Acta Haematologica Polonica 2023
Number 6, Volume 54, pages 342–371
DOI: 10.5603/ahp.97472
ISSN 0001–5814
e-ISSN 2300–7117

VIA MEDICA

Diagnostic and therapeutic recommendations of the Polish Society of Haematologists and Transfusiologists, and Polish Adult Leukemia Group-CLL for chronic lymphocytic leukemia in 2023

Iwona Hus[1*], Krzysztof Giannopoulos[2], Krzysztof Jamroziak[3], Dariusz Wołowiec[4], Jacek Roliński[5], Tadeusz Robak[6, 7]

[1]Department of Hematology, National Medical Institute of the Ministry of Internal Affairs and Administration, Warsaw, Poland

[2]Department of Experimental Hematooncology, Medical University of Lublin, Lublin, Poland

[3]Department of Hematology, Transplantology, and Internal Diseases, Central Clinical Hospital of UCK, Medical University of Warsaw, Warsaw, Poland

[4]Department and Clinic of Hematology, Blood Cancer and Bone Marrow Transplantation, Medical University of Wroclaw, Wrocław, Poland

[5]Department of Clinical Immunology, Medical University of Lublin, Lublin, Poland

[6]Department of Experimental Hematology, Medical University of Lodz, Łódź, Poland

[7]Department of Hematology, Medical University of Lodz, Provincial Specialist Hospital M. Kopernika in Lodz, Łódź, Poland

Abstract

Chronic lymphocytic leukemia (CLL) is a disease of the elderly, with a median age at diagnosis of c.70 years. The natural course of the disease varies greatly, and patients with non-progressive and asymptomatic leukemia do not require treatment. But advanced and progressive CLL do require treatment. The results of CLL treatment have improved significantly in recent years, mainly due to the introduction of new and more effective drugs, including B-cell receptor inhibitors and B-cell lymphoma 2 (BCL2) inhibitors. These new drugs are used continuously as monotherapy, or in combination schemes for specified periods. Venetoclax in combination with anti-CD20 antibodies is used for 24 (rituximab) or 12 (obinutuzumab) months, while treatment with ibrutinib and venetoclax lasts 15 months. The choice of treatment protocol should largely depend on the assessment of 17p deletion/*TP53* mutation, and in second treatment line immunoglobulin variable heavy chain (IGVH) mutation status, which correlate with a response to immunochemotherapy. The role played by immunochemotherapy has recently significantly decreased. It is still an option for first line treatment in patients without 17p deletion/*TP53* mutation, with mutated gene encoding IGVH and in good performance status. However, the results of recent studies have shown that these patients may also obtain major benefit from chemotherapy-free regimens. The remaining patients, both in the first and subsequent treatment lines, should receive new targeted therapies, which are currently available in Poland under the drug program. In this article, we present an update of the guidelines for the diagnosis and treatment of CLL, including the treatment of autoimmune complications, as well as the

*Address for correspondence: Iwona Hus, Clinical Department of Hematology, National Medical Institute of the Ministry of Internal Affairs and Administration, Wołoska 137, 02–507 Warszawa, Poland, e-mail: iwonach.hus@gmail.com

Received: 17.09.2023 Accepted: 29.10.2023 Early publication date: 29.12.2023

prophylaxis and treatment of infections, developed by the Polish Society of Haematologists and Transfusiologists and the Polish Adult Leukemia Group-CLL working group.

Key words: chronic lymphocytic leukemia, 17p deletion/*TP53* mutation, Bruton's kinase inhibitors, ibrutinib, acalabrutinib, zanubrutinib, BCL2 inhibitor, venetoclax, rituximab, obinutuzumab, fludarabine, bendamustine

Acta Haematologica Polonica 2023; 54, 6: 342–371

Introduction

Chronic lymphocytic leukemia (CLL) is a disease of the elderly, with a median age at diagnosis of 70–72 [1–3]. Apart from age, the only risk factor for developing CLL is family history [4, 5]. In CLL patients, the risk of secondary cancers is around three times greater than in the general population [6]. The advanced age of CLL patients was previously associated with a poor prognosis, mainly due to comorbidities and poor tolerance of more aggressive therapies [3]. In recent years, the treatment options for CLL have significantly expanded with the introduction of new groups of drugs i.e. B-cell receptor (BCR) signal transduction inhibitors including Bruton's kinase inhibitors (BTK), and B-cell lymphoma 2 (BCL2) inhibitors. These drugs are well tolerated by the elderly and highly effective also in patients with unfavorable prognostic factors such as 17p deletion (del17p)/*TP53* mutation and unmutated immunoglobulin heavy-chain variable region gene (IGVH) [7, 8].

Selecting the most appropriate treatment requires an assessment of the patient's clinical condition, age and comorbidities. Before treatment commences, it is recommended to assess factors of prognostic and predictive importance, primarily del17p/*TP53* mutation, and in cases of the first line of treatment also the *IGVH* mutation status, because lack of mutation correlates with a worse response to immunochemotherapy [7, 8]. The role played by immunochemotherapy has significantly decreased in recent years, and it is currently recommended in the first line only in patients without del17p/*TP53* mutation and mutated *IGVH,* although the results of recent studies show that these patients may also obtain a major benefit from chemotherapy-free regimens. The remaining patients should receive novel targeted therapies, which are currently available in Poland under the B.79 drug program.

In this article, we present an update of management standards in the diagnosis and treatment of CLL, including the treatment of autoimmune complications, as well as the prevention and treatment of infections, developed by the Polish Society of Haematologists and Transfusiologists and the PALG-CLL (the Polish Adult Leukemia Group — Chronic Lymphocytic Leukemia working group). The guidelines proposed in this paper were developed based on the results of clinical trials with different strengths of evidence and the authors' clinical experience.

Definition and epidemiology

Chronic lymphocytic leukemia is a lymphoid cancer which is characterized by clonal proliferation of B-cells, presenting on their surface CD5 antigen typical for the T line, and their accumulation in the peripheral blood, bone marrow, lymphoid organs, and, less frequently, in extralymphatic organs. According to the 5th edition of 2022 World Health Organization (WHO) classification [1] CLL is a type of neoplasm derived from mature B-cells. It is the most common leukemia in the western world, with just over five new cases per 100,000 population annually (SEER, Surveillance, Epidemiology, and End Results) [2]. The etiology of the disease is unknown, and there is no association between its occurrence and exposure to environmental or occupational factors. The incidence is 6.8/100,000 in males and 3.5/100,000 in females [2]. The disease is most common in the elderly between 65 to 74 years of age, 70% of patients are aged over 65, and only 10% are under 55. The median age at diagnosis is 72 [3]. CLL patients account for 1.3% of all cancer patients in the United States. Annual mortality from CLL is 1.1/100,000. Apart from age, the only risk factor for developing CLL is a family history. In first-degree relatives of CLL patients, the relative risk of developing CLL is up to 8.5 times higher than in the general population [4, 5]. In patients with CLL, the risk of secondary cancers is approximately three times that of the general population. The most common secondary neoplasms are skin cancer (an 8-times greater risk), lung cancer, gastrointestinal neoplasms, and hematological malignancies [6].

Diagnostic criteria

The main criterion for the diagnosis of CLL is the presence of at least 5 G/L clonal B lymphocytes in the peripheral blood, confirmed by immunophenotypic examination of light chains [kappa (κ), lambda (λ)] [7, 8]. Leukemic CLL cells are mostly small, mature lymphocytes, with a narrow border of cytoplasm and dense nuclear chromatin. This population also includes larger, atypical, nuclear-indented cells or prolymphocytes, the percentage of which should not exceed 55% of all peripheral blood lymphocytes. The presence of a higher percentage of prolymphocytes supports the diagnosis of chronic B-cell prolymphocytic leukemia (B-cell PLL) [7]. However, B-PLL as a separate disease entity was not included

in the WHO classification published in 2022 (5th ed.). Cases meeting WHO's previously used criteria should be diagnosed as other lymphoproliferative syndromes, especially mantle cell lymphoma or a disease newly defined by this classification called 'splenic lymphoma/splenic B-cell lymphoma/leukemia with prominent nucleoli'. B-PLL as a separate disease entity remained in the International Consensus Classification (ICC) published in 2022 [9].

CLL cells show typical co-expression of B-cell antigens (CD19, CD20) with T-cell antigen CD5 as well as CD23, CD43, and CD200 antigens [8]. Expression level of CD20, CD79a, and surface immunoglobulin antigens is lower than in normal B-cells. In 50% of cases, B-cell prolymphocytic leukemia cells do not express CD5, while CD20 and surface immunoglobulin are expressed [7]. According to the European Research Initiative on CLL (ERIC) and the European Society for Clinical Cell Analysis (ESCCA) expert panel recommendations, testing of CD19, CD5, CD20, CD23 antigens and κ and λ chains on peripheral blood lymphocytes is necessary and sufficient to establish the diagnosis in typical cases. The expert panel also recommends additional testing of CD43, CD79b, CD81, CD200, CD10 and ROR1, which may be helpful in establishing the diagnosis in more difficult cases [10].

Patients with lymphadenopathy and/or splenomegaly, with B-cells with typical CLL immunophenotype in the peripheral blood, but less than 5 G/L, meet the diagnostic criteria of small lymphocytic lymphoma (SLL) [7]. A final diagnosis of SLL requires histopathological examination of the affected tissue. According to the WHO classification, CLL and SLL are separate clinical manifestations of the same disease [1].

The presence of less than 5 G/L of clonal B cells in the peripheral blood, without accompanying lymphadenopathy or organomegaly, cytopenia or systemic symptoms, allows the diagnosis of monoclonal B-cell lymphocytosis (MBL). Annually, 1–2% of MBL cases progress to CLL [11].

A simplified diagram showing the cytometric differential diagnosis of CLL with leukemic forms of other B-cell lymphomas is presented in Figure 1.

Bone marrow examination is not needed to diagnose CLL. However, it should be performed in patients with cytopenia to diagnose its cause (e.g. displacement of normal hematopoietic cells by leukemic cells, drug toxicity or immunocytopenia), as well as in the case of inconclusive results of immunophenotyping [7, 8]. Typically, the bone marrow of CLL patients shows a diffuse or nodular infiltration of more than 30% of lymphoid cells. In patients with concomitant lymphadenopathy and an inconclusive immunophenotyping result, an open biopsy of the lymph node should be performed.

Patient evaluation at CLL diagnosis

Initial evaluation of a patient diagnosed with CLL should include a medical history, physical examination including lymph nodes, liver and spleen, laboratory tests, and, if necessary, diagnostic imaging. Attention should be paid to the general symptoms related to the disease (fever of unknown origin >38.0°C for ≥2 weeks, night sweats lasting ≥1 month, weight loss of more than 10% of the initial weight in the last six months, progressive weakness), recurrent infections and comorbidities that may influence therapeutic decisions. Laboratory tests include complete blood count with a manual blood smear review, biochemical tests with the assessment of kidney and liver function, three basic classes of immunoglobulins (IgA, IgG and IgM) blood levels, and a direct antiglobulin test (DAT) [7, 8]. In daily clinical practice, in asymptomatic patients it is not necessary to perform imaging diagnostics such as ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI). However, these tests are required in prospective clinical trials. Positron emission tomography (PET)/CT examination is recommended in patients with suspected Richter's transformation (RT) to determine the optimal biopsy site [7, 8].

During initial diagnostics, it is not necessary to perform cytogenetic and molecular tests, in particular the determination of *TP53* and IGHV mutation status [7, 8]. Cytogenetic tests may be helpful in cases of CLL with an atypical CD23 phenotype, which can accompany trisomy of chromosome 12.

During diagnostics, the clinical stage of CLL should be determined using one of the two equivalent clinical staging systems: Rai or Binet [12, 13]. Both classifications are based on the results of blood count and physical examination. According to the current recommendations, the modified 3-stage Rai staging system should be used rather than the original 5-stage system [7, 14]. The Binet staging system depends on the number of nodal areas involved, including:

1) enlarged lymph nodes in the head and neck, including Waldeyer's ring (counted as one area even if more than one node is enlarged at that location);
2) enlarged axillary lymph nodes (counted as one area even with bilateral involvement);
3) enlarged inguinal lymph nodes (counted as one area even with bilateral involvement);
4) spleen palpable on physical examination;
5) liver enlarged on physical examination.

The Rai and Binet classifications are set out in Table I [12–14].

Prognostic factors

The most important prognostic factors in CLL, the measurement of which before starting therapy is recommended by international guidelines [European Society For Medical Oncology (ESMO), National Cancer Center Network (NCCN), International Workshop on Chronic Lymphocytic Leukemia

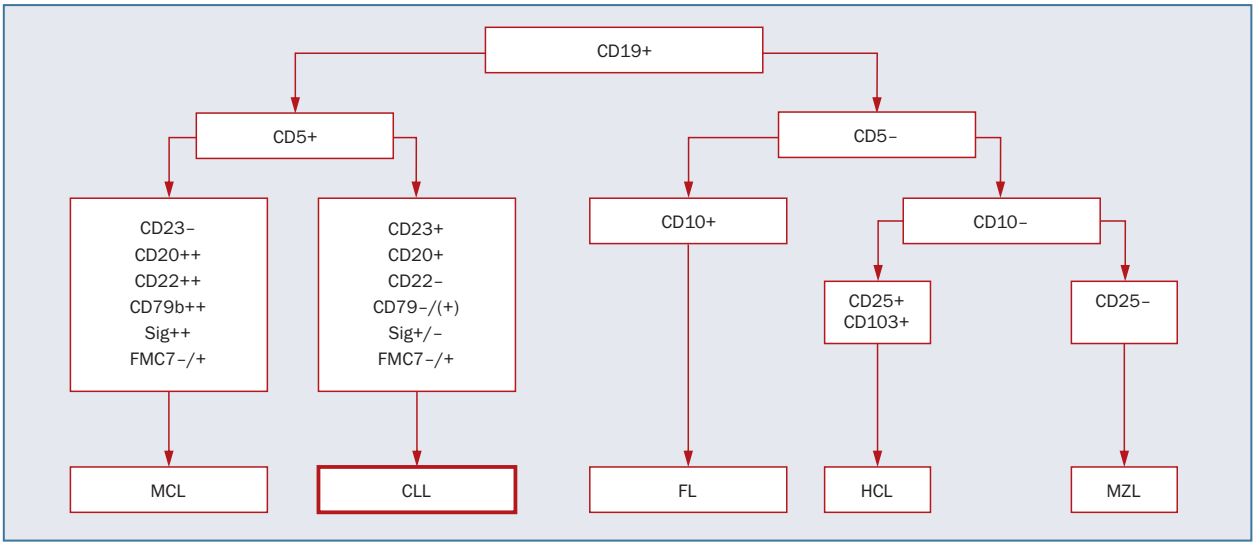

Figure 1. Simplified algorithm for the differential cytometric diagnosis of chronic lymphocytic leukemia (CLL); MCL — mantle cell lymphoma; FL — follicular lymphoma; HCL — hairy cell leukemia; MZL — marginal zone lymphoma

Table I. Clinical staging of chronic lymphocytic leukemia, according to Rai and Binet classifications (based on [10–12])

Classification	Clinical period/risk group		Criteria
Rai	0	Low risk	Lymphocytosis*
	I	Intermediate risk	Lymphocytosis + lymphadenopathy
	II		Lymphocytosis* + splenomegaly and/or hepatomegaly (with or without lymphadenopathy)
	III	High risk	Lymphocytosis* + anemia (Hb <11.0 g/dL)
	IV		Lymphocytosis* + thrombocytopenia (PLT <100.0 g/dL)
Binet	A		Involvement of <3 node areas/organs**
	B		Involvement of ≥3 node areas/organs**
	C		Anemia and/or thrombocytopenia (Hb <10 g/dL, and/or PLT <100 G/L)

*Absolute peripheral blood lymphocyte count >5,000/μL; **enlarged head and neck lymph nodes and/or axillary nodes and/or inguinal nodes and/or spleen and/or liver (see text for details); Hb — hemoglobin; PLT — platelets

(iwCLL)], include the main cytogenetic and molecular risk parameters, i.e. *TP53* gene disorders (17p deletions including the *TP53* gene locus and *TP53* gene mutations) and IGHV mutation status [7, 8]. *TP53* gene disorders and the IGHV mutation status have both prognostic and predictive value. This is particularly important in relation to the advisability of using classical immunochemotherapy, and therefore currently plays an important role in the decision as to which treatment method to choose. The presence of del17p/*TP53* mutation is associated with the worst prognosis in patients treated with immunochemotherapy, resulting in overall survival (OS) of 2–5 years [15–17]. The treatment outcomes of these patients improved significantly due to the introduction of targeted therapies with BCR and BCL2 inhibitors [18–20]; however, the prognosis still remains poor compared to patients without these mutations. The frequency of del17p/*TP53* mutation is c.10% in patients with indications to start first-line therapy, and increases with subsequent relapses of CLL if classical chemotherapy is used in the treatment. Del17p is determined by fluorescence *in situ* hybridization (FISH) and *TP53* mutations are determined by Sanger sequencing or next generation sequencing (NGS). The negative prognostic value of del11q (detected using FISH) has been significantly reduced due to the addition of rituximab to fludarbine and cyclophosphamide (FCR), and especially by new targeted therapies [17, 18, 21]. The second most important negative prognostic factor is the so-called unmutated status of the immunoglobulin variable heavy chain (IGVH) genes. IGHV genes are defined as unmutated when their germline variation is less than 2%. The unmutated state occurs in c.60% of patients with CLL who have indications for therapy [22] and does not change in the further course of the disease. The absence of IGVH mutations is associated with a more

aggressive course of CLL, shorter survival [22], more frequent occurrence of del17p and del 11q, and a short-term response to FCR immunochemotherapy [23–26], as well as, to a much lesser extent, to venetoclax therapy [21]. However, many separate analyses in patients treated in clinical trials with various BTK inhibitors have shown their activity regardless of the IGHV mutation status [27–30]. The presence of a complex karyotype, most often defined as the presence of three or more independent cytogenetic aberrations, also has a very important prognostic significance. However, due to the complexity of karyotype assessment in CLL, this factor is currently very rarely determined in clinical practice.

There are also other molecular prognostic and predictive factors known, but they have not yet found an established place in clinical practice. These include mutations of other relatively frequently mutated genes in CLL, for example: *NOTCH1, SF3B1, BIRC3, RPS15*, as well as so-called subtypes of immunoglobulin gene rearrangements. The prognostic value of these parameters in various modern treatment methods requires further prospective validation. However, it is very likely that some of them will be important in the future due to the tendency to further individualize CLL therapy.

The clinical stage according to Rai or Binet classification is still an important prognostic factor in patients with CLL, although its importance is decreasing with the introduction of more, and more effective, therapies. The only recognized biochemical prognostic factor is β_2-microglobulin level. Immunophenotyping of CD38 and ZAP-70 expression is currently of no importance in clinical practice.

