Disease (Tables 3 and 4)

Assessment of response should include a careful physical examination and evaluation of the blood and marrow. Imaging studies, in particular CT scans, generally are not required except to monitor the response to therapy in clinical trials (Table 3).

5.1. Complete remission (CR) requires all of the following criteria as assessed at least 3 months after completion of therapy:

5.1.1. Absence of clonal lymphocytes in the peripheral blood (as defined in section 1.1 and 1.2).

5.1.2. Absence of significant lymphadenopathy (e.g. lymph nodes greater than 1,5 cm in diameter) by physical examination. In clinical trials a CT scan of the abdomen, pelvis and thorax should be performed if previously abnormal. Lymph nodes should not be larger than 1,5 cm in diameter.

5.1.3. No hepatomegaly or splenomegaly by physical examination. In clinical trials a CT scan of the abdomen should be performed at response assessment if found to be abnormal prior to therapy or if physical exam is inconclusive at the time of evaluation.

5.1.4. Absence of constitutional symptoms.

5.1.5. Blood counts above the following values:

5.1.5.1. Polymorphonuclear leukocytes ≥ 1.500/µL.

5.1.5.2. Platelets > 100.000/µL.

5.1.5.3. Hemoglobin > 11,0 g/dL (untransfused).

5.1.6. For patients in clinical trials (Table 3): a marrow aspirate and biopsy should be performed at least 3 months after the last treatment and if clinical and laboratory results listed in 5.1.1 to 5.1.5 demonstrate that a CR has been achieved. The marrow should be analyzed by flow cytometry and/or immunohistochemistry to demonstrate that the marrow is free of clonal B-CLL cells. Cases with residual CLL cells by conventional (not 4-colour; see below) flow cytometry or immunohistochemistry are defined as PR.

In some cases, lymphoid nodules can be found (formerly used to define nodular PR), which often reflect residual disease.[55,56] Therefore, these nodules should be assessed by immunohistochemistry to define whether they are comprised primarily of T cells or lymphocytes other than CLL cells or of CLL cells. The category of "nodular PR" should no longer be used. If the marrow is found to be hypocellular, a repeat marrow biopsy should be performed after 4-6 weeks, provided that the blood counts have recovered, as defined in 5.1.5. Marrow biopsies should be compared with that of the pre-treatment marrow. In some cases, it is necessary to postpone the marrow biopsy until after all the other criteria to define a CR (5.1.1 to 5.1.5) have been satisfied. However, this time interval should not exceed 6 months after the last treatment.

5.1.7. A controversial issue is how best to categorize the response of patients who fulfill all the criteria for a CR (including the marrow examinations described in 5.16), but who have a persistent anemia or thrombocytopenia or neutropenia apparently unrelated to CLL, but related to drug toxicity. We recommend that these patients should be considered as a different category of remission, CR with incomplete bone marrow recovery (CRi). For the definition of this category, CRi, the marrow evaluation (see 5.1.6.) should be performed with scrutiny and not show any clonal infiltrate. In clinical trials, CRi patients should be monitored prospectively to determine whether their outcome differs from that of patients with detectable residual disease or with non-cytopenic CR.

5.2. Partial remission (PR) is defined by the criteria described in 5.21, 5.22, and/or 5.23 (if abnormal prior to therapy), as well as one or more of the features listed in 5.24. To define a PR at least one of these parameters needs to be documented for a minimal duration of 2 months (Table 4). Constitutional symptoms persisting for more than 1 month should also be documented.

5.2.1. A decrease in the number of blood lymphocytes by below 50% or more from the value prior to therapy.

5.2.2. Reduction in lymphadenopathy (to be assessed by CT scans in clinical trials[57] and by palpation in general practice) as defined by:

5.2.2.1 A decrease lymph node size by below 50% or more in

- the sum products of up to 6 lymph nodes, or
- in one lymph node diameter if only a single lymph node was present prior to therapy.

