

Real-life Diagnostic and Therapeutic Approach to CLL: A New Proposal from an Expert Panel in Tuscany Region

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ABSTRACT

BACKGROUND: In the last years genomic and somatic alterations have shown to play a pivotal role in the pathogenesis of chronic lymphocytic leukemia (CLL) and new prognostic factors have been identified accordingly.

AIM: To describe a real-life diagnostic and therapeutic approach to CLL that takes into account the role of genomic and somatic prognostic factors in the risk stratification of developing progressive disease, and treatment decision.

METHODS: This new proposal has been developed and validated by ten key opinion leaders from Tuscany Region during two Expert Meetings. The approach suggested comes from their experience in daily clinical practice and is supported by guidelines recommendations, clinical trials results, and drugs prescribing conditions in Italy.

RESULTS: Beside TP53 deletion or mutated status, the Expert Panel highlighted the importance of the IGHV mutation status characterization, since the diagnosis, in order to identify patients who will have a more aggressive progression. Furthermore, just before starting treatment, to obtain useful prognostic information and indication in the selection of the therapy, they recommend cytogenetic analysis for the detection of del(11q), trisomy 12, del(13q), del(17p), conventional karyotyping of stimulated CLL cells, TP53 sequencing, and molecular genetic analysis to detect IGHV mutation status.

CONCLUSIONS: The Expert Panel recognized the limitations associated with traditional staging systems in identifying patients who will have a more aggressive disease course and predicting response to treatment and suggested a real-life diagnostic and therapeutic approach to CLL to update the current patient management in light of recent advances that have improved understanding of CLL.

Keywords

Chronic lymphocytic leukemia; Prognostic factors; TP53 deletion; IGHV mutation status

BACKGROUND

Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disorder characterized by progressive accumulation of monomorphic B-cells in peripheral blood, bone marrow, spleen, and lymph nodes.

CLL represents the most common form of leukemia of adults in Western countries [1]. The Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute (NCI) reports an incidence of 4.9 new cases per 100,000 men and women per year [2]. CLL is most frequently diagnosed among people aged 65-74 with a median age at diagnosis of 70 years (about two third ≥ 65 years) and this malignancy is more common among men

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Stage	Description
Rai [4,5]	
0 (low risk risk)	Lymphocytosis in the peripheral blood and/or in the bone marrow.
I-II (intermediate risk)	Lymphocytosis in the peripheral blood + enlarged lymph nodes in any sites + splenomegaly and/or hepatomegaly.
III-IV (high risk)	Lymphocytosis with disease-related anemia (Hb <11 g/dL) or thrombocytopenia (platelets count <100×10 ⁹ /L).
Binet [6]	
A	Hb ≥10 g/dL + platelet count ≥100×10 ⁹ /L + <3 lymph node areas involved ¹ .
B	Hb ≥10 g/dL + platelet count ≥100×10 ⁹ /L + ≥3 lymph node areas involved ¹ .
C	Hb <10 g/dL and/or platelet count <100×10 ⁹ /L.

Table I. Staging systems [4-6]

¹ Presence of enlarged lymph nodes ≥1 cm in diameter or organomegaly. Areas of involvement considered are head and neck (including the Waldeyer ring), axillae, groin, liver, and spleen

Prognostic factors	10-year OS
Del(17p) vs non del(17p)	-87%
Serum β ₂ -microglobulin concentration >3.5 mg/L vs ≤3.5 mg/L	-73%
Unmutated vs mutated IGHV	-68%
Mutated vs unmutated TP53	-55%
Binet stage B vs A	-56.8%
Binet stage C vs A	-46.4%
Age >65 vs ≤65 years	-39%

Table II. Impact of the independent factors identified by the CLL-IPI. Elaboration from [12]

Risk group	Prognostic factors	10-year OS
Low	Mutated IGHV and no del(17p)/del(11p)	82%
Intermediate	Unmutated IGHV or del(17p)/del(11p)	52%
High	Unmutated IGHV and del(17p)/del(11p)	27%

Table III. Risk groups identified by the Barcelona score [13]

reflects the loss of TP53 gene, is frequently associated with the mutation of the remaining TP53 allele and worse outcomes and shorter survival [10,11]. Furthermore, the mutation of TP53 is associated with a reduction of overall and progression free survival (PFS), even in the absence of del(17p) [10,11].