An important dynamic prognostic factor (available during or after therapy) that is gaining importance is the negativity of measurable residual disease (MRD), defined as the presence of less than one CLL cell per 10,000 leukocytes. MRD can be assessed at various stages of treatment in the blood and marrow using standardized methods, including multicolor flow cytometry and ASO-PCR, and NGS at a deeper level [31]. Undetectable MRD indicates a profound response, which translates into longer progression-free survival (PFS) and overall survival (OS), as demonstrated in the CLL8 study in patients treated with FC regimens (fludarabine, cyclophosphamide) and FCR immunochemotherapy [32]. The results of a retrospective single-center analysis of patients treated between 1997 and 2006 showed a significant impact of MRD eradication on 10-year survival, regardless of therapy type [33]. A correlation between achieving MRD eradication and longer PFS has also been demonstrated in studies with venetoclax in combination with rituximab, anti-CD20 monoclonal antibodies (MURANO study), and obinutuzumab (CLL14 study) [21, 34, 35]. In both cases, MRD eradication rates were significantly higher compared to immunochemotherapy, and achieving MRD eradication was associated with longer PFS, regardless of the treatment method. However, MRD has no prognostic value in relation to therapies with BTK inhibitors. Currently, MRD assessment is only recommended in clinical trials, but it is believed that, in the future, MRD assessment is likely to have an impact on therapeutic decisions e.g. duration of treatment.

Indications for treatment initiation

The aim of treatment is to extend and improve quality of life for the patient. Despite enormous progress in understanding the biology of leukemia, increasing the possibility of correctly predicting an unfavorable prognosis, the basic indication for treatment still remains the disease stage assessed according to the Rai or Binet scales. The predictive value of some new genetic and biological markers for OS is lower in people over 75 years of age, i.e. the great majority of patients, but del17p, *TP53* mutation and IGHV mutational status should be taken into account when choosing therapy also in this group of patients. The criteria for treatment initiation in clinical trials may differ from those adopted in daily clinical practice. Except for clinical trials, treatment should not be initiated in patients with newly diagnosed CLL in the early stages (i.e. Rai stage 0 or Binet A stage) without evidence of disease progression. These patients should be followed up, with disease status monitored every 3–12 months [7, 8]. Patients in the intermediate stage of disease, i.e. Rai stage I and II or Binet B stage, require close monitoring of certain leukemia parameters every 3–9 months, and in this group treatment should be initiated in the presence of signs of active disease or progression. Patients with advanced CLL (Rai stage III/IV or Binet C stage) require anti-leukemic treatment. If cytopenia is caused solely by autoantibodies, immunosuppressive therapy (glucocorticosteroids) is indicated, and antileukemic therapy is indicated if immunosuppressive therapy is ineffective. The criteria proposed by Hallek et al. [7] should be used to assess indications for therapy. Initiation of anti-leukemic therapy is indicated if the symptoms set out in Table II are observed.

Pre-treatment evaluation

In patients with CLL who are offered initiation of treatment, the following tests are recommended [7, 8]:

- history and physical examination with assessment of lymph nodes, including Waldeyer's ring, liver, and spleen;
- assessment of general condition and comorbidities;
- complete blood count with manual blood smear review;
- bone marrow examination (fine needle biopsy / trephine biopsy) is indicated in cases of cytopenia of unknown cause and in clinical trials. Bone marrow biopsy may

Table II. Indications (at least 1 must occur) for chronic lymphocytic leukemia (CLL) initiation according to International Workshop on Chronic Lymphocytic Leukemia (iwCLL) (source [7])

1. Progressive bone marrow involvement as manifested by anemia and/or thrombocytopenia [assumed hemoglobin (Hb) cut-off point <10 g/dL (<6.21 mmol/L) or platelet count <100 G/L]. However, these parameters should be reproducible and systematically decreasing, because often, especially in platelet count, parameter is only slightly reduced, up to <100 G/L, but stable for a long time, which should not be considered an indication for treatment. In sudden and extremely low cytopenia, differential diagnosis should include autoimmune diseases, and appropriate laboratory workup should be planned
2. Significant (≥6 cm below costal margin), progressive or symptomatic splenomegaly
3. Significant (≥10 cm in long axis), progressive or symptomatic lymphadenopathy
4. Rapid increase in lymphocyte count — increase of >50% in two months or doubling of lymphocytosis in less than six months (if baseline lymphocyte count is ≥30 G/L). Other possible causes of sudden increase in lymphocyte count or progression of lymphadenopathy (including SARS-CoV-2 infection) should be ruled out. An absolute number of lymphocytes, even a very high number, without other symptoms, is not a sufficient indication for treatment initiation. This definition indicates necessity of examining patient and assessing blood count at least every six months
5. Autoimmune anemia and/or immune thrombocytopenia refractory to corticosteroid therapy or other standard treatments
6. One or more systemic symptoms depending on underlying disease, defined as:
   - unintentional weight loss of ≥10% in the last six months
   - significant fatigue (ECOG PS ≥2; inability to work or perform normal activities)
   - fever >38.0°C for ≥2 weeks or more with no other indication of infection
   - night sweats for more than one month without any other evidence of infection. A common problem in CLL patients is increased susceptibility to infection. Unless other symptoms of active disease coexist, it is not an indication for anti-leukemic treatment
7. Symptomatic extra-nodal localization

ECOG PS — the Eastern Cooperative Oncology Group of performance status; Hb — hemoglobin; PLT — platelets; SARS-CoV-2 — severe acute respiratory syndrome-related coronavirus 2

also be used as a baseline parameter in assessing response to treatment;

- biochemical tests to assess organ function (evaluation of liver and kidney function) and possibly exclude causes of anemia other than CLL;
- immunoglobulin serum levels (IgA, IgG, and IgM);
- DAT, haptoglobin level;
- diagnostic imaging (outside clinical trials, if needed): chest X-ray, abdominal ultrasound, CT/MRI; (as part of clinical trials): chest, abdomen, and pelvis CT. Diagnostic imaging (CT, MRI) may be helpful in clinical practice in assessing tumor mass and risk of tumor lysis syndrome, especially before starting venetoclax treatment, as well as in assessing response to treatment. In older patients, abdominal ultrasound and chest X-ray should be considered instead of CT [8];
- virological tests [HBs antigen, anti-HBc total, anti-hepatitis C virus (HCV), anti-human immunodeficiency virus (HIV) antibodies].

It is also advisable to perform other tests useful for assessing the risk of an unfavorable course of disease, including:

- cytogenetics (FISH) for del17p and molecular tests for *TP53* mutation (in absence of del17p): at least exons 4–10, recommended 2–11; <6 months before starting each line of treatment [8];
- IGVH mutation status [7, 8] before initiation of first line of treatment;
- serological markers: β_2-microglobulin, lactate dehydrogenase (LDH).

Treatment

Antileukemic drugs used in CLL

Alkylating agents

Chlorambucil, the drug with the longest history in CLL, allows for the reduction or resolution of symptoms in 30–70% of patients, but complete remission (CR) is observed only rarely (2–10%). Chlorambucil is used in various schedules (Table III). In British studies, the highest response rate and the longest PFS were observed with the use of chlorambucil at 10 mg/m^2 from days 1–7 of a 28-day cycle (Table III) [36]. Currently, chlorambucil monotherapy is used rarely, and only in patients whose old age and/or comorbidities do not allow the use of immunochemotherapy.

Purine analogs

Purine analogs (fludarabine, cladribine, pentostatin) are a group of cytostatics with the most pronounced therapeutic activity in CLL. However, they induce numerous adverse effects, including hematological complications (neutropenia, thrombocytopenia, anemia), autoimmune hemolytic anemia, increased incidence of infections, including opportunistic [*Pneumocystis jiroveci,* cytomegalovirus (CMV), varicella zoster virus (VZV)] associated with myelosuppressive and immunosuppressive effects, and an increased risk of secondary tumors, including hematopoietic malignancies (mainly acute myeloid leukemia and myelodysplastic syndrome). The risk of serious adverse events is greater in the elderly due to slower renal excretion of the fludarabine metabolites. The incidence of autoimmune complications

Table III. Selected treatment protocols used in patients with chronic lymphocytic leukemia

Protocol/drug	Dose	Administration route	Days	Notes	References
Chlorambucil	0.1 mg/kg bw		Continuous infusion	28-day cycles	[36]
	0.4–0.8 mg/bw	Oral	1 and 15	28-day cycles	
	10 mg/m^2		1–7	28-day cycles	
	40 mg/m^2		1	28-day cycles	
FCR				28-day cycles	[17]
F	25 mg/m^2; 40 mg/m^2	i.v./oral	1–3		
CY	250 mg/m^2	i.v., oral	1–3		
R	375 mg/m^2 (cycle 1)	i.v.	1		
	500 mg/m^2 (cycles 2–6)		1		
BR				28-day cycles	[37, 38]
B	90 (70)* mg/m^2	i.v.	1–2		
R	375 mg/m^2 (cycle 1)		1		
	500 mg/m^2 (cycles 2–6)				
Chlorambucil + rituximab					[39]
Chlorambucil	0.5 mg/kg bw or 10 mg/m^2	Oral	1, 15	28-day, up to six cycles	
	375 mg/m^2 (cycle 1)	Oral	1		
Rituximab	500 mg/m^2 (cycles 2–6)	i.v.	1		
Chlorambucil + obinutuzumab					[21]
Chlorambucil	0.5 mg/kg bw	Oral	1, 15	28-day, up to six cycles	
Obinutuzumab	1,000 mg	i.v.	1, 8, 15 (cycle 1)	One infusion over two days	
			1 (cycles 2–6)		
Ibrutinib	420 mg/day	Oral	Continuous treatment	Until progression or unacceptable toxicity	[40]
Venetoclax	400 mg after a 5 week titration period 20–400 mg	Oral	Continuous treatment	Until progression or unacceptable toxicity	[41]
Venetoclax + rituximab					[34]
Venetoclax	400 mg after the titration period 20–400 mg	Oral	24 months		
Rituximab	375 mg/m^2 (D1, C1)	i.v.	6 cycles		
	500 mg/m^2 (D1, C2–C6) every four weeks after end of titration period				
Venetoclax + obinutuzumab					[21]
Venetoclax	400 mg after a 5 week titration period 20–400 mg	Oral	12 cycles (28 days each), starting from day 1 of day 1 of cycle 1 of administration of obinutuzumab		
Obinutuzumab	1,000 mg every four weekns after end of titration period	i.v.	1, 8, 15 (cycle 1)		
			1 (cycles 2–6)		
Acalabrutinib	100 mg twice/day	Oral	Continuous treatment	Until progression or unacceptable toxicity	[42]
Ibrutinib + venetoclax					[43]
Ibrutinib	420 mg	Oral	15 months (1–15)		
Venetoclax	400 mg after the titration period 20–400 mg	Oral	12 months. (4–15)		
Zanubrutinib	320 mg/day or 160 mg twice/day	Oral	Continuous treatment	Until progression or unacceptable toxicity	[44]

*Treatment of relapsed disease; bw — body weight; B — bendamustine; FCR — fludarabine, cyclophosphamide, rituximab; i.v. — intravenous; BR — bendamustine, rituximab

is significantly lower when purine analogs are used in combination with cyclophosphamide and rituximab compared to monotherapy [15, 35, 36]. Fludarabine should not be used in patients with creatinine clearance <30 mL/min, and a dose reduction of 50% is indicated when the clearance is <70 mL/min. Particular attention should be paid to recurrent infections due to the strong immunosuppressive effect of fludarabine and poor functioning of the immune system in the elderly.

Bendamustine

Bendamustine is a cytostatic drug combining the properties of alkylating compounds and purine analogs. It is now widely used in the treatment of lymphoproliferative neoplasms, usually in combination with rituximab. The most important side effects of bendamustine are myelosuppression, infections, nausea, vomiting, and skin lesions. The hematological toxicity of bendamustine is greater than that of chlorambucil, but less than that of purine analogs. Bendamustine, unlike fludarabine, can be used in full doses in patients with renal failure. Modification of bendamustine dose is recommended only in cases of severe kidney disease (creatinine clearance <10 mL/min).

Immunochemotherapy

FCR (fludarabine, cyclophosphamide, rituximab)

FCR immunochemotherapy helps achieve significantly higher response rates and prolongation of PFS and OS compared to FC chemotherapy in younger patients, in good general condition, without significant diseases [CLL8 clinical trial (Table IV)] [17]. However, the FCR regimen is associated with significant toxicity, in particular with regard to cytopenias and infections. In the light of the European Organization for Research and Treatment of Cancer (EORTC) recommendations of 2011, and the NCCN recommendations, FCR is one of the regimens in which the risk of febrile neutropenia is more than 20%, which is an indication for primary prevention with granulopoiesis-stimulating factors [52, 53].

Immunochemotherapy is ineffective in patients with del17p/*TP53* mutation [17]. The results of FCR immunochemotherapy treatment are significantly worse also in patients with unmutated IGHV gene mutation status: the PFS rate is 33.1% compared to 66.6% of patients with IGHV mutation, in whom the median OS remained unreached [26]. The results of the CLL13 study show that in the group of patients without comorbidities, treatment with venetoclax in combination with obinutuzumab ± ibrutinib is characterized by greater effectiveness and lower toxicity compared to FCR immunochemotherapy [47].

Bendamustine and rituximab

A combination of bendamustine and rituximab (BR) allows for high response rates in both relapsed/refractory CLL and first-line treatment [37, 54]. The German group CLL10 has shown that FCR is more effective in inducing complete remissions (CR), and results in longer PFS (Table IV) and eradication of MRD in the first-line treatment [38]. In patients >65 years, the efficacy of both regimens in terms of PFS was comparable. The FCR regimen was significantly more toxic, including hematological toxicity (90% vs. 67%), severe neutropenia (84% vs. 59%) and infections (39% vs. 25%), especially in elderly patients. In patients treated with the BR regimen, routine primary prophylaxis of febrile neutropenia is not recommended, although it should be considered, especially when using the BR regimen in patients with relapsed/refractory CLL.

Chlorambucil in combination with anti-CD20 monoclonal antibodies

The results of the CLL11 study demonstrated that chlorambucil in combination with obinutuzumab is more effective than chlorambucil and rituximab in terms of CR, PFS, OS and MRD eradication [39, 55]. This regimen is currently rarely used due to the significantly greater effectiveness of venetoclax in combination with obinutuzumab (study CLL14) [21].

B-cell receptor signaling inhibitors

Inhibitors of BCR signaling approved in the European Union (EU) for the treatment of CLL include the BTK inhibitors ibrutinib, acalabrutinib and zanubrutinib, and δ isoform of phosphatidylinositol-3 kinase (PI3Kδ) — idelalisib and umbralisib. The summary of product characteristics (SmPC) indications for BTK inhibitors includes both first line and refractory/relapsed CLL treatment. The efficacy of ibrutinib in patients with relapsed/refractory CLL was assessed in a phase Ib/II study (PCYC-1102) [40] and a randomized phase III study (RESONATE) in which ofatumumab was used in the control arm (Table IV) [48]. The response rate in the PCYC-1102 study was 88%, including 2% CR, 68% partial remission (PR), and 18% partial response with lymphocytosis (PR-L). The response rates were similar regardless of the presence or absence of del17p/*TP53* mutation [48]. The median PFS was 52 months, and the OS rate after 7 years of follow-up was 55% [56]. In the RESONATE study, patients treated with ibrutinib had a very significantly higher response rate (63% vs. 4%, p <0.001) and a significantly longer PFS (44.1 vs. 8.1 months, p <0.001) [48]. An update of the RESONATE study results shows that the benefits of ibrutinib are maintained, and the risk of progression is reduced by 89%, compared to ofatumumab treatment. Median progression-free survival was significantly longer in patients randomized to the ibrutinib arm compared to ofatumumab (44.1 vs. 8.1 months). The benefits of ibrutinib versus ofatumumab were maintained in the high-risk population with del17p, *TP53* mutation, del11q and/or unmutated *IGVH* genes. Overall survival, censored for crossover, was longer on ibrutinib

Table IV. Selected phase III clinical trials in treatment of chronic lymphocytic leukemia

Study	Protocol	Number of participants	Median age	ORR [%]	CR [%]	PFS (months)	OS (months)	Reference
CLL8	FC	409	61	80	22	33	86	[17, 26]
Hallek (2010)	FCR	408	61	90*	44*	52*	NA* (after 6 years)	[38]
CLL10	FCR	282	62	95	40	55.2	91%	[39]
Eichhorst (2016)	BR	279	61	96	31* No difference in patients aged >65 years	41.7* No difference in patients aged >65 years	92% (after 3 years)	
CLL11	Chl	118	72	31.4*	0*	11.1*	ND	[39]
Goede (2014)	Rituximab + Chl	233	73	65.7*	7.3*	16.3*	73.1%	
	Obinutuzumab + Chl	238	74	77.7*	22.3*	26.7*	NA*	
RESONATE-2	Chl	133	73	37	2	15*	68%	[27]
Burger (2015)	Ibrutinib	136	72	92*	30	NA*	83% (after 5 years)	
ECOG1219	FCR	175	56.7	81.1	30.3	72.9%	91.5%	[30]
	Ibrutinib + rituximab	354	56.7	95.8*	17.2*	89.4%* (after 3 years)	98.8% (after 3 years)	
ALLIANCE	BR	183	70	81	26	74%	95%	[28]
	Ibrutinib	182	70	93	7	87%	90%	
	Ibrutinib + rituximab	182	71	94	12	88% (after 2 years)	94% (after 2 years)	
ILLUMINATE	Chl + obinutuzumab	116	72	88	8	19	86%	[29]
	Ibrutinib + obinutuzumab	113	70	73	19*	NA*	85% (after 30 months)	
CLL14	Obinutuzumab + Chl	216	72	71.3	23.1	35.4%	83.1	[21]
	Venetoclax + obinutuzumab	216	72	84.7	49.5	74% (after 48 months)	85.3 (after 48 months)	
ELEVATE TN	Obinutuzumab + Chl	177	71	79	5	22.6	92	[45]
	Acalabrutinib	179	71	86	1	NA	95	
	Acalabrutinib + obinutuzumab	179	71	94	13	NA 93 vs. 87 vs. 47 (after 24 months)	95 (after 24 months)	

→

Table IV (cont.). Selected phase III clinical trials in treatment of chronic lymphocytic leukemia

Study	Protocol	Number of participants	Median age	ORR [%]	CR [%]	PFS (months)	OS (months)	Reference
GLOW	Obinutuzumab + Chl	105	71	84.8	11.4	21 months (44.1% afters 24 months)	ND	[46]
	Ibrutinib + venetoclax	106	71	86.8	38.7	NA (84.4% after 25 months)	ND	
CLL 13 (GAIA)	FCR/BR	229	61	80.8	31	75.5% (after 3 years)	87.4% (after 3 years)	[47]
	VenR	237	62	93.3	49.4	80.8%	93%	
	VenG	229	62	96.1	56.8	87.7%	92.4%	
	VenGI	231	60	94.4	61.9	90.5%	98.4%	
SEQUOIA	Zanubrutinib	241	70	94.6	7%	85.6% (after 24 months)	94.3% (after 24 months)	[44]
	BR	238	70	85.3	15%	69.5%	94.6%	
RESONATE	Ofatumumab	196	67	4	0	8.1	65.1	[48, 49]
	Ibrutynib	195	67	91	11	44.1*	67.7*	
MURANO	BR	195	65	72.3	3.6	17	62.2	[34, 35]
	VenR	194	65	92.3*	8.2*	53.6* 84.9 36.3 (after 24 months)	82.1 (after 5 years)	
ASCEND	Investigator's choice**	155	68	81	ND	88	91	[42]
	Acalabrutinib	155	67	75	ND	68 (after 12 months)	94	
ELEVATE RR	Ibrutinib	265	65	77	ND	38.4	After 40.9 months 62.5%	[50]
	Acalabrutinib	268	66	81	ND	38.4	66.5%	
ALPINE	Ibrutinib	325	68	75.7	ND	34.2	NA	[51]
	Zanubrutinib	327	67	86.2	ND	NA	NA	

*Statistically significant difference, **BR — 36 patients, idelalisib + rituximab — 119 patients; ORR — overall response rate; CR — complete remission; PFS — progression-free survival; OS — overall survival; FC — fludarabine, cyclophosphamide; FCR — fludarabine, cyclophosphamide, rituximab; NA — not achived; BR — bendamustine, rituximab; Chl — chlorambucil; ND — no data; R — rituximab; VenR — venetoclax, rituximab; VenG — venetoclax, obinutuzumab; VenGI — venetoclax, obinutuzumab, imatinib

than ofatumumab [49]. The efficacy of ibrutinib was analyzed in patients with relapsed/refractory CLL progressing on their last treatment with venetoclax. Median PFS and OS after initiation of BTK inhibitors treatment were 34 and 42 months, respectively. BTK inhibitors (ibrutinib, n = 21; zanubrutinib, n = 2) have brought lasting benefits in patients with the *Gly101Val* mutation associated with resistance to venetoclax [57].

