Brief Overview about Chronic Lymphocytic Leukemia Markers and Diagnosis

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Abstract

Background: Chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL) is an indolent malignancy characterized by increased production of mature but dysfunctional B lymphocytes. CLL/SLL is defined as a monoclonal lymphoproliferative disease characterized by the proliferation and accumulation of morphologically mature but immunologically dysfunctional B-cell lymphocytes that are smudge cells, as noted on peripheral smear. The primary disease sites include peripheral blood, spleen, lymph nodes, and bone marrow. CLL and SLL are identical from a pathologic and immunophenotypic standpoint. Both CLL and SLL originate from B-cell lymphocytes but present with different manifestations depending on where the abnormal cells are found. Usually, the initial leukemic phase represents CLL, where the cells are present in the blood. This eventually progresses to the lymphoma phase, representing SLL, where the cells are found in the lymph nodes. The term SLL is commonly used to represent the lymphoproliferative process limited to the lymph nodes. The first and foremost laboratory abnormality found in CLL is lymphocytosis in peripheral blood and bone marrow. The first step in the diagnosis of CLL is a peripheral blood smear. The peripheral blood smear shows an absolute lymphocyte count of greater than 5000/mcL and smudge cells that confirm CLL. Although the diagnostic criteria for CLL are >=5000/mcL B lymphocytes on peripheral smear, a large proportion of patients present with an absolute lymphocyte count > 100,000/mcL. In patients with SLL, peripheral blood smear shows an absolute lymphocyte count of less than 5000/mcL along with lymphadenopathy but without cytopenias. However, lymph node biopsy is required to confirm the diagnosis of SLL. The peripheral blood smear shows leukemic cells, which are small, mature lymphocytes with a darkly stained nucleus, condensed chromatin, and indistinguishable nucleoli with a narrow rim of basophilic cytoplasm. Classical "smudge" cells ("basket" cells) are also seen on peripheral blood smear; these are pathognomic of CLL. The smudge cells are more fragile than normal lymphocytes that are disrupted during the process of being spread on a glass slide.

Keywords: Chronic Lymphocytic Leukemia, Diagnosis, Markers

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Introduction:

Chronic lymphocytic leukemia is a chronic lymphoproliferative disorder characterized by monoclonal B cell proliferation. It is the most common adult leukemia in Western populations and comprises 25 to 30 percent of leukemias in the United States. This activity reviews the evaluation and management of chronic lymphocytic leukemia and highlights the role of interprofessional team members in collaborating to provide well-coordinated care and enhance outcomes for affected patients. Chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL) is an indolent malignancy characterized by increased production of mature but dysfunctional B lymphocytes. CLL/SLL is defined as a monoclonal lymphoproliferative disease characterized by the proliferation and accumulation of morphologically mature but immunologically dysfunctional B-cell lymphocytes that are smudge cells, as noted on peripheral smear. The primary disease sites include peripheral blood, spleen, lymph nodes, and bone marrow. CLL and SLL are identical from a pathologic and immunophenotypic standpoint. Both CLL and SLL originate from B-cell lymphocytes but present with different manifestations depending on where the abnormal cells are found. Usually, the initial leukemic phase represents CLL, where the cells are present in the blood. This eventually progresses to the lymphoma phase, representing SLL, where the cells are found in the lymph nodes. The term SLL is commonly used to represent the lymphoproliferative process limited to the lymph nodes.

The exact etiology of CLL is unknown. Genetic factors, rather than environmental factors, are the most likely cause of CLL. However, few known risk factors for CLL include occupational causes by exposure to certain chemicals, radiation exposure, and tobacco users. Reports of farmers working around rubber manufacturing industries and workers with exposure to benzene and heavy solvent have shown an increased risk of CLL/SLL. However, these associations have not yet been proven. In atomic bomb survivors, no known increase in the incidence of CLL/SLL has been noted. However, there has been an increased risk of other types of leukemia. The uranium miner population, who suffer exposure to ionizing and non-ionizing radiation, has shown increased CLL incidence. Tobacco users and cigarette smokers show a significantly elevated risk of CLL compared to non-tobacco users. The Veterans Affairs recognizes CLL as having a relationship to exposure to Agent Orange or other herbicides during military service.

CLL comprises 25 to 30% of total leukemias in the United States. According to the American Cancer Society, there will be approximately 21,040 new CLL cases and about 4,060 deaths in the year of 2020. Worldwide, 191,000 cases and 61,000 deaths are attributed to CLL/SLL every year. CLL can affect adults as young as 30 years of age. However, it is mostly seen in adults with an average age of 70

years. CLL is extremely rare in children. The incidence is known to rapidly increase with increasing age. CLL has a slightly higher incidence in male populations than female populations (1.3 to 1 to 1.7 to 1). However, studies have shown that women can have a more aggressive form of the disease than men.