From these findings, new prognostic scores have been developed in order to overcome the limitations of the classical staging systems.

The international prognostic index for patients with chronic lymphocytic leukemia (CLL-IPI) [12] used data from 3472 patients and identified five independent prognostic factors impacting 5-year overall survival (OS): deleted or mutated TP53 status, unmutated IGHV, serum β₂-microglobulin >3.5 mg/L, Binet stage B/C or Rai I-IV, and age >65 years. Furthermore, the results of the univariate analyses showed the impact of each factor on CLL 10-year prognosis (Table II).

The Barcelona score [13] aimed at simplifying the CLL-IPI developing a biomarkers-only prognostic system based on the two most important prognostic factors: IGHV mutational status and fluorescence *in situ* hybridization (FISH) cytogenetics. Barcelona score identify three risk groups with different 10-year OS (Table III).

These novel prognostic factors aim at assisting patient management (i.e., define the follow-up strategy based on the risk of developing progressive disease, especially for patients with low tumor burden at diagnosis) and treatment decision [7]. The presence of del(17p) and/or mutated TP53 has in fact been associated with resistance to standard chemotherapy regi-

than women (6.8 vs 3.5 per 100,000 persons, respectively) [2].

The clinical course of the disease is extremely variable: while the majority of CLL patients is asymptomatic at diagnosis and becomes symptomatic within a few years, a part of patients remains asymptomatic for decades with a percentage of 20-30% who presents a life expectancy equal to that of the general population [3]. Due to this heterogeneity, it is very important to identify, since the diagnosis, which patients will have a more aggressive progression.

The oldest, and still most used, staging systems in CLL are Rai [4,5] and Binet [6] classifications. Both of them define three prognostic groups with different disease burden (Table I) and are only based on physical examination (lymph node involvement, hepatomegaly, and/or splenomegaly) and blood test results (presence of anemia or thrombocytopenia).

Despite their widespread use, these classifications have shown some limitations in identifying patients who will have a more aggressive disease course and predicting response to treatment [7].

Recently, genomic and somatic alterations have shown to play a pivotal role in the pathogenesis of CLL and new prognostic factors have been identified accordingly.

In particular, studies on the variable region of the immunoglobulin heavy chain gene (IGHV) have shown that the unmutated gene is associated with a worse prognosis and a significant reduction of survival compared to patients with mutated pattern [8,9]. Also, the advances on cytogenetic alterations allowed to identify patients with favorable [del(13q)] and unfavorable [del(11q), del(17p)] prognosis [10,11]. The presence of del(17p), which

mens (i.e., alkylating drugs and/or purine analogs) and poor response to chemoimmunotherapy [10], while better outcomes have been achieved with novel inhibitors [10]. Similarly, the presence of mutated IGHV genes identify patients who have long-term disease free-survival after the treatment with fludarabine, cyclophosphamide, and rituximab (FCR) [14], while in patients with the unmutated IGHV pattern the treatment with the novel BTK inhibitor ibrutinib is associated with better outcomes when compared with chlorambucil (CHL) [15-17].

Recently published international guidelines and expert recommendations [10,11,18-21] have already recognized the usefulness of the new diagnostic and prognostic factors in the management of patients with CLL.

AIM

Aim of this paper is to describe a real-life diagnostic and therapeutic approach to CLL proposed by an Italian Expert Panel with the objective to update the current patient management in light of recent advances that have improved understanding of CLL. This new proposal has been developed and validated by ten key opinion leaders from Tuscany Region during two Expert Meetings. The approach suggested comes from their experience in daily clinical practice and is supported by guidelines recommendations, clinical trials results, and drugs prescribing conditions in Italy.