5.2.2.2 No increase in any lymph node, and no new enlarged lymph node. In small lymph nodes (< 2 cm), an increase of < 25% is not considered to be significant.

5.2.3. A decrease in the size of the liver and/or spleen by 50% more as defined by CT scan in clinical trials or by palpation or ultrasound in general practice.

5.2.4. The blood count should show one of the following results:

5.2.4.1. Polymorphonuclear leukocytes at 1.500/µL or more or 50% improvement over baseline without G-CSF support.

5.2.4.2. Platelet counts greater than 100.000/µL or 50% improvement over baseline.

5.2.4.3. Hemoglobin greater than 11,0 g/dL or 50% improvement over baseline without red blood cell transfusions or erythropoietin support.

5.3. Progressive disease is characterized by at least one of the following:

5.3.1. Lymphadenopathy: progression of lymphadenopathy is discovered almost uniformly by blood counts. Therefore, imaging methods are not needed to follow CLL progression. Progression of lymphadenopathy may be documented by physical examination. Disease progression occurs, if one of the following events is observed:

- Appearance of any new lesion such as enlarged lymph nodes (> 1,5 cm), splenomegaly, hepatomegaly or other organ infiltrates.
- An increase by 50% or more in greatest determined diameter of any previous site. A lymph node of 1-1,5 cm must increase by 50% or more to a size greater than 1,5 cm in the longest axis. A lymph node of more than 1,5 cm must increase to more than 2,0 cm in the longest axis.
- An increase of 50% or more in the sum of the product of diameters of multiple nodes.

5.3.2. An increase in the liver or spleen size by 50% or more or the de novo appearance of hepatomegaly or splenomegaly.

5.3.3. An increase in the number of blood lymphocytes by 50% or more with at least 5,000 B lymphocytes per µL.

5.3.4. Transformation to a more aggressive histology (e.g. Richter's syndrome). Whenever possible, this diagnosis should be established by lymph node biopsy.

5.3.5. Occurrence of cytopenia (neutropenia, anemia or thrombocytopenia) attributable to CLL.

5.3.5.1 During therapy: cytopenias may occur as a side effect of many therapies and should be assessed according to Table 5. During therapy, cytopenias cannot be used to define disease progression. Each protocol should define the amount of drug(s) to be administered with such cytopenias.

5.3.5.2. Post treatment: The progression of any cytopenia (unrelated to autoimmune cytopenia), as documented by a decrease of Hb levels by more than 2 g/dl or to less than 10 g/dl, or by a decrease of platelet counts by more than 50% or to less than 100.000/µl, which occurs at least 3 months after treatment, defines disease progression, if the marrow biopsy demonstrates an infiltrate of clonal CLL cells.

5.4. Stable disease: Patients who have not achieved a CR or a PR, and who have not exhibited PD, will be considered to have stable disease (which is equivalent to a non-response).

5.5. Responses that should be considered clinically beneficial include CR and PR; all others (e.g. stable disease, non-response, progressive disease, or death from any cause) should be rated as a treatment failure.

5.6. Duration of response and progression-free survival

Duration of response should be measured from the end of the last treatment until evidence of progressive disease (as defined above). Progression-free survival (PFS) is defined as the interval between the first treatment day to the first sign of disease progression. Event-free survival is defined as the interval between the first treatment day to the first sign of disease progression, or treatment for relapse, or death (whichever occurs first). Survival duration is defined as the interval between the first treatment day to death.

5.7. Relapse

Relapse is defined as a patient who has previously achieved the above criteria (5.1-5.2) of a CR or PR, but *after* a period of 6 or more months, demonstrate evidence of disease progression (see 5.3).

5.8. Refractory disease

[0]Refractory disease is defined as treatment failure (as defined in 5.5) or disease progression *within* 6 months to the last anti-leukemic therapy. For the definition of "high risk CLL" justifying the use of allogeneic stem cell transplantation,[58] the disease should be refractory to a purine-analogue based therapy or to autologous hematopoietic stem cell transplantation.