Laboratory Finding:

Hematological Findings:

- **Peripheral blood:**

  - ***Lymphocytosis:***

The hallmark of the disease is the presence of an increased white blood cell (WBC) count with predominance of small, mature appearing lymphocytes. Usually the more advanced the disease the higher the WBC count ***(Wierda et al., 2008).***

The diagnosis of CLL requires a sustained monoclonal lymphocytosis greater than 5000/ul ($5x10^9$/L) for at least 3 months ***(Hallek et al., 2008).*** At diagnosis, the absolute lymphocyte count generally exceeds 10,000/ul and is sometimes greater than 100,000/ul. The majority of lymphocytes are small with scanty, bluish cytoplasm, clumped nuclear chromatin, inconspicuous nucleolus and absence of azurophilic granules in the cytoplasm ***(Amato et al., 2007).***

A patient affected by CLL shows the accumulation of small, homogeneous, mature looking lymphocytes, characterized by a peculiar fragility of the cell membrane that leads to frequent rupture of the leukemic cells while preparing the blood film, creating the so-called “smudge cells”, which are characteristic of the disease . Especially in those cases with a high lymphocyte count, medium–large cells with prominent nucleoli may be seen (prolymphocytes) <10%, but when they represent >10% of the cells in CLL patients' peripheral blood, they characterize the aggressive variant of this disease, named CLL/PLL **(*Dearden C, 2012).***

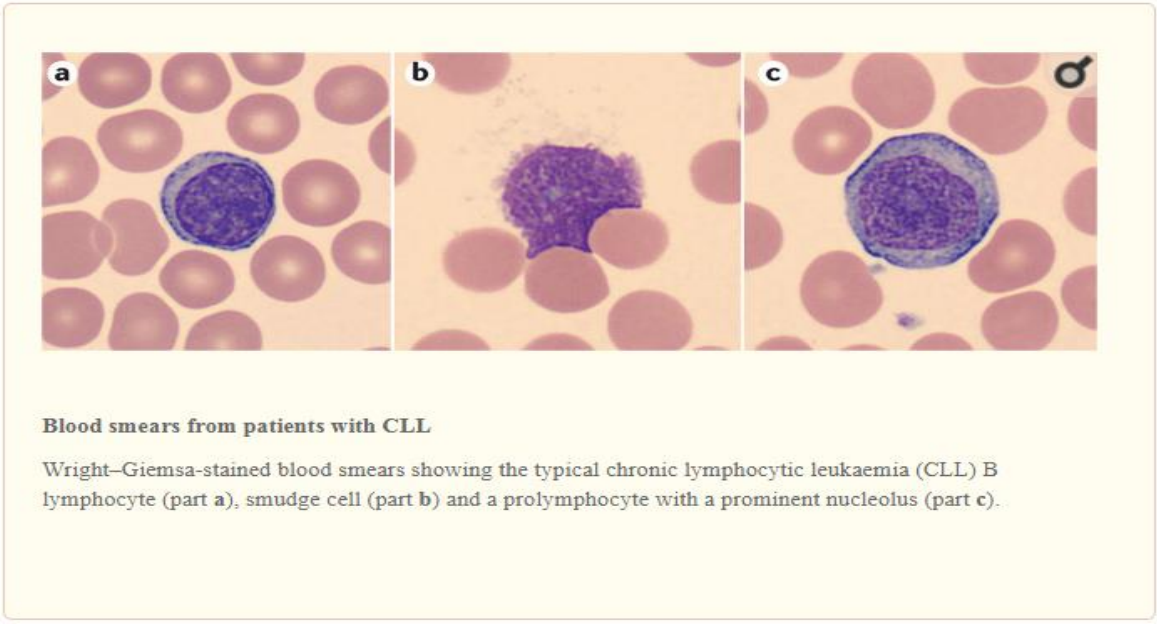


Figure (1) :Blood smear from CLL patient ***(Kipps et al., 2017)***

- ***Anemia:***

About one half of CLL patients are mildly anemic at presentation. The red cells are typically normocytic normochromic and hematocrit may be overestimated unless care is taken to exclude the expanded buffy coat due to extreme lymphocytosis ***(Kipps, 2006b).***

Anemia is an important prognostic feature used in staging, but other secondary causes of anemia (such as iron, folate or vitamin B12 deficiency) must be excluded before staging. Immune hemolytic anemia in B-CLL has more favorable prognosis than anemia caused by bone marrow failure. The incidence of a positive direct antiglobulin (Coomb's) test increases significantly with disease stage ***(Goldman and Mughal, 2005).***