DIAGNOSIS OF CLL

Onset of CLL is usually asymptomatic and the disease is discovered incidentally after a blood count evaluation performed for another reason. To differentiate CLL from MBL (monoclonal B-cell lymphocytosis), the diagnosis of CLL requires the presence, for at least three months, of $\geq 5 \times 10^9/L$ monoclonal B cell lymphocytes in the peripheral blood [10,11]. The clonality of B-cells must be confirmed by flow cytometry in order to detect the CLL peculiar immunophenotypic profile: clonal kappa or lambda light chain restriction, co-expression of the surface antigen CD5 and the B-cell antigens CD19, CD20, and CD23, high expression of CD200 [22] and low levels of surface immunoglobulin CD20 and CD79b [10,11].

Table IV reports the examinations recommended to confirm the diagnosis of CLL and predict patient prognosis [10,11].

In particular, molecular analysis to detect IGHV mutation status can provide useful prognostic information, especially in patients with low tumor burden at diagnosis, and enable physicians to provide more accurate patient counseling and define the frequency of follow-up. Furthermore, IGHV mutation status remains unchanged over time; thus it can inform therapy selection before starting treatment [7,10-13,23,24].

Time	Examinations recommended
At diagnosis	<ul style="list-style-type: none"> Detailed anamnesis with particular attention to prior or current malignancy. Blood tests: CBC, LDH, creatinine, total protein levels, serum protein electrophoresis, transaminases, bilirubin, β_2-microglobulin, IgG, IgA, and IgM levels. Microscopic examination of the peripheral blood smear. Immunophenotype: flow cytometry on peripheral blood lymphocytes. Physical examination (abdomen and palpable lymph nodes). Imaging: ultrasound and X-ray of chest. Molecular analysis: IGHV mutation status (Sanger or NGS).
At follow-up (asymptomatic patients)	<ul style="list-style-type: none"> Blood tests: CBC, LDH, creatinine, total protein levels, serum protein electrophoresis, transaminases, bilirubin, β_2-microglobulin, IgG, IgA, and IgM levels. Physical examination (abdomen and palpable lymph nodes). Imaging: abdomen ultrasound at least 1 time every 12 months¹.
Before treatment	<ul style="list-style-type: none"> Detailed anamnesis with particular attention to polypharmacy e comorbidity index (CIRS, ECOG, and Charlson). Blood tests: as at diagnosis + QuantiFERON, infectious disease status (HBV, HCV, HIV; if IgG < 500 mg/dl detection/quantification of viral genomes). Cytogenetic analysis: FISH on peripheral blood lymphocytes for chromosomes 11, 12, 13, and 17; conventional karyotyping of stimulated peripheral blood lymphocytes (aCGH). Molecular analysis: IGHV mutation status (if not performed at diagnosis), TP53 mutation status (Sanger or NGS, cut off > 10%). Heart tests: ECG, ECOCG.

Table IV. Examinations recommended at diagnosis, follow-up, and before treatment

¹ Timing to be defined according to clinical need

aCGH = array-based Comparative Genomic Hybridization; CBC = complete blood count; CIRS = Cumulative Illness Rating Scale; ECG = electrocardiogram; ECOCG = echocardiogram; ECOG = Eastern Cooperative Oncology Group; FISH = Fluorescence *in situ* hybridization; IGHV = Immunoglobulin Heavy Chain Variable Region; LDL = low-density lipoprotein; NGS = next generation sequencing

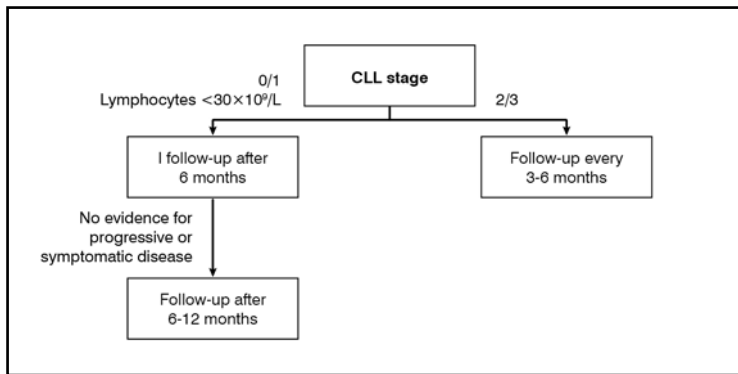


Figure 1. Monitoring of patients without active disease

MONITORING

According to IwCLL guidelines, patients with asymptomatic early-stage disease should be observed without therapy until disease progression or evidence of disease-related symptoms [10].