5.9. Minimal residual disease

The complete eradication of the leukemia is an obvious desired endpoint. New detection technologies such as multicolor flow cytometry and real-time quantitative PCR have determined that many patients who achieved a complete response by the 1996 NCI-WG guidelines have detectable minimal residual disease (MRD). While eradication of MRD may improve prognosis, prospective clinical trials are needed to define whether additional treatment intended solely to eradicate MRD provides a significant benefit to clinical outcome. The techniques for assessing MRD have undergone a critical evaluation and have become fairly standard.[59] Either four-color flow cytometry (MRD Flow) or allele-specific oligonucleotide PCR are reliably sensitive down to a level of approximately one CLL cell in 10.000 leukocytes. As such, patients will be defined as having a clinical remission in the absence of MRD when they have blood or marrow with less than one CLL cell per 10.000 leucocytes. The blood generally can be used for making this assessment except during the period within 3 months of completing therapy, particularly for patients treated with alemtuzumab, rituximab and other antibodies targeting CLL. In such cases it is essential to assess the marrow for MRD. Therefore, future clinical trials that aim toward achieving long-lasting complete remissions should include at least one test to assess MRD, because the lack of leukemia persistence using these sensitive tests seems to have a strong, positive prognostic impact [60-62].

6. Factors Requiring Stratification at Inclusion in a Clinical Phase III Trial

6.1. Patients ideally should be stratified with regard to previous treatment versus no previous treatment, and as purine analogue-sensitive versus purine-analogue refractory in studies for which prior therapy is allowed.

6.2. If more than one clinical stage is allowed, patients ideally should be stratified for stage.

6.3. Patients ideally should be stratified based upon whether or not they have leukemia cells with del(17p) or del(11q).

7. Assessment of Toxicity

Evaluation of treatment-related toxicity requires careful consideration of both the manifestations of the underlying disease and the anticipated adverse reactions to the agents used in therapy. For this reason, some of the conventional criteria used for assessing toxicity are not applicable to clinical studies involving patients with hematological malignancies in general, or CLL in particular. An example is hematological toxicity; patients with advanced CLL generally have cytopenias that may be caused by the underlying CLL and/or prior therapy. A few guidelines are presented to help evaluate for treatment-induced toxicity in CLL.

7.1. *Hematological Toxicity*

Evaluation of hematological toxicity in patients with CLL must take into consideration that many patients have low blood cell counts at the initiation of therapy. Therefore, the standard criteria used for solid tumors cannot be applied, as many CLL patients then would be considered to have grade II to IV hematological toxicity at the initiation of treatment. Furthermore, the absolute

blood neutrophil counts rarely are used at the initiation of therapy to modify the treatment dose, since these values typically are unreliable in CLL patients with lymphocytosis. However, the increasing use of more effective therapeutic agents, particularly those with neutropenia as a dose-limiting toxicity (e.g. nucleoside analogs), can result in clinically significant myelosuppression. Therefore, the 1996 guidelines proposed a new dose-modification scheme for quantifying hematological deterioration in patients with CLL, which included alterations in the dose of myelosuppressive agents based on the absolute neutrophil count. This dose modification scheme has proven very helpful in the context of several large prospective trials in CLL and should be retained (Table 5).

### *7.2. Infectious Complications*

Patients with CLL are at increased risk for infection because of compromised immune function, which might be related to the disease itself and/or to the consequences of therapy. Nevertheless, the rate(s) of infection following treatment can be used in assessing the relative immune-suppressive effects of a given therapy. The etiology of the infection should be reported and categorized as bacterial, viral, or fungal, and as proven or probable. The severity of infections should be quantified as minor (requiring either oral antimicrobial therapy or symptomatic care alone), major (requiring hospitalization and systemic antimicrobial therapy), or fatal (death as a result of the infection).