The efficacy of ibrutinib in first-line treatment was assessed in RESONATE-2, a randomized, phase III trial

performed in a population of patients aged ≥65. Ibrutinib was shown to be significantly more effective in terms of response rates, PFS, and OS compared to chlorambucil, regardless of the presence of del17p/*TP53* mutation and IGVH mutation status (Table IV) [58]. Moreover, a significant improvement in hematological parameters (anemia, thrombocytopenia) was observed more frequently in patients treated with ibrutinib [58]. In subsequent phase III clinical trials, ibrutinib regimens were compared to first line immunochemotherapy regimens. In the iLLUMINATE study, patients aged 65 or younger with comorbidities were treated with ibrutinib and obinutuzumab versus chlorambucil and obinutuzumab. The response rates (ORR, CR, MRD negativity) were significantly higher (91%, 41%, and 35% vs. 81%, 16%, and 25%, respectively), and median PFS (with a median follow-up of 45 months) was significantly longer, in patients treated with ibrutinib (not achieved after 19 months) irrespective of risk factors (del17p/*TP53* mutation, IGVH mutation status). There was no difference in PFS between patients with and without del17p/*TP53* mutation (77% vs. 74%) after 48 months of follow-up. Treatment tolerance was good, with no new safety data in the final analysis [29, 59]. In the E1912 trial, patients up to the age of 70 received first-line treatment of ibrutinib and rituximab or FCR immunochemotherapy. Both PFS and 3-year OS were significantly longer in patients treated with ibrutinib (89.4% vs. 72.9%, p <0.001; 98.8% vs. 91.5%, p <0.001), but subgroup analysis showed that that the real benefit of ibrutinib treatment was achieved by patients with unmutated *IGVH*. 3-year PFS in the group with mutated *IGVH* treated with ibrutinib was 87.7% compared to 88% in FCR-treated patients. In patients with unmutated *IGVH*, 3-year PFS was 90.7% versus 62.5%, respectively [30]. Long-term follow-up of patients participating in the E1912 study (median 6 years after randomization) showed that patients with both unmutated and mutated IGHV gene status benefitted in terms of PFS, and 60% of patients continued ibrutinib treatment. Treatment tolerance was good, with an atrial fibrillation rate of 4.5% [30, 60]. In the third study conducted by the ALLIANCE group, patients aged 65 or older received ibrutinib monotherapy, or ibrutinib in combination with rituximab, or the BR regimen as first-line treatment. After 55 months of follow-up, median PFS was not achieved in the ibrutinib arms, and was 44 months in the BR arm [61]. The PFS rate after 48 months was significantly higher in patients treated with ibrutinib regimens (76%, 76% and 47%), and no PFS benefit was demonstrated by adding rituximab to ibrutinib. Patients with del17p particularly benefited from ibrutinib treatment [28].

Ibrutinib is well tolerated. Most of the adverse reactions in clinical trials have been described as grades 1–2. The most common adverse effects are diarrhea, fatigue, muscle and joint pain, infections, bleeding complications, hypertension, and atrial fibrillation. In January 2020, acalabrutinib, a selective irreversible BTK inhibitor, was registered by the European Medicines Agency (EMA) for both first-line treatment (in monotherapy or in combination with obinutuzumab) and in patients who had received at least one previous therapy (in monotherapy).

In the ASCEND study, the efficacy and safety of acalabrutinib in the treatment of patients with relapsed/refractory CLL who had not previously received BTK and BCR inhibitors was compared to an investigator's choice therapy (BR or idelalisib and rituximab). The median PFS was significantly longer with acalabrutinib monotherapy (not reached) compared to the investigator's choice therapy (16.5 months, p <0.0001). The estimated 12-month PFS rate was 88% for acalabrutinib and 68% for the investigator's choice therapy [42].

In the ELEVATE TN study, acalabrutinib, or acalabrutinib in combination with obinutuzumab, was used in the first line for CLL patients aged ≥65 with a creatinine clearance of between 30 and 69 mL/min or co-morbidities [younger with a ClCr between 30 and 69 mL/min or disease comorbidities (Cumulative Illness Rating Scale {CIRS}) score >6]. A control group received obinutuzumab and chlorambucil. Median PFS was significantly longer in patients treated with acalabrutinib-based regimens (not achieved vs. 22.6 months, p <0.001). The estimated 2-year PFS rate was 93%, 87%, and 43%, respectively [45]. After 6 years of follow-up, median PFS was significantly longer in patients treated with acalabrutinib (not achieved vs. 27.8 months) regardless of risk factors (del17p/*TP53* mutation, IGVH mutation status). The efficacy of the drug was similar in patients with mutated and non-mutated IGVH mutation status, del17p/*TP53* mutation [45, 62]. The treatment was well tolerated. Most adverse reactions observed in clinical trials were grades 1–2. The most common adverse effects of acalabrutinib are headache, diarrhea, fatigue, nausea, and bleeding complications. The most common grade 4 adverse reactions are neutropenia, anemia, pneumonia, and thrombocytopenia. In the ASCEND study, serious adverse events occurred in 29% of patients (44 of 154) treated with acalabrutinib monotherapy, 56% (66 of 118) in the IR group, and 26% (9 of 35) in the BR group [42]. A phase III randomized trial ELEVATE RR directly head-to-head comparing acalabrutinib with ibrutinib in previously treated CLL patients showed similar efficacy of both drugs. Acalabrutinib was, however, better tolerated [50]. The incidence of any grade atrial fibrillation/atrial flutter was significantly lower with acalabrutinib compared to ibrutinib (9.4% vs. 16.0%; p = 0.02); among other selected secondary endpoints, grade 3 or higher infections (30.8% vs. 30.0%) and Richter's transformations (RT) (3.8% vs. 4.9%) were comparable between groups. Treatment discontinuation due to adverse events occurred in 14.7% of acalabrutinib-treated patients and in 21.3% of ibrutinib-treated patients [50].

Another BTK inhibitor that has been approved by the EMA is zanubrutinib. In the phase III SEQUOIA clinical trial, zanubrutinib (arm A) or BR regimen (arm B) was used as first-line treatment in patients without del17p. Median PFS was significantly longer in patients treated with zanubrutinib (not achieved vs. 28 months). The most common grade 3 or higher adverse event with zanubrutinib was neutropenia (11%), while the incidence of atrial fibrillation was less than 5%. The drug's effectiveness was similar in patients with mutated and unmutated IGHV mutation status. In arm C of the study (patients with del17p), median PFS was not achieved after 30 months of follow-up, and the percentage of patients without progression was 88.9% after 24 months of follow-up [44].

In the phase III ALPINE trial, two BTK inhibitors, ibrutinib versus zanubrutinib, were head-to-head compared in the treatment of relapsed or refractory CLL. After a median follow-up of 29.6 months, zanubrutinib was found to be superior to ibrutinib in terms of PFS among 652 patients. At 24 months, PFS was 78.4% in the zanubrutinib group and 65.9% in the ibrutinib group. Interestingly, among patients with del17p and/or *TP53* mutation, zanubrutinib also showed greater efficacy than ibrutinib in relation to the PFS. The safety profile of zanubrutinib was better than that of ibrutinib, with fewer adverse events leading to treatment discontinuation and fewer cardiac events, including fewer cardiac events leading to treatment discontinuation or death [51].

New BTK inhibitors in advanced clinical trials include pirtobrutinib and nemtabrutinib, which bind to BTK in a reversible and non-covalent manner. In the phase II BRUIN clinical trial, 82% of patients who had previously received treatment with another BTK inhibitor responded. The treatment was effective in patients with the BTK C481 mutation (associated with ibrutinib resistance) and was well tolerated [63].

BCL2 antagonists

Venetoclax is an oral, selective inhibitor of BCL2, the only drug in this group approved for the treatment of CLL. The current indication, according to the EMA registration, is first-line treatment in combination with obinutuzumab and for the treatment of relapsed/refractory CLL either alone or in combination with rituximab. Venetoclax alone enables 79% response rates in relapsed CLL [41]. Complete remission was observed in 20% of patients, and in 5% very deep responses with negative MRD. Venetoclax in monotherapy is used continuously, while in combination with monoclonal antibodies and BTK inhibitors, the therapy is administered for a limited time only. A venetoclax and rituximab (VenR) regimen was approved based on the results of the MURANO phase III clinical trial, in which venetoclax was administered together with rituximab (six doses) for two years, and the efficacy was compared to bendamustine and rituximab. The reduction in the risk of progression was 81% and the risk of death was 60% in patients treated with VenR compared to BR [34]. The median time to progression and time to the next treatment were 53.6 and 57.8 months in patients receiving venetoclax plus rituximab, and 17 and 23.9 months in the BR arm, respectively (Table IV) [34]. Undetectable MRD was achieved in as many as 63.8% of patients treated with VenR. An update of the results of the MURANO study after five years of follow-up, presented at the American Society of Hematology (ASH) meeting in 2020, showed that the benefits were maintained for PFS (57.3% and 4.6%) and OS (85.3% vs. 66.8%), despite using new targeted therapies in patients treated according to the BR regimen in subsequent lines of treatment. Particularly long responses were observed in patients who achieved MRD negativity after completing a VenR regimen [35].

An earlier study had proven the efficacy of venetoclax monotherapy in CLL patients with del17p. For all patients, the objective response rate was 77% and the estimated progression-free survival after 24 months was 54%. For 16 patients who had previously received kinase inhibitors, the objective response rate was 63% (10/16 patients) and the estimated 24-month PFS was 50% [20].

The efficacy of venetoclax has been assessed in patients receiving ibrutinib in their previous therapy. In total, 59/91 (65%) patients responded to treatment with venetoclax [64].

In the CLL14 study, venetoclax in combination with obinutuzumab was used in the first-line treatment in patients with comorbidities. Obinutuzumab in combination with chlorambucil was administered in the control arm. Treatment duration for both regimens was 12 months. At 24 months after randomization, the PFS rate was significantly higher in patients treated with the venetoclax-containing regimen (88.2% vs. 64.1%) (Table IV). A benefit in terms of PFS was also observed in patients with del17p and unmutated IGVH [21, 65].

The CLL13 study evaluated new chemotherapy-free and time-limited combination treatment regimens with venetoclax in patients eligible for intensive therapy [66]. The efficacy and safety of three regimens: venetoclax + + rituximab, venetoclax + obinutuzumab, and venetoclax + + obinutuzumab + ibrutinib were compared to FCR/BR regimens. The results of this study showed a higher percentage of patients with undetectable MRD and longer PFS in patients treated with venetoclax + obinutuzumab and venetoclax + obinutuzumab + ibrutinib regimens compared to immunochemotherapy [66]. The toxicity of immunochemotherapy was higher in terms of infectious complications and secondary malignancies.

In two clinical trials, a combination of venetoclax and ibrutinib was used in the first-line treatment of CLL. In the phase II CAPTIVATE trial, in the group of CLL patients eligible for intensive treatment, patients were divided into two

Table V. Tumor lysis syndrome risk assessment and pre-treatment prophylaxis (source [46])

Tumor lysis syndrome risk assessment		
Low risk	**Medium risk**	**High risk***
Enlarged lymph nodes <5 cm and peripheral blood lymphocyte count <25 G/L	Lymph nodes ≥5 cm and <10 cm or peripheral blood lymphocyte count ≥25 G/L	Lymph nodes >10 cm (in imaging) or peripheral blood lymphocyte count ≥25 G/L and lymph nodes ≥5 cm
Prophylaxis of tumor lysis syndrome		
Allopurinol 300–600 mg orally from 72 h before starting treatment Hydration 1.5 L orally from 48 h prior to treatment	Allopurinol 300–600 mg orally from 72 h before starting treatment Hydration 2–3 L orally from 24 h before start of treatment and consider intravenously during hospitalization	Allopurinol 300–600 mg orally from 72 h before starting treatment Hydration 2–3 L orally from 24 h before start of treatment and intravenously during hospitalization Rasburicase 0.05–0.2 mg/kw bw (depending on local procedures, necessary in patients with uric acid level >8.0 mg/dL)

*An additional risk factor for tumor lysis syndrome is renal failure with creatinine clearance <80 mL/min

cohorts. In both cases, ibrutinib monotherapy was used for the first three months, followed by combined treatment with ibrutinib and venetoclax for 12 months. In the first cohort ('FD, fixed-duration, cohort'), treatment was completed after 15 months. In the second cohort, ('MRD cohort'), the further course of treatment depended on the MRD assessment after 15 months of treatment. Patients with undetectable MRD were randomized to one of two groups: ibrutinib or a placebo, and patients with current MRD were randomized to treatment with venetoclax and ibrutinib, or ibrutinib alone. In the FD cohort, the post-treatment CR rate was 56% and the post-treatment PFS and OS rates were 95% and 98%, respectively. The uMRD rates during 27.9 months of follow-up reached 77% in peripheral blood and 60% in bone marrow [67]. Fixed-term treatment allowed a deep and lasting response to be achieved even in patients with a high genetic risk [43].

The phase III GLOW clinical trial included patients over 65 or under 65 with a CIRS score greater than 6 and/or creatinine clearance of less than 70 mL/min. The treatment included ibrutinib + venetoclax (three cycles of ibrutinib, then 12 cycles of ibrutinib and venetoclax) or chlorambucil and obinutuzumab (six cycles). After a median follow-up of 27.7 months, PFS was significantly longer in patients treated with IbrVen. The proportion of patients with undetectable MRD 3–12 months after treatment completion was 84.5% versus 29.3%. The most common adverse event in both arms was neutropenia: 34.9% and 49.5% [68].

The most common side effects of venetoclax are neutropenia, diarrhea, nausea, anemia, upper respiratory tract infection, thrombocytopenia and fatigue. Serious complications can include pneumonia, febrile neutropenia, hemolytic anemia, and metabolic disorders associated with TLS.

Table VI. Biochemical markers of tumor lysis syndrome (to make a diagnosis, ≥2 criteria must be met)

Parameter	Value	Change after treatment
Uric acid	>8 mg/dL	>25%
Potassium	>6 mg/dL	>25%
Inorganic phosphates	>1.45 mmol/L	>25%
Calcium	<1.75 mmol/L	>25%

In all patients, the risk of tumor lysis should be assessed, and appropriate prophylaxis and treatment should be applied if laboratory or clinical symptoms of TLS appear (Tables V, VI) [46].

Regimens used to treat patients with CLL and the results of phase III clinical trials regarding currently used regimens are set out in Tables III and IV.

Cellular immunotherapy

Allogeneic hematopoietic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is used much less often in the era of targeted therapies, but it remains the only method that can be used with intention to cure. However, because of the serious complications associated with this procedure, it is only recommended for high-risk patients. The introduction of new drugs has changed the site of allogeneic transplantation in the treatment of CLL. Currently, allo-HSCT is indicated in high-risk disease and after treatment failure with at least one BCR pathway inhibitor or a BCL2 antagonist [69, 70]. The decision should be made on an individual basis,

and patients with high-risk disease after novel BCR and BCL2 inhibitors failure should be carefully analyzed for alternative treatment options, risk of RT, complications, or transplant failure. A phase II study by a German group showed a 4-year survival rate of 65%, with no differences in the presence of negative cytogenetic prognosis or in patients refractory to previous treatment [71]. Similar results were obtained by other transplant groups, indicating a plateau of survival curves at a 40–50% level. Reduced-intensity conditioning protocols used by an American group resulted in 3-year survival in 59% of patients [72]. Long-term European Society for Blood and Marrow Transplantation (EBMT) analyses showed that 10-year event-free survival (EFS), OS, and non-relapse mortality (NRM) after allo-HSCT were 28%, 35%, and 40%, respectively [73].

New therapies in clinical and pre-clinical trials

The use of chimeric antigen receptor (CAR) T-cells is currently the most promising and dynamically developing cell therapy modality. Numerous CAR-T constructs are currently being evaluated in clinical trials that are at various stages of advancement, showing promising results in terms of therapeutic efficacy. In one long-term follow-up study, median PFS was 40.2 months in patients who achieved CR and did not reach median OS [74]. The addition of ibrutinib resulted in improved CAR-T efficacy in CLL patients. Novel BCL2 inhibitors (sonrotoclax, lisaftoclax), bispecific antibodies, BTK degraders, MDM2 antagonists (RG7112, RG7388), XPO1 inhibitors, and ATR inhibitors are being evaluated in clinical and preclinical studies.

First-line treatment

Currently there are three treatment strategies employed in first-line settings:

- continuous administration of targeted drugs: ibrutinib, acalabrutinib, zanubrutinib (reimbursement in Poland from January 2024);
- time-limited chemotherapy-free regimens: venetoclax + obinutuzumab, venetoclax + ibrutinib (not yet reimbursed in Poland; recommendations were being drawn up in December 2023);
- time-limited immunochemotherapy with anti-CD20 monoclonal antibodies.

Factors influencing choice of first-line treatment

The fundamental factors that should be taken into account when choosing the type of first-line treatment are genetic disorders with an unfavorable prognosis, del17p and/or *TP53* mutation, and the mutation status of the IGHV genes. Additionally, comorbidities, age, physical performance status [according to the Eastern Cooperative Oncology Group (ECOG), Karnofsky scales] and susceptibility to infections should be taken into account.