A “watch and wait” strategy until evidence of active disease is usually recommended also in patients with intermediate and high-risk disease.

As reported in Figure 1, asymptomatic patients with early-stage disease should be followed-up 6 months after diagnosis in order to exclude a rapid disease progression.

Afterwards, in case of stable disease, follow-up may take place every 6-12 months. The examinations recommended at each follow-up visit are reported in Table IV.

TREATMENT

Assessment before treatment

Table IV reports examinations recommended before treatment. In particular, cytogenetic analysis for the detection of del(11q) [10,11,13], trisomy 12 [10,11], del(13q) [10,11], del(17p) [10,11], conventional karyotyping of stimulated CLL cells [11,25], TP53 sequencing, and molecular genetic analysis to detect IGHV mutation status can provide useful prognostic information and may guide selection of therapy [10,11].

Since cytogenetic abnormalities can evolve over time, re-evaluation of FISH, stimulated karyotype, and of TP53 mutational status are recommended before each subsequent line of treatment.

Furthermore, the choice of treatment should take into account age, comorbidities, performance status, and creatinine clearance.

Indication for treatment

As reported above, treatment should only be started in patients with progressive or symptomatic disease (active disease) [10]. Active disease is defined by the presence of at least one of the criteria reported in Table V.

Neither the presence of del(17p), TP53 mutation, or other markers associated with poor prognosis, nor the absolute lymphocyte count, nor lymph node size, without the above mentioned criteria, should be used as indicator for treatment.

Assessment of response to treatment

In the recent years, the components of the panel acquired a huge experience about the employ of the ultrasound as fundamental tool for the response assessment. Indeed, according to the international guidelines, the quality of response is based on the disappearance/reduc-

Criteria for initiating treatment (at least 1 of the following should be met)
<ul style="list-style-type: none"> • Progressive lymphocytosis with an increase of $\geq 50\%$ over 2 months or LDT < 6 months (if lymphocytosis $> 30 \times 10^9/L$, a longer observation period may be required in patients with lymphocytosis $< 30 \times 10^9/L$). • Evidence of progressive marrow failure with development (or worsening) of anemia (Hb < 10 g/dL) and/or thrombocytopenia (platelet count $< 100 \times 10^9/L$). • Massive (≥ 6 cm from the costal arch) or progressive or symptomatic splenomegaly. • Massive (nodes with longest diameter ≥ 10 cm) or progressive or symptomatic lymphadenopathy. • Autoimmune anemia and/or thrombocytopenia resistant to corticosteroids. • Symptomatic of functional extranodal involvement. • Disease-related systemic symptoms: <ul style="list-style-type: none"> • Weight loss $\geq 10\%$ in the last 6 months • Significant asthenia/fatigue (ECOG ≥ 2) • Fever $\geq 38^\circ\text{C}$ for ≥ 15 days without evidence of infection • Night sweats for ≥ 1 months without evidence of infection

Table V. Criteria that define active disease [10]

LDT = Lymphocyte Doubling Time

tion or stability of the lymph nodes and spleen dimensions, assessed by CT scan and physical examination or by the physical examination only (in the general practice) [10]. In the routine, the physical examination is always subjective, with a high inter-individual variability, and CT scan exposes patient to a biological damage. On the contrary, the ultrasonography is a “biologically safe” technique, with lower variability of interpretation, able to measure with precision the lymph nodes and spleen dimensions and to distinguish the “reactive” from the “still pathological” masses. In conclusion, the experts suggest adding the ultrasound assessment to the physical examination in the clinical daily practice.

I line treatment algorithm

Figure 2 reports the treatment algorithm proposed by the Expert Panel for the treatment of patients with CLL.

The most important characteristics that guide the choice of therapy are the presence of del(17p) and/or mutated TP53, IGHV mutational status, the presence of del(11q), age, and comorbidities.