Particular attention should be given to monitoring for symptoms or laboratory evidence of infection with CMV in patients treated with agents like alemtuzumab (alone or in combination) or with allogeneic stem cell transplantation. In contrast, the infection rate seems low in patients younger than 65 years treated with fludarabine-based first line therapy, where no monitoring or routine anti-infective prophylaxis is required.[63]

### *7.3. Tumor lysis syndrome*

CLL patients rarely experience tumor lysis syndrome after therapy with a purine analogue based regimen.[64] However, this might not be the case following treatment with newer agents or novel treatment modalities. For this reason, patients in early phase clinical trials should be monitored for possible tumor lysis syndrome, which should be treated appropriately. If observed, the occurrence and severity of tumor lysis syndrome should be recorded in clinical trials.

### *7.4. Non-hematological Toxicities*

Other non-hematological toxicities should be graded according to the latest version of the NCI Common Toxicity Criteria (CTC).

## 8. Reporting of Clinical Response Data

Clear and careful reporting of data is an essential part of any clinical trial. In clinical studies involving previously treated patients, patients who are relapsed or refractory should be clearly distinguished. Relapse and refractory disease are defined above (5.7 and 5.8). For those patients who have relapsed, it is also useful to describe the quality and duration of their prior response.

## 9. Treatment endpoints

Given the recent increase of treatment options for CLL patients, the choice of treatment and the endpoints of clinical trials may depend on the fitness of the patients (see 3.1). For example, the number of MRD negative complete remissions or the overall survival might be appropriate endpoints in physically fit patients. In contrast, trials on patients with reduced physical fitness might choose the time to progression or health-related quality of life as trial endpoints. Moreover, recent data suggest that the quality of life in CLL patients is reduced as compared to the normal population and only moderately increased by some of the current treatment options.[65-68] Therefore, further studies assessing the health-related quality of life in CLL are strongly encouraged.

## 10. Supportive care and management of complications

### *10.1. Indications for growth factors in CLL*

While under myelosuppressive (chemo-)therapy, growth factors such as granulocyte-colony-stimulating factor (G-CSF) should be given according to the guidelines of the American Society of Clinical Onclogy.[69] The use of G-CSF also might benefit patients who experience prolonged cytopenias following treatment with alemtuzumab. Similarly, some CLL patients with anemia may benefit from erythropoiesis stimulating factors, if used according to recently published guidelines.[70,71] However, it should be pointed out that CLL-related cytopenias are often efficiently corrected by an appropriate anti-leukemic therapy.

### *10.2. Autoimmune hemolytic anemia (AIHA) or autoimmune thrombocytopenia (ITP)*

ITP and AIHA as a single abnormality caused by CLL initially should be treated with glucocorticoids and not chemotherapy. Second line treatment options for AIHA include splenectomy, intravenous immunoglobulins, and/or immunosuppressive therapy with agents such as cyclosporine A, azathioprin, or low-dose cyclophosphamide. Good responses also have been obtained with antibody therapy using agents as rituximab or alemtuzumab.[72-74] Treatment refractory autoimmune cytopenias can be an indication for chemotherapy or chemoimmunotherapy directed at the underlying CLL.[75] In this regard, the Binet or Rai staging systems do not distinguish between ITP/AIHA or marrow infiltration as the cause for anemia or thrombocytopenia that results in classifying a patient as having stage C or high-risk disease.

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## Authorship

Michael Hallek, Bruce D. Cheson, Daniel Catovsky, Federico Caligaris-Cappio, Guillaume Dighiero, Hartmut Döhner, Peter Hillmen, Michael Keating, Emili Montserrat, Kanti R. Rai, Thomas J. Kipps all wrote the paper.