CIRS is the most widely used tool to assess comorbidities. It involves the evaluation of 14 organs/systems using a 5-point scale, where zero means disease-free/ /normal organ function and four points mean a life-threatening condition [75, 76]. However, the importance of this scale in the choice of targeted therapy is less than that of immunochemotherapy. When choosing a therapeutic option, the patient's preferences should also be considered, after a detailed presentation of the potential benefits and side effects, the route of administration, and the need for hospitalization related to the given treatment method.

The presence of del17p/*TP53* mutation, correlated with resistance to alkylating drugs and purine analogs, and the unmutated state of IGHV genes, is associated with a short duration of response to immunochemotherapy. Tests should be performed before starting first-line treatment towards del17p, *TP53* mutations and IGHV gene mutation status.

When choosing between a time-limited treatment (in Poland in December 2023, the reimbursed regimen is venetoclax + obinutuzumab) and the continuous administration of BTK inhibitors, the following factors should be considered: toxicity profile (renal function and risk of TLS vs. atrial fibrillation and risk of bleeding); the administration route [intravenously (i.v.) + oral (*p.o., per os*) vs. only oral]; the frequency of follow-up visits (5-week period of increasing the dose of venetoclax); and patient preference [8].

Patients without del17p/*TP53* mutation and with mutated IGVH

Patients in good general condition without significant comorbidities

Patients in good general condition, without significant comorbidities and with normal kidney function, are currently the only patients in whom FCR immunochemotherapy is still considered an effective treatment method (Figure 1) [8]. Due to the results of the CLL13 and CAPTIVATE studies, the NCCN, German and French guidelines no longer recommend immunotherapy as first-line therapy for this group of patients. Similarly, in accordance with the PALG-PTHiT guidelines, in the treatment of this group of patients, treatment without immunochemotherapy should be considered first, i.e. venetoclax with obinutuzumab (based on the CLL13 study), or venetoclax and ibrutinib (based on the results of the CAPTIVATE study). However, this study did not show any differences in the survival time of patients treated with venetoclax and obinutuzumab as opposed to venetoclax and rituximab. Alternatives may be ibrutinib, acalabrutinib (in Poland not reimbursed in the drug program in this group of patients), or the venetoclax and obinutuzumab regimens. FCR immunochemotherapy is also a treatment option.

Patients with comorbidities not qualified for intensive immunochemotherapy

In patients not eligible for intensive immunochemotherapy, the currently recommended treatment standards are venetoclax combined with obinutuzumab, or BTK inhibitors: ibrutinib, acalabrutinib, zanubrutinib [8, 77]. Currently, in Poland, only the regimens of venetoclax and obinutuzumab, and of obinutuzumab and chlorambucil are reimbursed under the drug program. The latter regimen is currently rarely used due to the much greater efficacy of venetoclax in combination with obinutuzumab. From January 2024, zanubrutinib will be reimbursed for patients in this group.

In patients of very advanced age, in poor general condition, when the use of i.v. drugs is impossible, monotherapy with chlorambucil or cyclophosphamide may be used.

Patients without 17p deletion/*TP53* mutation with unmutated IGHV gene status

Patients in good general condition with no significant comorbidities

The recommended therapy for this group of patients is BTK inhibitors (ibrutinib, acalabrutinib, zanubrutinib) or venetoclax in combination with obinutuzumab or ibrutinib. Chemoimmunotherapy is not recommended due to poor survival rates. In Poland, treatment with BTK inhibitors as monotherapy: ibrutinib, acalabrutinib, zanubrutinib (this drug from January 2024 acc. to the rules indicated in the drug program) is reimbursed for this group of patients. A regimen of venetoclax and obinutuzumab will also be reimbursed from January 2024.

Patients in worse general condition with comorbidities

The recommended treatment regimen for this group of patients is venetoclax with obinutuzumab, ibrutinib, acalabrutinib, and zanubrutinib.

Currently, in Poland, the following are reimbursed for this group of patients: venetoclax and obinutuzumab, ibrutinib and acalabrutinib (under the B.79 drug program). From January 2024, zanubrutinib will also be reimbursed for patients in this group.

Patients with 17p deletion/*TP53* mutation

Patients with del17p/*TP53* mutation should not be treated with immunochemotherapy [8, 77]. BCR and BCL2 inhibitors are currently considered the most effective conventional regimens in patients with del17p/*TP53* mutation. The recommended first-line treatment regimens are BTK inhibitors. Venetoclax in combination with obinutuzumab, or venetoclax in monotherapy, could be alternatives. Idelalisib, according to the ESMO recommendation, can be used in the first line of CLL treatment in patients with del17p/*TP53* mutation who are ineligible for alternative treatments, and it is necessary to adhere to the recommendations to reduce the risk of infectious complications [8]. BTK inhibitors (ibrutinib, acalabrutinib, zanubrutinib — this drug from January 2024 acc. to the rules indicated in the drug program) are reimbursed in Poland. The current recommendations for selecting the first-line therapy are set out in Figure 2.

It should be underscored that when choosing first-line treatment (excluding patients with a del17p/mutation in the *TP53* gene), the type of therapy sometimes depends on the patient's preference and should be discussed with the patient, particularly when there is a high likelihood of non-adherence to therapy during long-term treatment with BTK inhibitors.

According to the current guidelines of international societies (NCCN, German guidelines, French guidelines), in all patients with CLL, regardless of genetic prognostic factors, the first-line therapy of choice is treatment without chemotherapy [77–79]. Currently, in Poland, targeted therapies registered in EU countries are not reimbursed to such a wide extent. In our recommendations for Polish hematologists, we take into account the availability of individual drugs in Poland, but we also present EMA registration indications.

Treatment of relapsed/refractory CLL

Indications for second and subsequent lines of treatment are the same as indications for first-line treatment. As in the case of the decision to start first-line treatment, also in patients with relapse, unfavorable prognostic biological features (LDH, β_2-microglobulins, chromosomal aberrations, unmutated IGHV gene status, *TP53* mutation) are not an indication to start treatment if the patient does not meet the above criteria demonstrating the progression of CLL. In the event of termination of treatment with a BTK inhibitor (e.g. due to side effects), it is not necessary to start another treatment immediately, especially if the leukemia is in remission. On the other hand, in a case of rapid progression during targeted therapies, an immediate change to another type of treatment is recommended.

Currently, in second- and subsequent-line treatment, the therapeutic decision depends to a greater or lesser extent on the duration of remission, the type of previous treatment, the presence of del17p/*TP53* mutations, the patient's general condition, any comorbidities, the patient's preference, and the availability of drugs.

According to the recommendations of international scientific societies, the optimal methods of treating patients with relapsed/refractory CLL are novel targeted therapies i.e. BCR and BCL2 inhibitors [8, 77].

One of two treatment options should be used:

1) venetoclax + rituximab (for 24 months); or
2) BTK inhibitors (as continuous treatment).

In a case of a relapse requiring therapy after first-line treatment according to the venetoclax + obinutuzumab

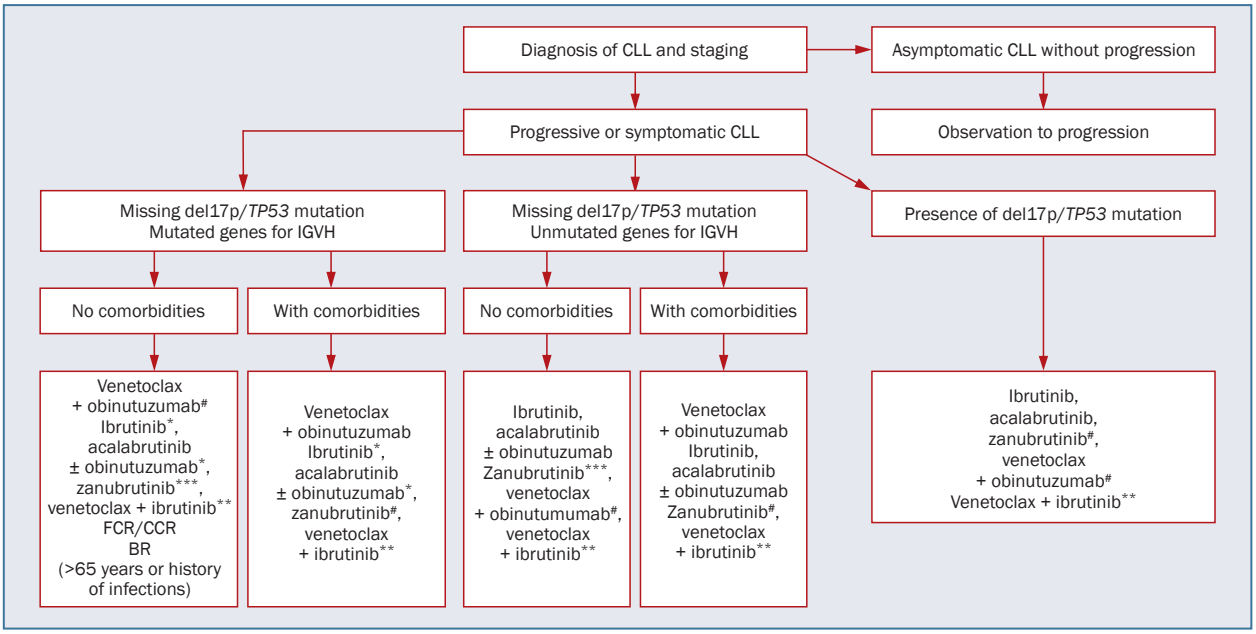

Figure 2. Recommendations for first-line treatment of patients with chronic lymphocytic leukemia (CLL) with indications to start therapy; #refund from January 2024; *not reimbursed in Poland for this indication; **not reimbursed in Poland; ***refund from January 2024, provided that: age ≥65 years or age 18–65 years and occurrence of in the last 2 years ≥1 severe infection (requiring hospitalisation or parenteral antibiotic therapy) or ≥3 infections (requiring oral antibiotic therapy) confirmed by the patient's medical records in the patient's medical records; order of Bruton tyrosine kinase (BTK) inhibitors by date of European Medicines Agency (EMA) registration. Note: Acalabrutinib is reimbursed in Poland only in monotherapy; BR — bendamustine, rituximab; CCR — cladribine, cyclophosphamide, rituximab; FCR — rituximab, fludarabine, cyclophosphamide; IGVH — immunoglobulin variable heavy chain

regimen, re-treatment with venetoclax + rituximab may be considered in cases of remission lasting 3+ years, or changing the therapy to a BTK inhibitor. If the time to symptomatic recurrence was shorter, and there are no contraindications to BTK therapy, the preferred choice will be a BTK inhibitor. When choosing another therapy after a BTK inhibitor, changing to another BTK inhibitor can be considered, especially in cases of intolerance, bearing in mind that data on the use of another BTK inhibitor after the previous one in the case of resistance indicates lower effectiveness than in the population of previously untreated patients. Another option will be therapy according to the venetoclax + rituximab regimen. However, there are no head-to-head comparisons indicating the choice of optimal therapy in the case of BTK inhibitor resistance i.e. switching to the venetoclax + rituximab regimen versus switching to another BTK inhibitor. Currently, studies are being conducted with new BTK inhibitors in this patient population.

A much less frequently used alternative is idelalisib in combination with rituximab (continuous treatment) or re-treatment with immunochemotherapy in patients with lack of del17p/*TP53* mutations and mutated IGHV gene status and if no other treatment options are available.

New targeted therapies should be used in patients with del17p or *TP53* mutation, regardless of the duration of response to first-line treatment:

- BTK inhibitors (ibrutinib, acalabrutinib, zanubrutinib);
- venetoclax in combination with rituximab or as monotherapy;
- idelalisib with rituximab.

In Poland, new targeted therapies are available under the B.79 drug program.

Ibrutinib, zanubrutinib (from January 2024) and venetoclax with rituximab are reimbursed in patients after one line of previous therapy, regardless of del17p/*TP53* mutation status.

Acalabrutinib may be used as part of the drug program in patients with resistant/relapsed CLL with del17p/*/TP53* mutation, and in patients with resistant/relapsed CLL who meet at least one of the following criteria:

- disease recurrence/progression after, or lack of response to, treatment with a regimen containing venetoclax in combination with an anti-CD20 antibody;
- medical contraindications to the use of a regimen containing venetoclax in combination with an anti-CD20 antibody (i.e. failure to meet the appropriate qualification criteria for therapy with venetoclax with an anti-CD20 antibody);
- toxicity not allowing continuation of treatment with venetoclax and anti-CD20 antibody.

The introduction of BCR inhibitors and BCL2 antagonists has significantly improved the treatment options for patients with refractory/relapsed CLL, and has changed the

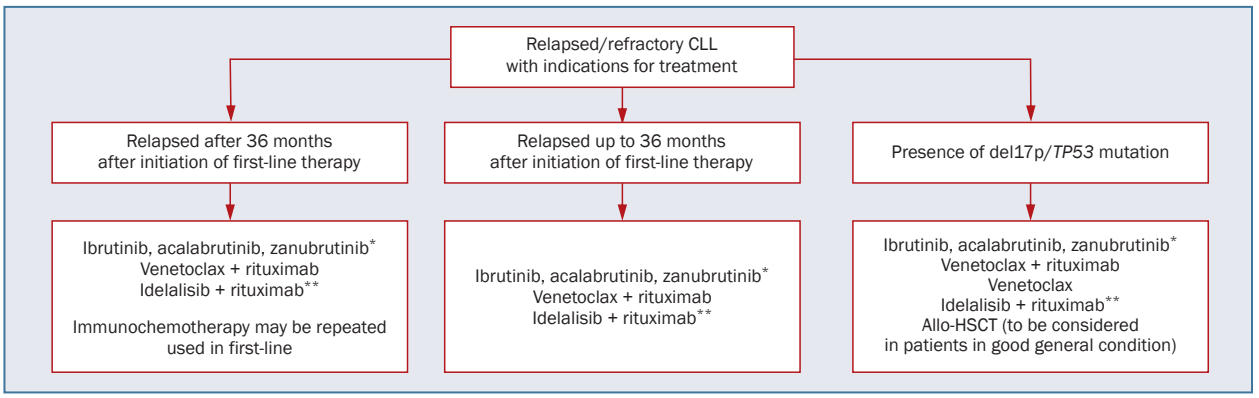

Figure 3. Recommendations for the treatment of patients with refractory or relapsed chronic lymphocytic leukemia (CLL); *refunded from January 2024; **not reimbursed in Poland; order of Bruton tyrosine kinase (BTK) inhibitors according to the date of registration with the European Medicines Agency (EMA); allo-HSCT — allogeneic hematopoietic stem cell transplantation

indications for allo-HSCT, which is currently recommended in these two clinical situations:

- resistance to new targeted therapies;
- RT clonally related to CLL after achieving remission after pharmacological treatment [8].

To summarize the available therapeutic options, the use of BCR and BCL2 inhibitors (in combination with rituximab or as monotherapy) should be considered in subsequent treatment lines).

In selected patients, especially those with a poor prognosis who are resistant to targeted therapies, allogeneic hematopoietic cell transplantation should be considered. Current recommendations regarding the selection of therapy in patients with refractory or relapsed CLL are set out in Figure 3.

Richter's transformation

RT is one of the most serious complications of CLL. RT is defined as the occurrence of secondary aggressive B-cell lymphoma in a patient diagnosed with CLL [80]. The most common histological subtype, accounting for 80–95% of all cases, is diffuse large B-cell lymphoma (DLBCL) [81]. The second and much less common form is transformation to classical Hodgkin's lymphoma (HL), often called the Hodgkin's lymphoma variant of Richter's transformation (HLvRT) [82]. This variant affects 5–15% of all cases of RT.

Despite the widespread belief, RT is neither a very rare nor a late complication. Based on many observational studies, RT occurs in up to 5–15% of patients with CLL. The median time from the diagnosis of CLL to the onset of RT is 2–4 years, and in rare cases both tumors are diagnosed simultaneously [81]. It should be emphasized that the percentage of patients with RT in a given center depends significantly on the frequency of surgical biopsies of lymph nodes in the event of rapid progression of CLL [83]. A more intensive biopsy strategy should be considered, especially in patients with risk factors for RT (Table VII).

Table VII. Risk factors associated with Richter's syndrome in course of chronic lymphocytic leukemia

Patient dependent factors
CD38 gene polymorphism
LPR-4 gene polymorphism
BCL2 gene polymorphism
Age (controversial)
Environmental factors
EBV reactivation (controversial)
Treatment with purine analogs (controversial)
Factors associated with leukemia biology
Karyotype (lack of del13q14)
Lack of IGHV mutation
Stereotyped BCR
Short telomeres
High expression of CD38
Clinical factors
Lymphadenopathy >3 cm
Stage of advancement according to Rai III/IV

BCR — B-cell receptor; EBV — Epstein-Bárr virus; IGVH — immunoglobulin variable heavy chain

The pathomechanism of RT has not been definitively elucidated, but the molecular basis has been quite well characterized [84–86]. Molecular analyses of a series of patients with RT revealed, among other things, a high frequency of defects in genes directly or indirectly regulating the course of the cell cycle, including *TP53*, NOTCH1 and CDKN2A/B [87]. Two types of transformation have been distinguished, and they are characterized by different clinical courses. In the first, RT occurs as a result of clonal evolution of CLL (this is known as RT 'clonally related to CLL'), while in the remaining patients the aggressive lymphoma comes from another lymphocytic clone (RT 'clonally unrelated to CLL').

RT clonally related to CLL is much more frequent (80–90%) and has a very unfavorable prognosis [84]. RT clonally unrelated to CLL occurs less frequently, but its prognosis is similar to DLBCL and *de novo* HL. In one analysis, the median survival of patients with RT clonally related to CLL was only 14 months, compared to 62 months in a group of patients with clonally unrelated RT [87].

Clinically, RT is usually characterized by a deterioration in the general condition, often with the appearance of systemic symptoms such as weight loss, fever, and night sweats, plus rapidly progressive local or generalized lymph node enlargement or, less frequently, extranodal lesions [81]. To diagnose RT, histopathological evaluation of a surgical specimen of the lymph node or the involved extranodal organ is required. Histopathological diagnosis is crucial in order to differentiate RT from similar clinical conditions such as progression of CLL and prolymphocytic transformation. It is recommended to take the node with the largest diameter or the one that is growing the fastest. PET/CT imaging may be of significant assistance, and the most metabolically active node should be sampled [88]. Exceptionally, if surgical biopsy of the node is impossible, the diagnosis can also be made by an experienced diagnostician based on cytological examination with cytometric immunophenotyping. After the diagnosis of RT, standard tests should be performed to assess its severity, similarly to primary DLBCL and HL. However, staging is difficult due to the impossibility of distinguishing nodal from organ lesions resulting from RT and CLL in imaging studies.