Patients with TP53 mutation and/or del(17p)

Since chemoimmunotherapy showed poor outcome, patients with del(17p) and/or TP53 mutation should be treated with novel inhibitors.

In particular, in the absence of contraindication, ibrutinib, an inhibitor of Bruton’s tyrosine kinase (BTK), is the preferred treatment option [11,26].

Adult patients who are not eligible for chemoimmunotherapy could be considered for therapy with idelalisib (an inhibitor of phosphatidylinositol 3-kinase p110δ), in combination with rituximab [27]; while patients who are unsuitable for B-cell receptor pathway inhibitors, could be considered for therapy with venetoclax (a BCL2 inhibitor) [28].

Patients without TP53 mutation or del(17p)

In these patients, IGHV mutational status and age are the main drivers to define treatment strategy.

Age ≥65 years

Unmutated IGHV. In the RESONATE-2 study [15-17], ibrutinib showed significantly higher overall response rate (ORR) and longer PFS compared to CHL and thus it is now the first choice of treatment. For patients who are not eligible to receive ibrutinib (in patients <70 years ibrutinib is indicated if at least one of the following criteria are satisfied: creatinine clearance <70 mL/min, ECOG 1-2, anemia <10 g/dL or thrombocytopenia <100.000/μL [29]) the second choice of treatment is the combination of the new anti-CD20 monoclonal antibody obinutuzumab and chlorambucil (G-CHL).

Mutated IGHV. As reported above, ibrutinib is associated with better outcomes compared to CHL [15-17]. Furthermore, the Alliance North American Intergroup Study showed

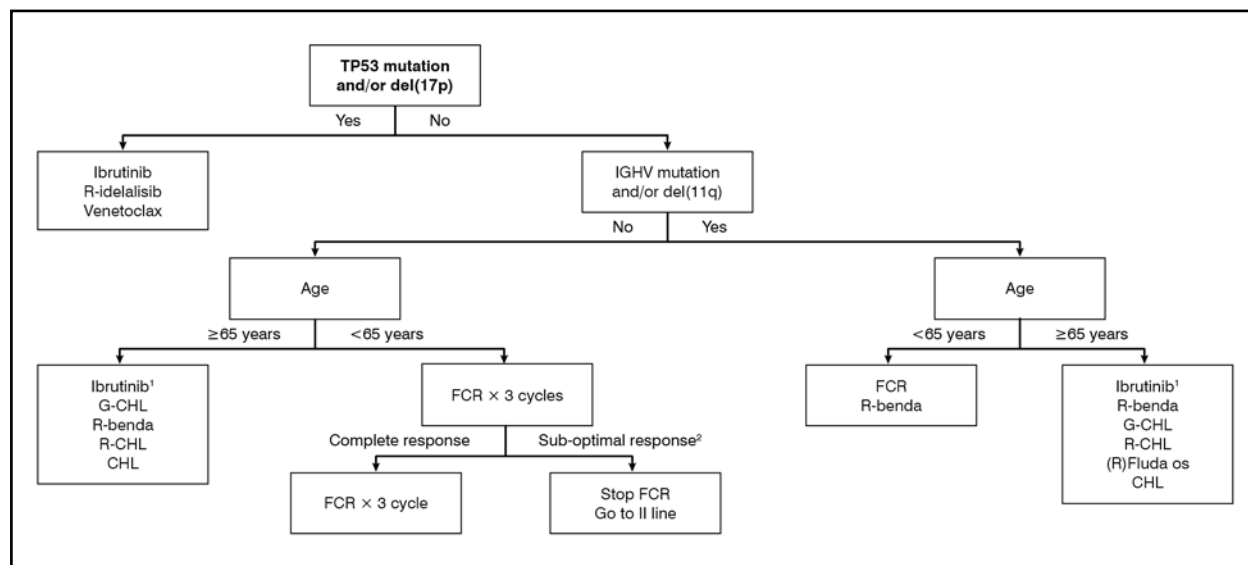


Figure 2. Treatment algorithm for patients with CLL

¹ In patients <70 years ibrutinib is indicated if at least one of the following criteria are satisfied: creatinine clearance <70 mL/min, ECOG 1-2, anemia <10 g/dL or thrombocytopenia <100.000/μL [29]; ² Evaluated at the discretion of the clinician