**Conflict of Interest Disclosure**
The authors declare no competing financial interests.

TABLES

**Table 1. Pretreatment Evaluation of Patients with CLL**

| Diagnostic test | Section of guidelines | General practice | Clinical trial |
|---|---|---|---|
| Tests to establish the diagnosis | 1. | | |
| Complete blood count and differential count | 1.1 | Always | Always |
| Immunophenotyping of lymphocytes | 1.2. | Always | Always |
| Assessment prior to treatment | 3.5.1 | | |
| History and physical, performance status | 3.5.1.1, 3.5.1.2 | Always | Always |
| Complete blood count and differential | 3.5.1.3 | Always | Always |
| Marrow aspirate and biopsy | 3.5.1.4 | Desirable | Desirable |
| Serum chemistry, serum immunoglobulin, direct antiglobulin test | 3.5.1.5, 3.5.1.6, 3.5.1.7 | Always | Always |
| Chest radiograph | 3.5.1.8 | Always | Always |
| Infectious disease status | 3.3 | Always | Always |
| Additional tests prior to treatment | 3.5.2 | | |
| Cytogenetics (FISH) for del(13q), del(11q), del(17p), add(12), del(6q) in the peripheral blood lymphocytes | 3.5.2.1 | Desirable | Always |
| IgVH mutational status, ZAP-70, and CD38 | 1.2 | No | Always |
| CT scan of chest, abdomen, and pelvis | 3.5.2.2 | No | Desirable; always if CR is the desired endpoint. |
| MRI, Lymphangiogram, gallium scan, PET scans | 3.5.2.3 | No | No |
| Abdominal ultrasound | 3.5.2.4 | Possible | No |

General practice is defined as the use of accepted treatment options for a CLL patient not enrolled on a clinical trial.

Abbreviations: No, not generally indicated; RQ, indicated if a research question; PBS, peripheral blood smear; MRI, magnetic resonance imaging; PET, positron emission tomography; FISH, fluorescence in situ hybridization.

**Table 2. Recommendations Regarding Indications for Treatment in CLL**

| | General practice | Clinical trial |
|---|---|---|
| Treat with Rai stage 0 | No* | RQ |
| Treat with Binet stage A | No* | RQ |
| Treat with Binet stage B or Rai stage I or Rai stage II | Possible* | Possible* |
| Treat with Binet stage C or Rai stage III or Rai stage IV | Yes | Yes |
| | | |
| Treatment of active/progressive disease | Yes | Yes |
| Treat without active/progressive disease | No | RQ |

General practice is defined as the use of accepted treatment options for a CLL patient not enrolled on a clinical trial.

Abbreviations: No, not generally indicated; RQ, indicated if a research question.

*Treatment is indicated, if the disease is active as defined in section 4.

**Table 3. Recommendations regarding the response assessment in CLL patients**

| Diagnostic test | Section of guidelines | General practice | Clinical trial |
|---|---|---|---|
| History, physical examination | 5.1.2, 5.1.3, 5.1.4, 5.2.2, 5.2.3, 5.3.1, 5.3.2 | Always | Always |
| Immunophenotyping of peripheral blood lymphocytes | 5.1.1 | If clinical and hematological response indicates CR | If clinical response and hematological response indicates CR |
| CBC and differential count | 5.1.5, 5.2.4, 5.3.3, 5.3.5 | Always | Always |
| Marrow aspirate and biopsy | 5.1.6 | At cytopenia of uncertain cause | At CR or cytopenia of uncertain cause |
| Assessment for minimal residual disease | 5.9 | No | If a long-lasting CR is the desired endpoint |
| Ultrasound of the abdomen | 5.1.2, 5.1.3, 5.2.2, 5.2.3, 5.3.1, 5.3.2 | Possible, if previously abnormal | No |
| CT scans of chest, pelvis, and abdomen | 5.1.2, 5.1.3, 5.2.2, 5.2.3, 5.3.1, 5.3.2 | No | Indicated if previously abnormal and otherwise in CR |

General practice is defined as the use of accepted treatment options for a CLL patient not enrolled on a clinical trial.