RT is most often characterized by an aggressive course, resistance to treatment, and short survival [81]. In the first line of treatment in patients with RT of the DLBCL type, the R-CHOP regimen is most often used, although the effectiveness of such treatment is not satisfactory [87]. Another option is the DA-EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab) regimen, but there is no direct evidence that it is more effective. The use of stronger chemotherapy regimens has been shown to allow for an increase in the rate and depth of response, but it was associated with significantly greater toxicity, and did not generally lead to an improvement in prognosis. In phase II studies, intensive OFAR-1, OFAR-2, R-hyper-CVAD regimens (rituximab, cyclophosphamide, vincristine, doxorubicin, dexamethasone) allowed CR to be achieved in 39–51% of patients, but median survival was only 6–10 months [89, 90]. New targeted therapies, which have resulted in a dramatic improvement in the prognosis of refractory/relapsed CLL, have not yet demonstrated a satisfactory rate of durable responses in patients with RT. However, clinical trials are still being conducted on the optimal use of monotherapy or combination therapy with drugs such as BTK inhibitors, PI3K inhibitors, BCL2 inhibitors (venetoclax) and programmed death receptor 1/programmed death-ligand 1 (PD-1/PD-L1) checkpoint inhibitors [91–93]. In a phase II clinical trial, Davis et al. used venetoclax in combination with DA-EPOCH-R, achieving CR in 50% of patients with a median PFS and OS of 10.1 and 19.6 months, respectively [94].

Initial trials of using CAR-T immunotherapy are also underway [95].

Due to the low incidence of RT, which makes it impossible to conduct randomized trials, no standard treatment has yet been developed. Moreover, due to the often advanced age and poor performance status of patients, it is often necessary to reduce the intensity of chemotherapy in clinical practice. Currently, in each patient with a new diagnosis of RT, it is first recommended to establish a clonal relationship with CLL by comparing immunoglobulin gene rearrangements of CLL cells and aggressive lymphoma infiltration. In patients with RT clonally unrelated to CLL (c.20% of patients), treatment should be conducted in accordance with the standard of therapy for *de novo* DLBCL. In RT clonally related to CLL or when it is impossible to ascertain a clonal relationship, there is no effective treatment method and participation in a clinical trial should be the first choice. If this is impossible, immunochemotherapy with an anti-CD20 antibody should be used, but the R-CHOP regimen still seems to be a rational choice. Given the expected short response time, the next step in all patients who achieve at least a partial response to chemotherapy and are in good clinical condition and age should be consolidation of the response using high-dose chemotherapy with hematopoietic stem cell transplantation (HSCT) [96]. The preferred method of consolidation, especially in younger patients, is allo-HSCT, but autologous hematopoietic stem cell transplantation (auto-HSCT) may also improve the prognosis in some patients [97]. It should be emphasized that due to the clinical context, allo-HSCT can only be performed in 10–15% of patients diagnosed with RT [97].

Patients with HLvRT are usually given chemotherapy according to the ABVD regimen (adriamycin, bleomycin, vinblastine, dacarbazine). The results obtained are better than in the clonally dependent form of ZR-DLBCL, but worse than in *de novo* HL [81, 82]. Therefore, if the patient cannot be qualified for a clinical trial, the recommended therapy is the ABVD regimen. The importance of consolidation with HSCT in this type of transformation is not yet established.

The treatment of resistant and relapsed forms is not standardized and is mainly based on combination chemotherapy used in aggressive lymphomas. The results of treatment are usually unfavorable. Therefore, the preferred option should always be for the patient to participate in a clinical trial. The prognosis for patients with RT is unfavorable. In most published reports, median survival of patients with RT of the DLBCL type ranges from six to 18 months after transformation [85, 97]. Patients who developed TR due to untreated CLL have a longer expected

Table VIII. Richter syndrome risk score (adapted from [97])

Parameters with independent negative predictive value for survival	
ECOG performance status >1	
LDH >1.5 upper limit of normal	
PLT <100 G/L	
Largest node or non-nodal lesion >5 cm	
Number of previous lines of therapy >1	
Prognostic index	
Score	**Estimated survival time**
0–1	13 months
2	11 months
3	4 months
4–5	1 month

ECOG — Eastern Cooperative Oncology Group; LDH — lactate dehydrogenase; PLT — platelets

survival than patients previously treated with chemotherapy for CLL [98]. Most reports indicate that the prognosis in HLvRT is better than in patients with classic transformation to DLBCL, although the available data on this subject is inconclusive [81, 82]. For a more detailed assessment of the prognosis of RT, a simple prognostic system has been developed based on basic clinical and laboratory parameters (Table VIII) [97].

Diagnosis and treatment of autoimmune complications

Autoimmune complications in patients with CLL are the result of disorders in the immune system that lead to the production of antibodies directed against self-antigens, usually located on blood cells or their precursors. These disorders lead to autoimmune cytopenias, primarily autoimmune hemolytic anemia (AIHA) and immunological thrombocytopenia (IT). The coexistence of AIHA and IT is called Evans syndrome, which has an estimated prevalence of 5–10%. It is caused by warm class IgG autoantibodies detected by a DAT or, less commonly, by cold class IgG autoantibodies.

Autoimmune hemolytic anemia is the most common autoimmune cytopenia reported in patients with CLL. Its incidence is estimated at 5–10%. It is caused by warm-type IgG autoantibodies detected in the DAT or, less commonly, by cold-type IgG autoantibodies [99, 100]. A positive DAT result is also the most important risk factor for the development of AIHA, although it does not guarantee its occurrence. Similarly, a negative DAT result does not exclude the occurrence of AIHA in the future (positive predictive value c. 30%, negative predictive value c.90%) [101].

Autoimmune cytopenias can also occur during cytoreductive treatment [102]. In particular, it has been observed that treatment with purine analogs as monotherapy can increase the risk of AIHA [103–105]. The incidence of autoimmune cytopenias during treatment with ibrutinib or venetoclax as monotherapy and in combination with rituximab is small, and in most studies does not exceed 5% [34, 106–109].

The basis for the diagnosis of AIHA is the detection of laboratory signs of hemolysis (increased free bilirubin concentration, increased LDH activity, decreased haptoglobin concentration, and an increased number of reticulocytes). However, it should be remembered that each of these indicators has significant limitations in sensitivity and specificity. An increase in the number of reticulocytes may not occur when the red blood cell system in the marrow is suppressed. Elevated LDH activity is a very non-specific laboratory symptom and can also result from progression of the underlying disease, while indirect hyperbilirubinemia requires differentiation from Gilbert's syndrome — testing for *UGT1A1* gene mutations is helpful here. An important diagnostic element is a positive DAT result detecting IgG immunoglobulins and/or complement component C3, which is observed in more than 90% of patients [101].

The mainstay of treatment for AIHA is glucocorticosteroids, usually prednisone or prednisolone as monotherapy or in combination with rituximab, at a dose of 1 mg/kg body weight (bw), increased to 1.5 mg/kg bw if there is no response. Prednisone treatment remains effective in most patients, and it is recommended to maintain the therapeutic dose of corticosteroid for 2–6 weeks and then gradually discontinue the drug over three months. To obtain a faster response to treatment, methylprednisolone can be used in a single dose of 1.0 g or immunoglobulin i.v. at a dose of 0.4 g/kg bw/day for 4–5 days. There is no generally accepted standard of second-line treatment in patients who do not respond to prednisone treatment or whose hemolysis recurs after attempting to discontinue it. In such cases, four weekly administrations of rituximab at a dose of 375 mg/m^2 (if it was not administered in the first-line of treatment) and cyclosporine at a dose of 5–8 mg/kg bw/day are recommended to achieve a serum drug concentration of 100–150 ng/mL, or mycophenolate mofetil, cyclophosphamide or azathioprine can also be used orally [110–112]. The ineffectiveness of drug therapy is an indication for splenectomy. Dearden [110] proposed an algorithm for the management of patients who do not respond to corticosteroid therapy or with recurrence of hemolysis when trying to reduce the dose. In a case of ineffectiveness of two-week administration of prednisone at a dose of 1.5 mg/kg bw, rituximab at a dose of 375 mg/m^2 should be used, and after obtaining a response, supportive treatment with cyclosporine or mycophenolate mofetil should be used. However, the ineffectiveness of rituximab justifies recommending splenectomy. Recurrence of hemolysis when reducing the dose of prednisone can be controlled by

adding cyclosporine at a dose of 5–8 mg/kg bw/day. A response is expected within six weeks. Once such a response has been achieved, then maintenance treatment with cyclosporine or mycophenolate mofetil or rituximab should be considered, followed by splenectomy. Maintenance treatment with cyclosporine or mycophenolate mofetil is also recommended after splenectomy [110]. To maintain the response, the dose of cyclosporine can be reduced to 3 mg/kg bw/day — so that its serum concentration does not exceed 100 µg/L. Both cyclosporine and mycophenolate mofetil can be administered chronically. However, patients should be monitored for adverse effects when using cyclosporine, especially for nephrotoxicity and hypertension.

Due to the risk of alloimmunization, red cell concentrate transfusions should only be used in cases of profound [hemoglobin (Hb) concentration <6 g/dL] and/or symptomatic anemia. In situations of the rapid development of life-threatening hemolysis, methylprednisolone in an intravenous bolus is used, and immunoglobulins may also be given at a dose of 0.4 g/kg bw for five days or 1 g/kg bw for two days.

Autoimmune hemolytic syndrome unresponsive to or poorly controlled by immunosuppressive treatment is an indication for cytoreductive treatment. Regimens with increased immunosuppressive potential, developed for other lymphoproliferative diseases, are preferred. RCD is most often used (rituximab 375 mg/m^2 i.v. on day 1, cyclophosphamide 750 mg/m^2 on day 2, dexamethasone 12 mg i.v. on days 1 and 2, then *p.o.* on days 3–7, cycles repeated every 3–4 weeks) or R-COP (cyclophosphamide 750 mg/m^2, vincristine 1.4 mg/m^2, maximum 2 mg, rituximab 375 mg/m^2 on day 1, prednisone 40 mg/m^2 on days 1–5 every 21 days) [113, 114]. Treatment with purine analogs as monotherapy can increase the risk of AIHA, especially if they are used as monotherapy [38]. However, cases of hemolysis and/or DAT negativity restoration have been observed during treatment with regimens containing purine analogs [115]. The combination of bendamustine with rituximab is also highly effective [116]. Treatment with ibrutinib or idelalisib may have a beneficial effect on the course of autoimmune cytopenia [107, 108, 117]. Individual reports also suggest that venetoclax may have a similar effect [118, 119], although cases of AIHA induction in CLL patients treated with venetoclax have also been described [120, 121].

Immunothrombocytopenia is observed less frequently than AIHA, with an incidence of 1–5% [122–125]. It should be taken into account in every case of a sudden decrease in the number of platelets not explained by other reasons, especially disease progression or treatment. The diagnosis of immunothrombocytopenia is indicated by a rapid (< 2 weeks) and significant (< 100 G/L and or by at least half of the initial value) reduction in the number of platelets, normal or increased megakaryopoiesis in the bone marrow, the absence of splenomegaly, and not having received cytostatic treatment in the previous month [123]. Due to the lack of sufficiently sensitive tests detecting antiplatelet antibodies in clinical practice, the diagnosis of IT is most often a diagnosis by exclusion.

The goal of immunothrombocytopenia treatment is to maintain the platelet count above the hemostatic safety threshold, i.e. above 20–30 G/L. The principles of management are similar to those in AIHA and essential immunothrombocytopenia. The basis of first-line treatment remains corticosteroid therapy, including prednisone at a dose of 1 mg/kg bw, dexamethasone at a dose of 40 mg/day for 4 days every 2–3 weeks, or a single dose of methylprednisolone at a dose of 1 g. There is no clear data regarding the preferability of one of these steroid therapy methods over the others.

In a case of resistance or relapse, when trying to reduce the dose of corticosteroids, cyclosporine with prednisone, vincristine at a dose of 1 mg weekly for 4–6 weeks, rituximab monotherapy or RCD are suggested [110, 126–128]. Another option is the use of thrombopoietin receptor agonists, eltrombopag or romiplostim [129–131]. Failure of conservative treatment is a justification for splenectomy.

Pure red cell aplasia (PRCA) and autoimmune neutropenia are the rarest autoimmune complications in CLL, occurring in less than 1% of patients. In clinical practice, their diagnosis is most often a diagnosis of exclusion. This requires a bone marrow trephine biopsy, which in the case of PRCA shows atrophy of the red blood cell system with preserved granulopoiesis and thrombopoiesis, while in autoimmune neutropenia no precursors of granulopoiesis are detected. PRCA shows an Hb concentration not exceeding 11 g/dL in the absence of hemolysis, absolute reticulocytopenia, and a normal number of granulocytes and platelets. A viral background to aplasia should also be ruled out. The diagnosis of autoimmune granulocytopenia should be considered in the case of prolonged neutropenia below 0.5 G/L in a patient who has not received cytostatic treatment in the preceding eight weeks. As yet, there are no generally accepted rules for the management of these cytopenias. In the treatment of PRCA, in addition to transfusions of red blood cell concentrates, prednisone, cyclosporine, rituximab monotherapy or RCD are suggested [114, 125–128, 132, 133]. The basis of treatment in immunological neutropenia is prevention and combating infection.

It should be emphasized that the occurrence of isolated autoimmune cytopenia is not an indication for cytostatic treatment. Such an indication is AIHA or immunothrombocytopenia that is resistant to treatment or accompanied by the progression of the underlying disease.

In the course of CLL, autoimmune phenomena affecting other organs may occur, which can be manifested by the presence of autoantibodies, such as antinuclear antibodies

or rheumatoid factor, as well as the coexistence of autoimmune diseases [99]. Non-hematological autoimmune complications of CLL include paraneoplastic pemphigus, glomerulonephritis, and acquired angioedema. Due to its rarity, there are no established standards of care.

Prevention and treatment of infections

Chronic lymphocytic leukemia is a disease classified as a secondary immunodeficiency. The clinical picture in 50% of patients (regardless of the stage of CLL) is dominated by recurrent infections, often severe, and more than one in three deaths is infection-related [134–137]. Infections in patients with CLL result not only from immune disorders related to the leukemia itself, but also from the advanced age of the patients, the presence of comorbidities (e.g. diabetes, circulatory failure) and — in people undergoing therapy — from immunosuppression caused by anticancer treatment. The pathogenic factors responsible for the development of infections in CLL patients are dominated by bacteria (67%), to a lesser extent viruses (25%), and, rarely, fungi (7%) [138–140]. Immune disorders in the course of CLL in some patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lead to impaired elimination of the virus from the body. Positive PCR and antigen tests lasting even more than 8–12 weeks, or recurrence of infection shortly after obtaining negative test results for SARS-CoV-2 infection, have been observed in many CLL patients [141, 142, our own observations].

Prevention of infection

Prevention of infections and related complications is an important element of the treatment of patients with CLL. Prophylaxis of *Pneumocystis jirovecii* pneumonia is recommended in patients receiving treatment regimens containing fludarabine, cladribine, bendamustine or idelalisib. Cotrimoxazole is most often administered at a dose of 960 mg every other day during treatment with the above-mentioned drugs, and then for a minimum 3–6 months after completion of treatment. Prophylaxis against *Pneumocystis jirovecii* infection is not required when using BTK inhibitors and venetoclax. Prevention of *Herpes simplex* and *Herpes zoster* viral infections is recommended in patients treated with fludarabine, bendamustine, and anti-CD20 antibodies, especially in patients with a history of recurrent infections with these viruses and with a low percentage/number (<0.2 G/L) of lymphocytes CD4+ T [136]. Prophylactic use of antiviral drugs, such as acyclovir or valacyclovir, should continue for 2–6 months after the end of chemotherapy or until the CD4+ T-cell count is greater than 0.2 G/L, if it is possible to measure it. In patients treated with anti-CD20 monoclonal antibodies who have anti-HBc antibodies and/or a positive HBs antigen in their blood serum, a PCR test for the presence of hepatitis B virus (HBV) DNA should be performed. HBsAg-positive patients with or without detectable HBV DNA, and HBsAg-negative/anti-HBc-positive patients, should also start HBV reactivation prophylaxis with entecavir or tenofovir [142]. Screening and prevention of reactivation of HBV infection are also recommended in patients treated with ibrutinib [143, 144].

Antifungal prophylaxis in the form of fluconazole, and in the case of suspected *Aspergillus* infection — itraconazole, voriconazole, posaconazole or caspofungin — is recommended in patients at high risk of infection, with a low number of CD4+ T lymphocytes, receiving purine analogs or alemtuzumab. Ibrutinib increases the risk of developing invasive mycosis (especially aspergillosis) and pneumocystis (*Pneumocystis jiroveci*) in the first months of use (median three) [145, 146]. Yet despite this, prophylactic use of antifungal drugs is not recommended, and concomitant use of ibrutinib with corticosteroids or other immunosuppressive therapy should be avoided.

Prophylactic and therapeutic use of immunoglobulins

Prophylactic use of immunoglobulins in patients with CLL can reduce the frequency of bacterial infections, but does not affect the frequency of viral and fungal infections, or prolong survival [147, 148]. Recurrent or severe infections, especially with encapsulated bacteria, despite prophylactic antibiotic therapy *p.o.* in patients with a serum IgG concentration below 5 g/L, is an indication for immunoglobulin substitution [a procedure reimbursed by the National Health Fund [NHF]) i.v. or subcutaneously (s.c.)]. Human immunoglobulin preparations can be administered i.v. every 3–4 weeks, at an initial dose of 0.4 g/kg bw or every two weeks in an s.c. infusion [136]. Preparations for s.c. infusions are better tolerated and very rarely cause the side effects such as fever, chills, and symptoms of anaphylaxis that occur when using i.v. preparations. Ultimately, such treatment should lead to IgG concentrations exceeding 6–8 g/L after four months of treatment [149]. The dose of immunoglobulin should be adjusted according to the clinical response and the achieved antibody titer. Maintaining higher trough concentrations may be beneficial in patients with coexisting chronic bronchial and pulmonary diseases [150, 151]. If a decision is made to discontinue human immunoglobulin replacement therapy, this should occur during the summer months and IgG levels should be checked before the onset of winter. Treatment should be discontinued if no reduction in the frequency or severity of bacterial infections is observed after 12 months [152]. Hypogammaglobulinemia does not significantly affect the clinical course of coronavirus disease 2019 (COVID-19) [153], although CLL patients with low IgG concentration in blood serum may be more likely to develop secondary bacterial infections, which can cause sepsis and death [154, 155].

Protective vaccinations

It has been shown that one of the important factors influencing the frequency and severity of infections in some patients with CLL, apart from the reduced IgG concentration, is the simultaneous low titer of specific antibodies against polysaccharides contained in the pneumococcal capsule [154]. This indicates the possibility of a beneficial effect of vaccinations against *Streptococcus pneumoniae* in this group of patients. An assessment of the post-vaccination response in CLL patients found that they show a weaker response to immunization against pneumococci and influenza virus than healthy people [156–159]. Numerous studies have shown that protective vaccinations in patients with CLL are safe and some of them respond properly, especially to conjugate vaccines against *Streptococcus pneumoniae* and *Haemophilus influenzae* type B, administered immediately after the diagnosis of the disease, at least two weeks before the start of treatment [160]. Seasonal influenza vaccination in patients who have not responded to the first immunization should be administered in a two-dose program, with a minimum interval of one month between vaccinations [161].