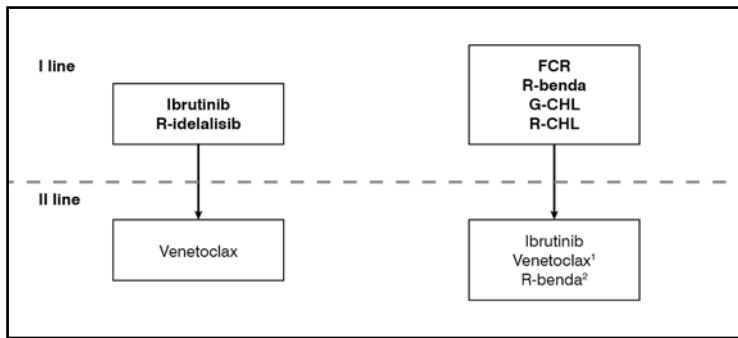


Figure 3. Relapsed therapy

¹ The use of R-venetoclax will be considered after the publication of reimbursement criteria in the Italian Official Gazette

² Re-treatment with R-bendamustine should be considered after at least 36 months from relapse only in patients with unmutated TP53 and mutated IGHV status

that, among older patients (≥ 65 years), treatment with ibrutinib was superior to the combination of rituximab and bendamustine (R-bendamustine) in terms of PFS [30].

Although OS was not statistically different, chemoimmunotherapy could still be a good option for these patients, when deletion of chromosome 11 has been excluded. The regimens more frequently adopted by the experts resulted: R-bendamustine, low-dose of FCR [31] and G-chlorambucil.

Age <65 years

Unmutated IGHV. Recent studies highlighted the limits of treatment with FCR in young unmutated IGHV patients in favor of

ibrutinib [32]. Waiting for the approval of ibrutinib for this indication, chemoimmunotherapy with FCR remains the standard of care in these patients [11,14,33]. Patients who complete 6 cycles of FCR achieve better outcomes [14]; however, based on our clinical experience, we recommend assessing the response already after 3 cycles. In case of complete response (CR), we suggest continuing with FCR regimen for other 3 cycles, otherwise, in case of sub-optimal response (evaluated at the discretion of the clinician), it is recommended to discontinue FCR treatment and move to the II line treatment.

Mutated IGHV. In these patients, the treatments recommended are FCR and R-bendamustine. FCR is associated with better outcomes, while R-bendamustine is associated with less toxic effects [34]; therefore, the choice of type and duration of treatment should be made at the discretion of the clinician.

Relapsed therapy

According to IwCLL guidelines relapse is defined as «evidence of disease progression in a patient who has previously achieved the above criteria of complete or partial remission for ≥ 6 months» [10].

Figure 3 reports the recommended relapsed therapies.

CONCLUSIONS

The Expert Panel, consisting of clinicians with experience in management of CLL, recognized the limitations associated with traditional staging systems in identifying patients who will have a more aggressive disease course and predicting response to treatment.

Therefore, based on their clinical practice, guidelines recommendations, clinical trials results, and drugs prescribing conditions, they suggested a new diagnostic and therapeutic approach that takes into account the role of genomic and somatic prognostic factors in the risk stratification of developing progressive disease, and treatment decision. In particular, beside TP53 deletion or mutated status, they highlighted the importance of the IGHV mutation status characterization, since the diagnosis, in order to identify patients who will have a more aggressive progression. Furthermore, just before starting treatment, to obtain useful prognostic information and indication in the selection of the therapy, they agreed with other groups and guidelines to recommend cytogenetic analysis for the detection of del(11q), trisomy 12, del(13q), del(17p), conventional karyotyping of stimulated CLL cells, TP53 sequencing, and molecular genetic analysis to detect IGHV mutation status.

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Conflicts of Interest

All authors have nothing to disclose regarding this work.

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