**Table 4 - Response definition after treatment for CLL patients, using the parameters of Tables 1 and 3**

| Parameter | CR | PR | PD | SD |
|---|---|---|---|---|
| Lymphadenopathy [1)] | None above 1,5 cm | Decrease ≥ 50% | Increase ≥ 50% | Change of -49% to +49% |
| Liver and/or spleen size | Normal size | Decrease ≥ 50% | Increase ≥ 50% | Change of -49% to +49% |
| Constitutional symptoms | None | Any | Any | Any |
| Polymorphonuclear leukocytes | > 1500/µl | > 1500/µl or > 50% improvement over baseline | Any | Any |
| Circulating clonal B-lymphocytes | Nil | Decrease ≥ 50% from baseline | Increase ≥ 50% over baseline | Change of -49% to +49% |
| Platelet count | > 100.000/µl | > 100.000/µl or increase ≥ 50% over baseline | Decrease of ≥ 50% from baseline secondary to CLL | Change of -49 to +49% |
| Hemoglobin | > 11,0 g/dl (untransfused and without erythropoietin) | > 11 g/dl or increase ≥ 50% over baseline | Decrease of > 2 g/dl from baseline secondary to CLL | Increase < 11,0 g/dl or < 50% over baseline, or decrease < 2 g/dl |
| Marrow | Normocellular, < 30% lymphocytes, no B-lymphoid nodules. Hypocellular marrow defines CRi (5.1.6.). | ≥ 30% lymphocytes, or B-lymphoid nodules, or not done | Increase of lymphocytes to more than 30% from normal | No change in marrow infiltrate |
| | | | | |

1) sum of the products of multiple lymph nodes (as evaluated by CT scans in clinical trials, or by physical exam or ultrasound in general practice).

CR: complete remission, all of the criteria have to be met; PR: partial remission, at least one of the criteria has to be met; PD: progressive disease: at least one of the above criteria has to be met; SD; all of the above criteria have to be met.

**Table 5. Grading Scale for Hematological Toxicity in CLL Studies**

| Grade[#] | Decrease in Platelets* or Hb° (nadir) From Pretreatment value (%) | absolute neutrophil count/µL§ (nadir) |
|---|---|---|
| 0 | No change to 10% | ≥ 2,000 |
| 1 | 11%-24% | ≥ 1,500 and < 2,000 |
| 2 | 25%-49% | ≥ 1,000 and < 1,500 |
| 3 | 50%-74% | ≥ 500 and < 1,000 |
| 4 | ≥ 75% | < 500 |

* Platelet counts must be below normal levels for grades 1-4. If, at any level of decrease the platelet count is <20.000/µL, this will be considered grade 4 toxicity, unless a severe or life-threatening decrease in the initial platelet count (e.g., 20.000/µL) was present pretreatment, in which case the patient is not evaluable for toxicity referable to platelet counts.

° Hb levels must be below normal levels for grades 1-4. Baseline and subsequent Hb determinations must be performed before any given transfusions. The use of erythropoietin is irrelevant for the grading of toxicity, but should be documented.

# Grades: 1, mild; 2, moderate; 3, severe; 4, life-threatening; 5, fatal. Death occurring as a result of toxicity at any level of decrease from pretreatment will be recorded as grade 5.

§ If the absolute neutrophil count (ANC) reaches less than 1,000/µL, it should be judged to be grade 3 toxicity. Other decreases in the white blood cell count, or in circulating granulocytes, are not to be considered, since a decrease in the white blood cell count is a desired therapeutic end point. A gradual decrease in granulocytes is not a reliable index in CLL for stepwise grading of toxicity. If the ANC was less than 1,000/µL prior to therapy, the patient is not evaluable for toxicity referable to the ANC. The use of G-CSF is irrelevant for the grading of toxicity, but should be documented.

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