The vaccination schedule should be adapted to the planned treatment, with particular emphasis on anti-CD20 antibody therapy, which leads to the depletion of B lymphocytes and may cause hypogammaglobulinemia. It has been shown that CLL patients do not achieve protective antibody titers after influenza vaccination when vaccination was performed more than two weeks before, or during, or up to six months after, rituximab treatment [162]. If the patient received an unconjugated pneumococcal vaccine many years ago and if the titers of specific antibodies against *Streptococcus pneumoniae* remain low, re-vaccination is recommended, preferably before the initiation of substitution therapy with human immunoglobulin.

According to CDC guidelines, the recombinant shingles vaccine, available in Poland, is recommended for people with immune disorders [163].

Recommendations regarding vaccinations

Vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae* type B is recommended immediately after diagnosis and before treatment. Patients who, despite an initial response to vaccination, demonstrate a decrease in the specific antibody titer that leads to the development of infection, should be re-vaccinated. It is recommended to vaccinate against seasonal influenza annually (September/October) with vaccines containing the current strains of this virus in that particular season. In patients with CLL, vaccinations with live vaccines against tuberculosis (BCG) and measles, rubella, mumps, chickenpox/*Herpes zoster*, polio myelitis (Sabin and Koprowski vaccine), and yellow fever should be avoided. Vaccinations should not be administered less than two weeks before the start of chemoimmunotherapy, or during its duration, or up to six months after the end of treatment. Protective vaccinations are also not used during serious infections or acute diseases with fever. Mild infections (colds) should not be a reason to postpone vaccinations. Table IX sets out the recommended vaccinations for patients with CLL and the methods of their administration.

Recommendations regarding vaccination against SARS-CoV-2 in patients with CLL

Many questions regarding vaccination against SARS-CoV-2 in patients with CLL remain unanswered because cancer patients were not included in clinical trials. Currently, the only absolute contraindication to administering vaccines is hypersensitivity to the active substance or to any of the excipients in the vaccine preparation. In people with a history of severe allergic reactions, vaccination decisions should be made individually. Taking into account the risk of severe complications in the course of COVID-19 in cancer patients, and the good safety profile of vaccines, then according to the opinion of experts from international scientific societies [European Hematology Associacion (EHA), ASH, NCCN, ESMO], vaccination against SARS-CoV-2 is recommended in cancer patients, including CLL. Anticancer treatment is not a contraindication to vaccination. The challenge is to obtain an effective protective response to vaccination in patients with CLL, especially in patients undergoing immunochemotherapy with anti-CD20 antibodies, treatment with BTK inhibitors or high-dose glucocorticosteroids. The protective effect of the vaccine will depend on the degree of immunosuppression associated with the disease and/or treatment of cancer. Patients with CLL should be vaccinated as soon as possible due to the fact that they are more vulnerable than the general population to hospitalization or death due to severe COVID-19. This also applies to patients several years after completing oncological treatment [164]. On 31 August, 2023, a new monovalent vaccine targeting the XBB.1.5 variant was approved in the EU, used as a single dose regardless of previous vaccination history. Further doses of the vaccine may be administered to immunosuppressed patients depending on national recommendations [165].

Treatment of infections

Treatment of infections in patients with CLL depends not only on the type of etiopathogenetic factor, but also on the patient's general condition and risk factors for the development of life-threatening infectious complications, such as hypogammaglobulinemia (including IgG subclass deficiency) and neutropenia [139]. In many countries, antibiotic prophylaxis is used in patients with CLL, despite a lack of evidence as to the effectiveness of such treatment. Especially in patients with bronchiectasis, prophylactic

Table IX. Vaccinations recommended in patients with chronic lymphocytic leukemia (CLL)

Type of vaccine	Method of administration
13-valent conjugate vaccine against *Streptococcus pneumoniae* (PCV13): (Prevenar 13®) 20-valent conjugate vaccine against *Streptococcus pneumoniae* (PCV20): (Apexxnar®)	Vaccination should be performed as soon as diagnosis of CLL is made. PCV13 and PCV20 are administered in a single dose, intramuscularly (i.m.) into deltoid muscle. Currently, there is no data on need to repeat vaccination
Polysaccharide vaccine against *Streptococcus pneumoniae* (PPSV23): only Pneumovax 23® is available in Poland	Not earlier than two months after PCV13; PPSV23 should be administered i.m. or subcutaneously (s.c.) into deltoid muscle or s.c. Booster dose should be administered after 3–5 years, earlier administration may be considered depending on response to vaccine (it is not in line with SmPC); monitoring of antibody levels is advisable. PPSV23 vaccination is not used in patients previously vaccinated with PCV20
Vaccine against *Hemophilus influenzae* type B (HiB)	Vaccine against HiB is administered in a single dose, i.m. into deltoid muscle or s.c. There is currently no data on need for repeated vaccination
Flu vaccine Multivalent inactivated vaccines against strains recommended each year by WHO for vaccine production. These products are available in Poland: • Influvac® — inactivated sub-unit vaccine containing influenza virus surface antigens • Vaxigrip® — inactivated split vaccine with split influenza virion as an antigen • IDflu® — inactivated split vaccine with split influenza virion as an antigen	Intramuscularly into deltoid muscle or s.c. vaccination should be repeated annually before flu season (preferably in September) in patients with secondary immunodeficiency (especially severe hypogammaglobulinemia <5 g/L) and poor response to vaccination (if a titer of specific antibodies against influenza antigens is not doubled, revaccination after one month may be considered
Vaccine against HBV	Primary vaccination according to this schedule: 0; 1; 6 months in previously unvaccinated patients, preferably straight after diagnosis In patients undergoing immunosuppressive therapy, it is recommended to maintain antibody levels >100 IU/L. Antibody control is performed every six months; when concentration drops below <100 IU/L, a double dose of vaccine should be administered. In patients with profound immunodeficiency (hypogammaglobulinemia IgG requiring IVIG/SCIG supplementation), when concentration of HBs antibodies is <10 IU/L after primary immunization, it is recommended to administer another 1–3 doses of vaccine. If antibody concentration is still <10 IU/L, no further vaccinations are performed
SARS-CoV-2 vaccine	It is recommended to administer one or two doses of vaccine, depending on preparation and manufacturer's recommendations

*According to authors, in patients with blood diseases, it is better to use sub-unit vaccines containing surface subunits (hemagglutinin and influenza virus neuraminidase). In our practice, after administration of split vaccines containing split virion in patients with secondary immunodeficiency, more side effects were observed; SmPC — summary of product characteristics; WHO — World Health Organization; HBV — hepatitis B virus; IVIG/SCIG — intravenous immunoglobulin/subcutaneous immunoglobulin; SARS-CoV-2 — severe acute respiratory syndrome-related coronavirus 2

administration of azithromycin at a dose of 250 mg three times a week should be considered [137]. Patients who are not at risk of sepsis and with an absolute neutrophil count above 0.5 G/L may be treated with antibiotics with a narrower range of action directed against the most likely pathogen previously detected in cultures from biological material [140].

Suspicion of the development of sepsis and/or an absolute neutrophil count of below 0.5 G/L should be treated as a life-threatening condition, and treatment with i.v. antibiotics should be initiated as soon as possible until the results of bacteriological tests with a broad spectrum of action are received [140]. *Herpes simplex* and *Herpes zoster* infections often occur in patients with advanced CLL, and complicate the use of anti-leukemic therapy. The course of the infection is usually mild, and oral antiviral drugs are sufficient. If DNA CMV is detected, antiviral treatment with gancyclovir at a dose of 5 mg/kg bw should be initiated i.v. twice daily for at least two weeks or valgancyclovir at a dose of 900 mg twice daily. In patients refractory to this treatment, foscarnet or cidofovir are recommended. If CMV DNA is detected, antiviral treatment should be initiated with gancyclovir 5 mg/kg bw i.v. twice daily for at least two weeks or valgancyclovir at a dose of 900 mg twice daily. In patients resistant to this treatment, foscarnet or cidofovir are indicated.

Article information and declarations

Acknowledgements
Not applicable.

Conflict of interest
IH — research funding: AstraZeneca, Roche; honoraria lecture fees/for Scientific Advisory Board: AbbVie, AstraZeneca, Beigene, Janssen-Cilag, Roche; travel grants: AbbVie, AstraZeneca, Janssen-Cilag. KJ — scientific support: AbbVie, Janssen-Cilag; lecture/travel honoraria. Scientific Advisory Board: Abbvie, AstraZeneca, Beigene, Janssen, Roche. TR — consultancy and advisory: AstraZeneca, Beigene; research funding — AbbVie, AstraZeneca, Beigene, Janssen-Cilag, Octapharma, Regeneron; honoraria: AbbVie, AstraZeneca, Beigene, Janssen-Cilag; travel, accommodation, expenses: AstraZeneca, Janssen-Cilag. JR, DW, KG declare no conflict of interest.

Funding
None.

Supplementary files
None.

References

1. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022; 36(7): 1703–1719, doi: 10.1038/s41375-022-01613-1, indexed in Pubmed: 35732831.
2. Cancer Stat Facts: NHL — Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL). https://seer.cancer.gov/statfacts/html/cllsll.html (April 23, 2021).
3. Hallek M. Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. Am J Hematol. 2019; 94(11): 1266–1287, doi: 10.1002/ajh.25595, indexed in Pubmed: 31364186.
4. Goldin LR, Slager SL, Caporaso NE. Familial chronic lymphocytic leukemia. Curr Opin Hematol. 2010; 17(4): 350–355, doi: 10.1097/MOH.0b013e328338cd99, indexed in Pubmed: 20389242.
5. Sud A, Chattopadhyay S, Thomsen H, et al. Analysis of 153 115 patients with hematological malignancies refines the spectrum of familial risk. Blood. 2019; 134(12): 960–969, doi: 10.1182/blood.2019001362, indexed in Pubmed: 31395603.
6. Robak T. Second malignancies and Richter's syndrome in patients with chronic lymphocytic leukemia. Hematology. 2004; 9(5–6): 387–400, doi: 10.1080/10245330400018599, indexed in Pubmed: 15763979.
7. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood. 2018; 131(25): 2745–2760, doi: 10.1182/blood-2017-09-806398, indexed in Pubmed: 29540348.
8. Eichhorst B, Robak T, Montserrat E, et al. ESMO Guidelines Committee. Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2021; 32(1): 23–33, doi: 10.1016/j.annonc.2020.09.019, indexed in Pubmed: 33091559.
9. Campo E, Jaffe ES, Cook JR, et al. The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee. Blood. 2022; 140(11): 1229–1253, doi: 10.1182/blood.2022015851, indexed in Pubmed: 35653592.
10. Rawstron AC, Kreuzer KA, Soosapilla A, et al. Reproducible diagnosis of chronic lymphocytic leukemia by flow cytometry: an European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation project. Cytometry B Clin Cytom. 2018; 94(1): 121–128, doi: 10.1002/cyto.b.21595, indexed in Pubmed: 29024461.
11. Rawstron AC, Bennett FL, O'Connor SJM, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. N Engl J Med. 2008; 359(6): 575–583, doi: 10.1056/NEJMoa075290, indexed in Pubmed: 18687638.
12. Rai KR, Sawitsky A, Cronkite EP, et al. Clinical staging of chronic lymphocytic leukemia. Blood. 1975; 46(2): 219–234, indexed in Pubmed: 1139039.
13. Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981; 48(1): 198–206, doi: 10.1002/1097-0142(19810701)48:1<198::aid-cncr2820480131>3.0.co;2-v, indexed in Pubmed: 7237385.
14. Rai KR. A critical analysis of staging in CLL. In: Gale RP, Rai KR. ed. Chronic lymphocytic leukemia: recent progress and future directions. Liss, New York 1987: 253–264.
15. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000; 343(26): 1910–1916, doi: 10.1056/NEJM200012283432602, indexed in Pubmed: 11136261.
16. Zenz T, Eichhorst B, Busch R, et al. TP53 mutation and survival in chronic lymphocytic leukemia. J Clin Oncol. 2010; 28(29): 4473–4479, doi: 10.1200/JCO.2009.27.8762, indexed in Pubmed: 20697090.
17. Hallek M, Fischer K, Fingerle-Rowson G, et al. International Group of Investigators, German Chronic Lymphocytic Leukaemia Study Group. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet. 2010; 376(9747): 1164–1174, doi: 10.1016/S0140-6736(10)61381-5, indexed in Pubmed: 20888994.
18. O'Brien S, Furman RR, Coutre S, et al. Single-agent ibrutinib in treatment-naïve and relapsed/refractory chronic lymphocytic leukemia: a 5-year experience. Blood. 2018; 131(17): 1910–1919, doi: 10.1182/blood-2017-10-810044, indexed in Pubmed: 29437592.
19. Jones J, Mato A, Coutre S, et al. Evaluation of 230 patients with relapsed/refractory deletion 17p chronic lymphocytic leukaemia treated with ibrutinib from 3 clinical trials. Br J Haematol. 2018; 182(4): 504–512, doi: 10.1111/bjh.15421, indexed in Pubmed: 29873072.
20. Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax for patients with chronic lymphocytic leukemia with 17p deletion: results from the full population of a phase II pivotal trial. J Clin Oncol. 2018; 36(19): 1973–1980, doi: 10.1200/JCO.2017.76.6840, indexed in Pubmed: 29715056.
21. Fischer K, Al-Sawaf O, Bahlo J, et al. Venetoclax and obinutuzumab in patients with CLL and coexisting conditions. N Engl J Med. 2019; 380(23): 2225–2236, doi: 10.1056/NEJMoa1815281, indexed in Pubmed: 31166681.
22. Hamblin TJ, Davis Z, Gardiner A, et al. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999; 94(6): 1848–1854, indexed in Pubmed: 10477713.

23. Lin K, Sherrington PD, Dennis M, et al. Relationship between p53 dysfunction, CD38 expression, and IgV(H) mutation in chronic lymphocytic leukemia. Blood. 2002; 100(4): 1404–1409, doi: 10.1182/blood-2001-11-0066, indexed in Pubmed: 12149224.
24. Stilgenbauer S, Bullinger L, Lichter P, et al. German CLL Study Group (GCLLSG). Genetic and chronic lymphocytic leukemia: genomic aberrations and V(H) gene mutation status in pathogenesis and clinical course. Leukemia. 2002; 16(6): 993–1007, doi: 10.1038/sj.leu.2402537, indexed in Pubmed: 12040431.
25. Kröber A, Bloehdorn J, Hafner S, et al. Additional genetic high-risk features such as 11q deletion, 17p deletion, and V3-21 usage characterize discordance of ZAP-70 and VH mutation status in chronic lymphocytic leukemia. J Clin Oncol. 2006; 24(6): 969–975, doi: 10.1200/JCO.2005.03.7184, indexed in Pubmed: 16418492.
26. Fischer K, Bahlo J, Fink AM, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. Blood. 2016; 127(2): 208–215, doi: 10.1182/blood-2015-06-651125, indexed in Pubmed: 26486789.
27. Burger JA, Tedeschi A, Barr PM, et al. RESONATE-2 Investigators. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. N Engl J Med. 2015; 373(25): 2425–2437, doi: 10.1056/NEJMoa1509388, indexed in Pubmed: 26639149.
28. Woyach JA, Ruppert AS, Heerema NA, et al. Ibrutinib regimens versus chemoimmunotherapy in older patients with untreated CLL. N Engl J Med. 2018; 379(26): 2517–2528, doi: 10.1056/NEJMoa1812836, indexed in Pubmed: 30501481.
29. Moreno C, Greil R, Demirkan F, et al. Ibrutinib plus obinutuzumab versus chlorambucil plus obinutuzumab in first-line treatment of chronic lymphocytic leukaemia (iLLUMINATE): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol. 2019; 20(1): 43–56, doi: 10.1016/S1470-2045(18)30788-5, indexed in Pubmed: 30522969.
30. Shanafelt TD, Wang XV, Hanson CA, et al. Ibrutinib-Rituximab or chemoimmunotherapy for chronic lymphocytic leukemia. N Engl J Med. 2019; 381(5): 432–443, doi: 10.1056/NEJMoa1817073, indexed in Pubmed: 31365801.
31. Ghia P, Rawstron A. Minimal residual disease analysis in chronic lymphocytic leukemia: a way for achieving more personalized treatments. Leukemia. 2018; 32(6): 1307–1316, doi: 10.1038/s41375-018-0109-x, indexed in Pubmed: 29700386.
32. Böttcher S, Ritgen M, Fischer K, et al. Minimal residual disease quantification is an independent predictor of progression-free and overall survival in chronic lymphocytic leukemia: a multivariate analysis from the randomized GCLLSG CLL8 trial. J Clin Oncol. 2012; 30(9): 980–988, doi: 10.1200/JCO.2011.36.9348, indexed in Pubmed: 22331940.
33. Kwok M, Rawstron AC, Varghese A, et al. Minimal residual disease is an independent predictor for 10-year survival in CLL. Blood. 2016; 128(24): 2770–2773, doi: 10.1182/blood-2016-05-714162, indexed in Pubmed: 27697770.
34. Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-rituximab in relapsed or refractory chronic lymphocytic leukemia. N Engl J Med. 2018; 378(12): 1107–1120, doi: 10.1056/NEJMoa1713976, indexed in Pubmed: 29562156.
35. Kater AP, Kipps T, Eichhorst B, et al. Five-year analysis of Murano study demonstrates enduring undetectable minimal residual disease (uMRD) in a subset of relapsed/refractory chronic lymphocytic leukemia (R/R CLL) patients (pts) following fixed-duration venetoclax-rituximab (VenR) therapy (tx). Blood. 2020; 136(Suppl 1): 19–21, doi: 10.1182/blood-2020-136109.
36. Catovsky D, Else M, Richards S. Chlorambucil — still not bad: a reappraisal. Clin Lymphoma Myeloma Leuk. 2011; 11(Suppl 1): S2–S6, doi: 10.1016/j.clml.2011.02.006.
37. Fisher K, Cramer P, Busch R, et al. German CLL Study Group. Bendamustine combined with rituximab in patients with relapsed and/or refractory chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. J Clin Oncol. 2011; 29(26): 3559–3566, doi: 10.1200/JCO.2010.33.8061, indexed in Pubmed: 21844497.
38. Eichhorst B, Fink AM, Bahlo J, et al. international group of investigators, German CLL Study Group (GCLLSG). First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial. Lancet Oncol. 2016; 17(7): 928–942, doi: 10.1016/S1470-2045(16)30051-1, indexed in Pubmed: 27216274.
39. Goede V, Fischer K, Busch R, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. N Engl J Med. 2014; 370(12): 1101–1110, doi: 10.1056/NEJMoa1313984, indexed in Pubmed: 24401022.
40. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med. 2013; 369(1): 32–42, doi: 10.1056/NEJMoa1215637, indexed in Pubmed: 23782158.
41. Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. N Engl J Med. 2016; 374(4): 311–322, doi: 10.1056/NEJMoa1513257, indexed in Pubmed: 26639348.
42. Ghia P, Pluta A, Wach M, et al. ASCEND: phase III, randomized trial of acalabrutinib versus idelalisib plus rituximab or bendamustine plus rituximab in relapsed or refractory chronic lymphocytic leukemia. J Clin Oncol. 2020; 38(25): 2849–2861, doi: 10.1200/JCO.19.03355, indexed in Pubmed: 32459600.
43. Tam CS, Allan JN, Siddiqi T, et al. Fixed-duration ibrutinib plus venetoclax for first-line treatment of CLL: primary analysis of the CAPTIVATE FD cohort. Blood. 2022; 139(22): 3278–3289, doi: 10.1182/blood.2021014488, indexed in Pubmed: 35196370.
44. Tam CS, Brown JR, Kahl BS, et al. Zanubrutinib versus bendamustine and rituximab in untreated chronic lymphocytic leukaemia and small lymphocytic lymphoma (SEQUOIA): a randomised, controlled, phase 3 trial. Lancet Oncol. 2022; 23(8): 1031–1043, doi: 10.1016/S1470-2045(22)00293-5, indexed in Pubmed: 35810754.
45. Sharman JP, Egyed M, Jurczak W, et al. Acalabrutinib with or without obinutuzumab versus chlorambucil and obinutuzmab for treatment-naive chronic lymphocytic leukaemia (ELEVATE TN): a randomised, controlled, phase 3 trial. Lancet. 2020; 395(10232): 1278––1291, doi: 10.1016/S0140-6736(20)30262-2, indexed in Pubmed: 32305093.
46. Fischer K, Al-Sawaf O, Hallek M. Preventing and monitoring for tumor lysis syndrome and other toxicities of venetoclax during treatment of chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program. 2020; 2020(1): 357–362, doi: 10.1182/hematology.2020000120, indexed in Pubmed: 33275717.
47. Eichhorst B, Niemann CU, Kater AP, et al. GCLLSG, the HOVON and Nordic CLL Study Groups, the SAKK, the Israeli CLL Association, and Cancer Trials Ireland. First-Line venetoclax combinations in chronic lymphocytic leukemia. N Engl J Med. 2023; 388(19): 1739–1754, doi: 10.1056/NEJMoa2213093, indexed in Pubmed: 37163621.

48. Byrd JC, Brown JR, O'Brien S, et al. RESONATE Investigators. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. N Engl J Med. 2014; 371(3): 213–223, doi: 10.1056/NEJMoa1400376, indexed in Pubmed: 24881631.
49. Munir T, Brown JR, O'Brien S, et al. Final analysis from RESONATE: up to six years of follow-up on ibrutinib in patients with previously treated chronic lymphocytic leukemia or small lymphocytic lymphoma. Am J Hematol. 2019; 94(12): 1353–1363, doi: 10.1002/ajh.25638, indexed in Pubmed: 31512258.
50. Byrd JC, Hillmen P, Ghia P, et al. Acalabrutinib versus ibrutinib in previously treated chronic lymphocytic leukemia: results of the first randomized phase III trial. J Clin Oncol. 2021; 39(31): 3441–3452, doi: 10.1200/JCO.21.01210, indexed in Pubmed: 34310172.
51. Brown JR, Eichhorst B, Hillmen P, et al. Zanubrutinib or ibrutinib in relapsed or refractory chronic lymphocytic leukemia. N Engl J Med. 2023; 388(4): 319–332, doi: 10.1056/nejmoa2211582, indexed in Pubmed: 36511784.
52. Aapro MS, Bohlius J, Cameron DA, et al. European Organisation for Research and Treatment of Cancer. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. Eur J Cancer. 2011; 47(1): 8–32, doi: 10.1016/j.ejca.2010.10.013, indexed in Pubmed: 21095116.
53. Lukenbill J, Kalaycio M. Fludarabine: a review of the clear benefits and potential harms. Leuk Res. 2013; 37(9): 986–994, doi: 10.1016/j.leukres.2013.05.004, indexed in Pubmed: 23787174.
54. Fischer K, Cramer P, Busch R, et al. Bendamustine in combination with rituximab for previously untreated patients with chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. J Clin Oncol. 2012; 30(26): 3209–3216, doi: 10.1200/JCO.2011.39.2688, indexed in Pubmed: 22869884.
55. Goede V, Fischer K, Dyer M, et al. Overall survival benefit of obinutuzumab over rituximab when combined with chlorambucil in patients with chronic lymphocytic leukemia and comorbidities: final survival analysis of the CLL11 study Study EHA Congress, 2018. Abstract S151.
56. Byrd JC, Furman RR, Coutre SE, et al. Ibrutinib treatment for first-line and relapsed/refractory chronic lymphocytic leukemia: final analysis of the pivotal phase Ib/II PCYC-1102 study. Clin Cancer Res. 2020; 26(15): 3918–3927, doi: 10.1158/1078-0432.CCR-19-2856, indexed in Pubmed: 32209572.
57. Lin VS, Lew TE, Handunnetti SM, et al. BTK inhibitor therapy is effective in patients with CLL resistant to venetoclax. Blood. 2020; 135(25): 2266–2270, doi: 10.1182/blood.2020004782, indexed in Pubmed: 32244251.
58. Burger JA, Barr PM, Robak T, et al. Long-term efficacy and safety of first-line ibrutinib treatment for patients with CLL/SLL: 5 years of follow-up from the phase 3 RESONATE-2 study. Leukemia. 2020; 34(3): 787–798, doi: 10.1038/s41375-019-0602-x, indexed in Pubmed: 31628428.
59. Moreno C, Greil R, Demirkan F, et al. First-line treatment of chronic lymphocytic leukemia with ibrutinib plus obinutuzumab chlorambucil plus obinutuzumab: final analysis of the randomized, phase III iLLUMINATE trial. Haematologica. 2022; 107(9): 2108–2120, doi: 10.3324/haematol.2021.279012, indexed in Pubmed: 35021599.
60. Shanafelt TD, Wang XV, Hanson CA, et al. Long-term outcomes for ibrutinib-rituximab and chemoimmunotherapy in CLL: updated results of the E1912 trial. Blood. 2022; 140(2): 112–120, doi: 10.1182/blood.2021014960, indexed in Pubmed: 35427411.
61. Woyach JA, Ruppert A, Heerema N, et al. Long-term results of alliance A041202 show continued advantage of ibrutinib-based regimens compared with bendamustine plus rituximab (BR) chemoimmunotherapy. Blood. 2021; 138(Suppl 1): 639–639, doi: 10.1182/blood-2021-153146.
62. Sharman JP, Egyed M, Jurczak W, et al. Acalabrutinib ± obinutuzumab vs obinutuzumab + chlorambucil in treatment-naive chronic lymphocytic leukemia: 6-year follow-up of ELEVATE-TN: a randomised, controlled, phase 3 trial. Presented at the American Society of Hematology (ASH) Annual Meeting; December 9–12, 2023. https://ash.confex.com/ash/2023/webprogram/Paper174750.html (December 10, 2023).
63. Shah NN, Wang ML, Brown JR, et al. Safety and tolerability of pirtobrutinib monotherapy in patients with B-cell malignancies who were previously intolerant to a covalent BTK inhibitor: results from the phase 1/2 BRUIN study. Blood. 2022; 140(Suppl 1): 4127–4132, doi: 10.1182/blood-2022-159035.
64. Jones JA, Mato A, Wierda W, et al. Venetoclax for chronic lymphocytic leukaemia progressing after ibrutinib: an interim analysis of a multicentre, open-label, phase 2 trial. Lancet Oncol. 2018; 19(1): 65–75, doi: 10.1016/s1470-2045(17)30909-9.
65. Al-Sawaf O, Zhang C, Robecht S, et al. Clonal dynamics after venetoclax-obinutuzumab therapy: novel insights from the randomized, phase 3 CLL14 trial. ASH, 2020. Abstract 127.
66. Eichhorst B, Niemann CU, Kater AP, et al. GCLLSG, the HOVON and Nordic CLL Study Groups, the SAKK, the Israeli CLL Association, and Cancer Trials Ireland. First-Line venetoclax combinations in chronic lymphocytic leukemia. N Engl J Med. 2023; 388(19): 1739–1754, doi: 10.1056/NEJMoa2213093, indexed in Pubmed: 37163621.
67. Wierda WG, Allan JN, Siddiqi T, et al. Ibrutinib plus venetoclax for first-line treatment of chronic lymphocytic leukemia: primary analysis results from the minimal residual disease cohort of the randomized phase II CAPTIVATE study. J Clin Oncol. 2021; 39(34): 3853–3865, doi: 10.1200/JCO.21.00807, indexed in Pubmed: 34618601.
68. Kater AP, Owen C, Moreno C, et al. Fixed-duration ibrutinib-venetoclax in patients with chronic lymphocytic leukemia and comorbidities. NEJM Evidence. 2022; 1(7), doi: 10.1056/evidoa2200006.
69. Dreger P, Schetelig J, Andersen N, et al. European Research Initiative on CLL (ERIC) and the European Society for Blood and Marrow Transplantation (EBMT). Managing high-risk CLL during transition to a new treatment era: stem cell transplantation or novel agents? Blood. 2014; 124(26): 3841–3849, doi: 10.1182/blood-2014-07-586826, indexed in Pubmed: 25301705.
70. Dreger P, Michallet M, Bosman P, et al. Ibrutinib for bridging to allogeneic hematopoietic cell transplantation in patients with chronic lymphocytic leukemia or mantle cell lymphoma: a study by the EBMT Chronic Malignancies and Lymphoma Working Parties. Bone Marrow Transplant. 2019; 54(1): 44–52, doi: 10.1038/s41409-018-0207-4, indexed in Pubmed: 29728701.
71. Dreger P, Döhner H, Ritgen M, et al. German CLL Study Group. Allogeneic stem cell transplantation provides durable disease control in poor-risk chronic lymphocytic leukemia: long-term clinical and MRD results of the German CLL Study Group CLL3X trial. Blood. 2010; 116(14): 2438–2447, doi: 10.1182/blood-2010-03-275420, indexed in Pubmed: 20595516.
72. Shea T, Johnson J, Westervelt P, et al. Cancer and Leukemia Group B. Reduced-intensity allogeneic transplantation provides high event-free and overall survival in patients with advanced indolent B cell malignancies: CALGB 109901. Biol Blood Marrow Transplant. 2011; 17(9): 1395–1403, doi: 10.1016/j.bbmt.2011.01.016, indexed in Pubmed: 21296675.

73. van Gelder M, de Wreede LC, Bornhäuser M, et al. Long-term survival of patients with CLL after allogeneic transplantation: a report from the European Society for Blood and Marrow Transplantation. Bone Marrow Transplant. 2017; 52(3): 372–380, doi: 10.1038/bmt.2016.282, indexed in Pubmed: 27941763.
74. Frey NV, Gill S, Hexner EO, et al. Long-term outcomes from a randomized dose optimization study of chimeric antigen receptor modified t cells in relapsed chronic lymphocytic leukemia. J Clin Oncol. 2020; 38(25): 2862–2871, doi: 10.1200/JCO.19.03237, indexed in Pubmed: 32298202.
75. Linn BS, Linn MW, Gurel L. Cumulative illness rating scale. J Am Geriatr Soc. 1968; 16(5): 622–626, doi: 10.1111/j.1532-5415.1968.tb02103.x, indexed in Pubmed: 5646906.
76. Salvi F, Miller MD, Grilli A, et al. A manual of guidelines to score the modified cumulative illness rating scale and its validation in acute hospitalized elderly patients. J Am Geriatr Soc. 2008; 56(10): 1926–1931, doi: 10.1111/j.1532-5415.2008.01935.x, indexed in Pubmed: 18811613.
77. https://www.nccn.org/professionals/physician_gls/pdf/cll.pdf (September 17, 2023).
78. Update: the Onkopedia recommendations on chronic lymphocytic leukemia (CLL). https://www.mll.com/en/update-the-onkopedia-recommendations-on-chronic-lymphocytic-leukemia-cll (September 17, 2023).
79. LLC — Les recommandations du FILO 2023. https://www.filo-leucemie.org/actualites/traitements/llc-les-recommandations-du-filo-2023/ (September 17, 2023).
80. Richter MN. Generalized reticular cell sarcoma of lymph nodes associated with lymphatic leukemia. Am J Pathol. 1928; 4(4): 285–292.7, indexed in Pubmed: 19969796.
81. Tsimberidou AM, Keating MJ. Richter syndrome: biology, incidence, and therapeutic strategies. Cancer. 2005; 103(2): 216–228, doi: 10.1002/cncr.20773, indexed in Pubmed: 15578683.
82. Bockorny B, Codreanu I, Dasanu CA. Hodgkin lymphoma as Richter transformation in chronic lymphocytic leukaemia: a retrospective analysis of world literature. Br J Haematol. 2012; 156(1): 50–66, doi: 10.1111/j.1365-2141.2011.08907.x, indexed in Pubmed: 22017478.
83. Jamroziak K, Grzybowska-Izydorczyk O, Jesionek-Kupnicka D, et al. Poor prognosis of Hodgkin variant of Richter transformation in chronic lymphocytic leukemia treated with cladribine. Br J Haematol. 2012; 158(2): 286–288, doi: 10.1111/j.1365-2141.2012.09127.x, indexed in Pubmed: 22500554.
84. Chigrinova E, Rinaldi A, Kwee I, et al. Two main genetic pathways lead to the transformation of chronic lymphocytic leukemia to Richter syndrome. Blood. 2013; 122(15): 2673–2682, doi: 10.1182/blood-2013-03-489518, indexed in Pubmed: 24004666.
85. Fabbri G, Khiabanian H, Holmes AB, et al. Genetic lesions associated with chronic lymphocytic leukemia transformation to Richter syndrome. J Exp Med. 2013; 210(11): 2273–2288, doi: 10.1084/jem.20131448, indexed in Pubmed: 24127483.
86. Rossi D, Spina V, Forconi F, et al. Molecular history of Richter syndrome: origin from a cell already present at the time of chronic lymphocytic leukemia diagnosis. Int J Cancer. 2012; 130(12): 3006–3010, doi: 10.1002/ijc.26322, indexed in Pubmed: 21796624.
87. Tsimberidou AM, O'Brien S, Khouri I, et al. Clinical outcomes and prognostic factors in patients with Richter's syndrome treated with chemotherapy or chemoimmunotherapy with or without stem-cell transplantation. J Clin Oncol. 2006; 24(15): 2343–2351, doi: 10.1200/JCO.2005.05.0187, indexed in Pubmed: 16710033.
88. Papajík T, Mysliveček M, Urbanová R, et al. 2-[18F]fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography examination in patients with chronic lymphocytic leukemia may reveal Richter transformation. Leuk Lymphoma. 2014; 55(2): 314–319, doi: 10.3109/10428194.2013.802313, indexed in Pubmed: 23656196.
89. Tsimberidou AM, O'Brien SM, Cortes JE, et al. Phase II study of fludarabine, cytarabine (Ara-C), cyclophosphamide, cisplatin and GM-CSF (FACPGM) in patients with Richter's syndrome or refractory lymphoproliferative disorders. Leuk Lymphoma. 2002; 43(4): 767–772, doi: 10.1080/10428190290016872, indexed in Pubmed: 12153163.
90. Rogers KA, Huang Y, Ruppert AS, et al. A single-institution retrospective cohort study of first-line R-EPOCH chemoimmunotherapy for Richter syndrome demonstrating complex chronic lymphocytic leukaemia karyotype as an adverse prognostic factor. Br J Haematol. 2018; 180(2): 259–266, doi: 10.1111/bjh.15035, indexed in Pubmed: 29193006.
91. Jain N, Ferrajoli A, Basu S, et al. et al.. A phase II trial of nivolumab combined with ibrutinib for patients with Richter transformation [abstract]. Blood. 2018; 132(Suppl 1): 296.
92. Younes A, Brody J, Carpio C, et al. Safety and activity of ibrutinib in combination with nivolumab in patients with relapsed non-Hodgkin lymphoma or chronic lymphocytic leukaemia: a phase 1/2a study. Lancet Haematol. 2019; 6(2): e67–e78, doi: 10.1016/S2352-3026(18)30217-5, indexed in Pubmed: 30642819.
93. Ding W, LaPlant BR, Call TG, et al. Pembrolizumab in patients with CLL and Richter transformation or with relapsed CLL. Blood. 2017; 129(26): 3419–3427, doi: 10.1182/blood-2017-02-765685, indexed in Pubmed: 28424162.
94. Davids MS, Rogers KA, Tyekucheva S, et al. Venetoclax plus dose-adjusted R-EPOCH for Richter syndrome. Blood. 2022; 139(5): 686–689, doi: 10.1182/blood.2021011386, indexed in Pubmed: 34788401.
95. Xia L, Wang Yi, Li T, et al. The clinical study on treatment of CD19--directed chimeric antigen receptor-modified T cells in a case of refractory Richter syndrome. Cancer Med. 2019; 8(6): 2930–2941, doi: 10.1002/cam4.2193, indexed in Pubmed: 31050207.
96. Cwynarski K, van Biezen A, de Wreede L, et al. Autologous and allogeneic stem-cell transplantation for transformed chronic lymphocytic leukemia (Richter's syndrome): a retrospective analysis from the chronic lymphocytic leukemia subcommittee of the chronic leukemia working party and lymphoma working party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 2012; 30(18): 2211–2217, doi: 10.1200/JCO.2011.37.4108, indexed in Pubmed: 22547610.
97. Wang Y, Tschautscher MA, Rabe KG, et al. Clinical characteristics and outcomes of Richter transformation: experience of 204 patients from a single center. Haematologica. 2020; 105(3): 765–773, doi: 10.3324/haematol.2019.224121, indexed in Pubmed: 31197071.
98. Tsimberidou AM, O'Brien S, Kantarjian HM, et al. Hodgkin transformation of chronic lymphocytic leukemia: the M. D. Anderson Cancer Center experience. Cancer. 2006; 107(6): 1294–1302, doi: 10.1002/cncr.22121, indexed in Pubmed: 16902984.
99. Barcellini W, Capalbo S, Agostinelli RM, et al. GIMEMA Chronic Lymphocytic Leukemia Group. Relationship between autoimmune phenomena and disease stage and therapy in B-cell chronic lymphocytic leukemia. Haematologica. 2006; 91(12): 1689–1692, indexed in Pubmed: 17145607.
100. Zent CS, Kay N. Autoimmune complications in chronic lymphocytic leukaemia (CLL). Best Pract Res Clin Haematol. 2010; 23(1): 47–59, doi: 10.1016/j.beha.2010.01.004.

101. Dearden C, Wade R, Else M, et al. UK National Cancer Research Institute (NCRI), Haematological Oncology Clinical Studies Group, NCRI CLL Working Group. The prognostic significance of a positive direct antiglobulin test in chronic lymphocytic leukemia: a beneficial effect of the combination of fludarabine and cyclophosphamide on the incidence of hemolytic anemia. Blood. 2008; 111(4): 1820–1826, doi: 10.1182/blood-2007-07-101303, indexed in Pubmed: 18055869.
102. Lewis FB, Schwartz RS, Dameshek W. X-radiation and alkylating agents as possible „trigger" mechanisms in the autoimmune complications of malignant lymphophroliferative disease. Clin Exp Immunol. 1966; 1(1): 3–11, indexed in Pubmed: 5953036.
103. Montillo M, Tedeschi A, Leoni P. Recurrence of autoimmune thrombocytopenia after treatment with fludarabine in a patient with chronic lymphocytic leukemia. Leuk Lymphoma. 1994; 15(1–2): 187–188, doi: 10.3109/10428199409051696, indexed in Pubmed: 7858499.
104. Weiss RB, Freiman J, Kweder SL, et al. Hemolytic anemia after fludarabine therapy for chronic lymphocytic leukemia. J Clin Oncol. 1998; 16(5): 1885–1889, doi: 10.1200/JCO.1998.16.5.1885, indexed in Pubmed: 9586905.
105. Myint H, Copplestone JA, Orchard J, et al. Fludarabine-related autoimmune haemolytic anaemia in patients with chronic lymphocytic leukaemia. Br J Haematol. 1995; 91(2): 341–344, doi: 10.1111/j.1365-2141.1995.tb05300.x, indexed in Pubmed: 8547072.
106. Rogers KA, Ruppert AS, Bingman A, et al. Incidence and description of autoimmune cytopenias during treatment with ibrutinib for chronic lymphocytic leukemia. Leukemia. 2016; 30(2): 346–350, doi: 10.1038/leu.2015.273, indexed in Pubmed: 26442611.
107. Montillo M, O'Brien S, Tedeschi A, et al. Ibrutinib in previously treated chronic lymphocytic leukemia patients with autoimmune cytopenias in the RESONATE study. Blood Cancer J. 2017; 7(2): e524, doi: 10.1038/bcj.2017.5, indexed in Pubmed: 28157216.
108. Hampel PJ, Larson MC, Kabat B, et al. Autoimmune cytopenias in patients with chronic lymphocytic leukaemia treated with ibrutinib in routine clinical practice at an academic medical centre. Br J Haematol. 2018; 183(3): 421–427, doi: 10.1111/bjh.15545, indexed in Pubmed: 30117139.
109. Davids MS, Hallek M, Wierda W, et al. Comprehensive safety analysis of venetoclax monotherapy for patients with relapsed/refractory chronic lymphocytic leukemia. Clin Cancer Res. 2018; 24(18): 4371–4379, doi: 10.1158/1078-0432.CCR-17-3761, indexed in Pubmed: 29895707.
110. Dearden C. Disease-specific complications of chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program. 2008: 450–456, doi: 10.1182/asheducation-2008.1.450, indexed in Pubmed: 19074125.
111. Cortes J, O'Brien S, Loscertales J, et al. Cyclosporin A for the treatment of cytopenia associated with chronic lymphocytic leukemia. Cancer. 2001; 92(8): 2016–2022, doi: 10.1002/1097-0142(20011015)92:8<2016::aid-cncr1539>3.0.co;2-e, indexed in Pubmed: 11596014.
112. D'Arena G, Laurenti L, Capalbo S, et al. Rituximab therapy for chronic lymphocytic leukemia-associated autoimmune hemolytic anemia. Am J Hematol. 2006; 81(8): 598–602, doi: 10.1002/ajh.20665, indexed in Pubmed: 16823816.
113. Bowen DA, Call TG, Shanafelt TD, et al. Treatment of autoimmune cytopenia complicating progressive chronic lymphocytic leukemia/small lymphocytic lymphoma with rituximab, cyclophosphamide, vincristine, and prednisone. Leuk Lymphoma. 2010; 51(4): 620–627, doi: 10.3109/10428191003682767, indexed in Pubmed: 20302386.
114. Kaufman M, Limaye SA, Driscoll N, et al. A combination of rituximab, cyclophosphamide and dexamethasone effectively treats immune cytopenias of chronic lymphocytic leukemia. Leuk Lymphoma. 2009; 50(6): 892–899, doi: 10.1080/10428190902887563, indexed in Pubmed: 19391041.
115. Robak T, Błasińska-Morawiec M, Krykowski E, et al. Autoimmune haemolytic anaemia in patients with chronic lymphocytic leukaemia treated with 2-chlorodeoxyadenosine (cladribine). Eur J Haematol. 1997; 58(2): 109–113, doi: 10.1111/j.1600-0609.1997.tb00933.x, indexed in Pubmed: 9111592.
116. Quinquenel A, Willekens C, Dupuis J, et al. Bendamustine and rituximab combination in the management of chronic lymphocytic leukemia-associated autoimmune hemolytic anemia: a multicentric retrospective study of the French CLL intergroup (GCFLLC/MW and GOELAMS). Am J Hematol. 2015; 90(3): 204–207, doi: 10.1002/ajh.23909, indexed in Pubmed: 25428829.
117. Quinquenel A, Godet S, Dartigeas C, et al. Ibrutinib and idelalisib in the management of CLL-associated autoimmune cytopenias: a study from the FILO group. Am J Hematol. 2019; 94(7): E183–E185, doi: 10.1002/ajh.25480, indexed in Pubmed: 30945328.
118. Lacerda MP, Guedes NR, Yamakawa PE, et al. Treatment of refractory autoimmune hemolytic anemia with venetoclax in relapsed chronic lymphocytic leukemia with del(17p). Ann Hematol. 2017; 96(9): 1577–1578, doi: 10.1007/s00277-017-3039-1, indexed in Pubmed: 28597168.
119. Galindo-Navarro P, Delgado-García A, Rodríguez-Gil MA, et al. Venetoclax for treating refractory autoimmune hemolytic anemia in chronic lymphocytic leukemia: report of two cases in Spain. Haematologica. 2023; 108(8): 2261–2264, doi: 10.3324/haematol.2022.281850, indexed in Pubmed: 36861400.
120. Hadjiaggelidou C, Douganiotis G, Tsirou K, et al. A rare case of autoimmune hemolytic anemia during venetoclax therapy for relapsed chronic lymphocytic leukemia. Leuk Lymphoma. 2021; 62(12): 3054–3056, doi: 10.1080/10428194.2021.1941932, indexed in Pubmed: 34151719.
121. Abdel-Samad N, Sughayar R. Can treatment with venetoclax for chronic lymphocytic leukemia (CLL) result in autoimmune hemolytic anemia? Am J Case Rep. 2021; 22: e928514, doi: 10.12659/AJCR.928514, indexed in Pubmed: 33750754.
122. Zent CS, Ding W, Schwager SM, et al. The prognostic significance of cytopenia in chronic lymphocytic leukaemia/small lymphocytic lymphoma. Br J Haematol. 2008; 141(5): 615–621, doi: 10.1111/j.1365-2141.2008.07086.x, indexed in Pubmed: 18373706.
123. Visco C, Ruggeri M, Laura Evangelista M, et al. Impact of immune thrombocytopenia on the clinical course of chronic lymphocytic leukemia. Blood. 2008; 111(3): 1110–1116, doi: 10.1182/blood-2007-09-111492, indexed in Pubmed: 17986663.
124. Kyasa MJ, Parrish RS, Schichman SA, et al. Autoimmune cytopenia does not predict poor prognosis in chronic lymphocytic leukemia/small lymphocytic lymphoma. Am J Hematol. 2003; 74(1): 1–8, doi: 10.1002/ajh.10369, indexed in Pubmed: 12949883.
125. Hamblin TJ, Oscier DG, Young BJ. Autoimmunity in chronic lymphocytic leukaemia. J Clin Pathol. 1986; 39(7): 713–716, doi: 10.1136/jcp.39.7.713, indexed in Pubmed: 3488334.
126. D'Arena G, Capalbo S, Laurenti L, et al. Chronic lymphocytic leukemia-associated immune thrombocytopenia treated with rituximab: a retrospective study of 21 patients. Eur J Haematol. 2010; 85(6): 502–507, doi: 10.1111/j.1600-0609.2010.01527.x, indexed in Pubmed: 20846302.

127. Rossignol J, Michallet AS, Oberic L, et al. Rituximab–cyclophosphamide–dexamethasone combination in the management of autoimmune cytopenias associated with chronic lymphocytic leukemia. Leukemia. 2011; 25(3): 473–478, doi: 10.1038/leu.2010.278, indexed in Pubmed: 21127498.
128. Cesana C, Carlo-Stella C, Mangoni L, et al. Response to cyclosporin A and recombinant human erythropoietin in a case of B cell chronic lymphocytic leukemia and pure red cell aplasia. Leukemia. 1996; 10(8): 1400–1401, indexed in Pubmed: 8709652.
129. Koehrer S, Keating MJ, Wierda WG. Eltrombopag, a second-generation thrombopoietin receptor agonist, for chronic lymphocytic leukemia-associated ITP. Leukemia. 2010; 24(5): 1096–1098, doi: 10.1038/leu.2010.45, indexed in Pubmed: 20339441.
130. Visco C, Rodeghiero F, Romano A, et al. Eltrombopag for immune thrombocytopenia secondary to chronic lymphoproliferative disorders: a phase 2 multicenter study. Blood. 2019; 134(20): 1708–1711, doi: 10.1182/blood.2019001617, indexed in Pubmed: 31570488.
131. Paul S, Jain N, Ferrajoli A, et al. A phase II trial of eltrombopag for patients with chronic lymphocytic leukaemia (CLL) and thrombocytopenia. Br J Haematol. 2019; 185(3): 606–608, doi: 10.1111/bjh.15581, indexed in Pubmed: 30406944.
132. Chikkappa G, Pasquale D, Zarrabi MH, et al. Cyclosporine and prednisone therapy for pure red cell aplasia in patients with chronic lymphocytic leukemia. Am J Hematol. 1992; 41(1): 5–12, doi: 10.1002/ajh.2830410103, indexed in Pubmed: 1503099.
133. Ghazal H. Successful treatment of pure red cell aplasia with rituximab in patients with chronic lymphocytic leukemia. Blood. 2002; 99(3): 1092–1094, doi: 10.1182/blood.v99.3.1092, indexed in Pubmed: 11807020.
134. Abbott BL. Chronic lymphocytic leukemia: recent advances in diagnosis and treatment. Oncologist. 2006; 11(1): 21–30, doi: 10.1634/theoncologist.11-1-21, indexed in Pubmed: 16401710.
135. Oscier D, Dearden C, Eren E, et al. British Committee for Standards in Haematology. Guidelines on the diagnosis, investigation and management of chronic lymphocytic leukaemia. Br J Haematol. 2012; 159(5): 541–564, doi: 10.1111/bjh.12067 , indexed in Pubmed: 23057493.
136. Molica S. Infections in chronic lymphocytic leukemia: risk factors, and impact on survival, and treatment. Leuk Lymphoma. 1994; 13(3-4): 203–214, doi: 10.3109/10428199409056283, indexed in Pubmed: 8049645.
137. Quinquenel A, Aurran-Schleinitz T, Clavert A, et al. Diagnosis and Treatment of Chronic Lymphocytic Leukemia: recommendations of the French CLL Study Group (FILO). Hemasphere. 2020; 4(5): e473, doi: 10.1097/HS9.0000000000000473, indexed in Pubmed: 33062946.
138. Molteni A, Nosari A, Montillo M, et al. Multiple lines of chemotherapy are the main risk factor for severe infections in patients with chronic lymphocytic leukemia with febrile episodes. Haematologica. 2005; 90(8): 1145–1147, indexed in Pubmed: 16079122.
139. Morrison VA. Management of infectious complications in patients with chronic lymphocytic leukemia. Hematology. 2007; 2007(1): 332–338, doi: 10.1182/asheducation-2007.1.332.
140. Rai KR, Keating MJ. Overview of the complications of chronic lymphocytic leukemia. https://www.uptodate.com/contents/overview-of-the-complications-of-chronic-lymphocytic-leukemia (March 30, 2021).
141. Ye X, Xiao X, Li B, et al. Low humoral immune response and ineffective clearance of SARS-CoV-2 in a COVID-19 patient with CLL during a 69-day follow-up. Front Oncol. 2020; 10: 1272, doi: 10.3389/fonc.2020.01272, indexed in Pubmed: 32719750.
142. Kos I, Balensiefer B, Roth S, et al. Prolonged course of COVID-19-associated pneumonia in a B-cell depleted patient after rituximab. Front Oncol. 2020; 10: 1578, doi: 10.3389/fonc.2020.01578, indexed in Pubmed: 32984017.
143. Pawłowska M, Flisiak R, Gil L, et al. Prophylaxis of hepatitis B virus (HBV) infection reactivation - recommendations of the Working Group for prevention of HBV reactivation. Clin Exp Hepatol. 2019; 5(3): 195–202, doi: 10.5114/ceh.2019.87631, indexed in Pubmed: 31598555.
144. Hammond SP, Chen K, Pandit A, et al. Risk of hepatitis B virus reactivation in patients treated with ibrutinib. Blood. 2018; 131(17): 1987–1989, doi: 10.1182/blood-2018-01-826495, indexed in Pubmed: 29490923.
145. Ryan CE, Cheng MP, Issa NC, et al. Pneumocystis jirovecii pneumonia and institutional prophylaxis practices in CLL patients treated with BTK inhibitors. Blood Adv. 2020; 4(7): 1458–1463, doi: 10.1182/bloodadvances.2020001678, indexed in Pubmed: 32282880.
146. Ghez D, Calleja A, Protin C, et al. Early-onset invasive aspergillosis and other fungal infections in patients treated with ibrutinib. Blood. 2018; 131(17): 1955–1959, doi: 10.1182/blood-2017-11-818286.
147. Besa EC. Use of intravenous immunoglobulin in chronic lymphocytic leukemia. Am J Med. 1984; 76(3A): 209–218, doi: 10.1016/0002-9343(84)90344-9, indexed in Pubmed: 6424454.
148. Griffiths H, Brennan V, Lea J, et al. Crossover study of immunoglobulin replacement therapy in patients with low-grade B-cell tumors. Blood. 1989; 73(2): 366–368, indexed in Pubmed: 2492832.
149. Wasserman RL, Church JA, Peter HH, et al. IgPro10 in PID Study group. Pharmacokinetics of a new 10% intravenous immunoglobulin in patients receiving replacement therapy for primary immunodeficiency. Eur J Pharm Sci. 2009; 37(3–4): 272–278, doi: 10.1016/j.ejps.2009.02.014, indexed in Pubmed: 19491015.
150. Lucas M, Hugh-Jones K, Welby A, et al. Immunomodulatory therapy to achieve maximum efficacy: doses, monitoring, compliance, and self-infusion at home. J Clin Immunol. 2010; 30(Suppl 1): S84–S89, doi: 10.1007/s10875-010-9400-y, indexed in Pubmed: 20387103.
151. Maarschalk-Ellerbroek LJ, Hoepelman IM, Ellerbroek PM. Immunoglobulin treatment in primary antibody deficiency. Int J Antimicrob Agents. 2011; 37(5): 396–404, doi: 10.1016/j.ijantimicag.2010.11.027, indexed in Pubmed: 21276714.
152. Provan D, Chapel HM, Sewell WA, et al. UK Immunoglobulin Expert Working Group. Prescribing intravenous immunoglobulin: summary of Department of Health guidelines. BMJ. 2008; 337: a1831, doi: 10.1136/bmj.a1831, indexed in Pubmed: 18936060.
153. Scarfò L, Chatzikonstantinou T, Rigolin GM, et al. COVID-19 severity and mortality in patients with chronic lymphocytic leukemia: a joint study by ERIC, the European Research Initiative on CLL, and CLL Campus. Leukemia. 2020; 34(9): 2354–2363, doi: 10.1038/s41375-020-0959-x, indexed in Pubmed: 32647324.
154. Hosoda T, Harada S, Okamoto K, et al. COVID-19 and fatal sepsis caused by hypervirulent Klebsiella pneumoniae, Japan, 2020. Emerg Infect Dis. 2021; 27(2): 556–559, doi: 10.3201/eid2702.204662, indexed in Pubmed: 33320080.

155. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020; 395(10229): 1054–1062, doi: 10.1016/S0140-6736(20)30566-3, indexed in Pubmed: 32171076.
156. Griffiths H, Lea J, Bunch C, et al. Predictors of infection in chronic lymphocytic leukaemia (CLL). Clin Exp Immunol. 1992; 89(3): 374–377, doi: 10.1111/j.1365-2249.1992.tb06965.x, indexed in Pubmed: 1516254.
157. Sinisalo M, Aittoniemi J, Käyhty H, et al. Vaccination against infections in chronic lymphocytic leukemia. Leuk Lymphoma. 2003; 44(4): 649–652, doi: 10.1080/1042819031000063408, indexed in Pubmed: 12769342.
158. Sinisalo M, Vilpo J, Itälä M, et al. Antibody response to 7-valent conjugated pneumococcal vaccine in patients with chronic lymphocytic leukaemia. Vaccine. 2007; 26(1): 82–87, doi: 10.1016/j.vaccine.2007.10.053, indexed in Pubmed: 18053620.
159. Pollyea DA, Brown JMY, Horning SJ. Utility of influenza vaccination for oncology patients. J Clin Oncol. 2010; 28(14): 2481––2490, doi: 10.1200/JCO.2009.26.6908, indexed in Pubmed: 20385981.
160. Hartkamp A, Mulder AH, Rijkers GT, et al. Antibody responses to pneumococcal and haemophilus vaccinations in patients with B-cell chronic lymphocytic leukaemia. Vaccine. 2001; 19(13–14): 1671–1677, doi: 10.1016/s0264-410x(00)00409-6, indexed in Pubmed: 11166890.
161. de Lavallade H, Garland P, Sekine T, et al. Repeated vaccination is required to optimize seroprotection against H1N1 in the immunocompromised host. Haematologica. 2011; 96(2): 307–314, doi: 10.3324/haematol.2010.032664, indexed in Pubmed: 20971824.
162. Yri OE, Torfoss D, Hungnes O, et al. Rituximab blocks protective serologic response to influenza A (H1N1) 2009 vaccination in lymphoma patients during or within 6 months after treatment. Blood. 2011; 118(26): 6769–6771, doi: 10.1182/blood-2011-08-372649, indexed in Pubmed: 22058114.
163. CDC. Centers for Disease Control and Prevention. Altered immunocompetence. General best practice guidelines for immunization. https://www.cdc.gov/vaccines/hcp/acip-recs/general-recs/immunocompetence.html (August 1, 2023).
164. Garassino MC, Vyas M, de Vries EGE, et al. European Society for Medical Oncology. The ESMO call to action on COVID-19 vaccinations and patients with cancer: Vaccinate. Monitor. Educate. Ann Oncol. 2021; 32(5): 579–581, doi: 10.1016/j.annonc.2021.01.068, indexed in Pubmed: 33582237.
165. European Commission. Commission implementing decision of 31.8.2023 amending the marketing authorisation granted by Decision C(2022) 7342(final) for "Comirnaty — tozinameran, COVID-19 mRNA vaccine (nucleoside-modified)", a medicinal product for human use. https://ec.europa.eu/health/documents/community-register//2023/20230831160389/dec_160389_en.pdf (August 31, 2023).