- ***Thrombocytopenia:***

Platelets count below 100,000 is a feature of poor prognosis and it indicates terminal disease unless it is a result of immune phenomenon or splenic pooling and however, at any stage patients can develop immune thrombocytopenia due to antiplatelet antibodies ***(O'Brien and Keating, 2005).***

- ***Neutropenia:***

Rarely, patients with a white blood cell count of less than $5x10^9$/L may be found to have CLL based on phenotyping of the lymphocytes. Neutropenia is seldom severe in CLL until terminal stages of marrow replacement ***(O'Brien and Keating, 2005).***

- ***Bone marrow aspiration:***

In a bone marrow aspirate, an increased lymphocytic infiltration of more than or equal to 30% of all nucleated cells is characteristic of CLL ***(Sah et al., 2003).***

Erythroid hyperplasia may be a striking feature if hemolytic anemia occurs. Also a relative increase in erythroid precursors and/or megakaryocytes in the bone marrow is a feature of hypersplenism even in the presence of lymphocytic infiltration ***(O'Brien and Keating, 2005).***

- ***Bone marrow biopsy:***

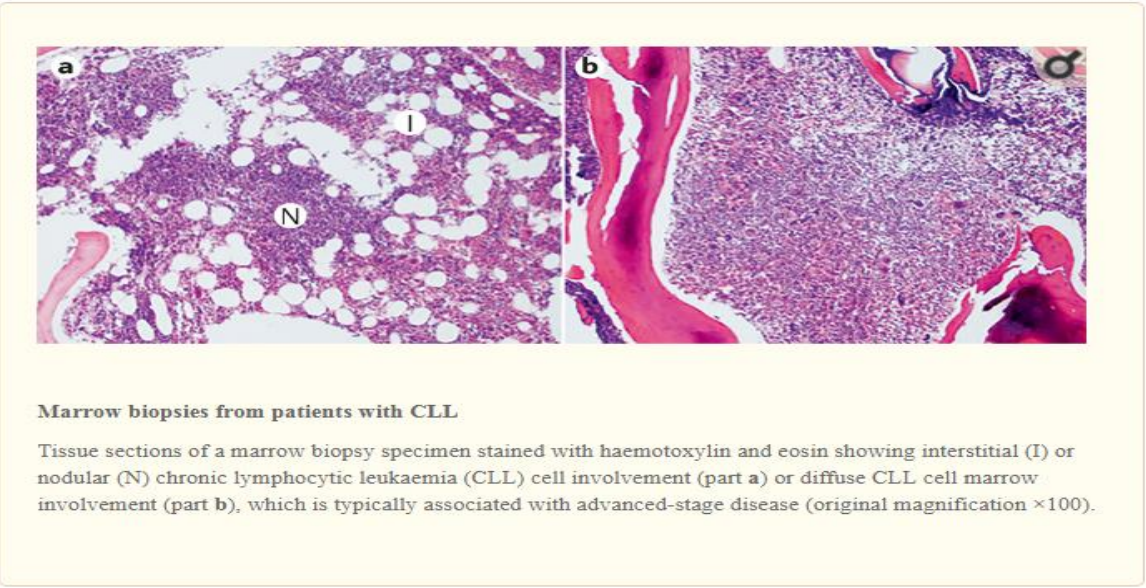
Trephine biopsy is more important than aspirate and is required for; confirmation of diagnosis and exclusion of other lymphoproliferative disorders e.g. follicular lymphoma and hairy cell leukemia; to assess the bone marrow hemopoietic capacity and to define the patterns of bone marrow infiltration ***(Goldman and Mughal, 2005).***

At least 4 patterns of bone marrow infiltration have been established

- Interstitial or lacy pattern (in one third of patients; indicates better prognosis and early stage disease).
- Nodular pattern (10% of patients).
- Mixed nodular interstitial pattern (25% of patients). Both nodular and mixed patterns are seen in relatively early stages of the disease.
- Extensive marrow replacement producing diffuse pattern associated with advanced clinical stage and more aggressive disease ***(Wolowiec et al., 2006).***

In addition, the marrow usually shows reduced numbers of myeloid and erythroid cells, which otherwise have normal maturation. ***(Kipps et al., 2017)***

Although the type of marrow infiltration (which may be nodular, interstitial, mixed nodular and interstitial, or diffuse) reflects the tumor burden and provides some prognostic information, it is recommended to repeat a marrow biopsy in patients with persisting cytopenia after treatment to uncover disease- versus therapy- related causes ***(Michael et al., 2008).***

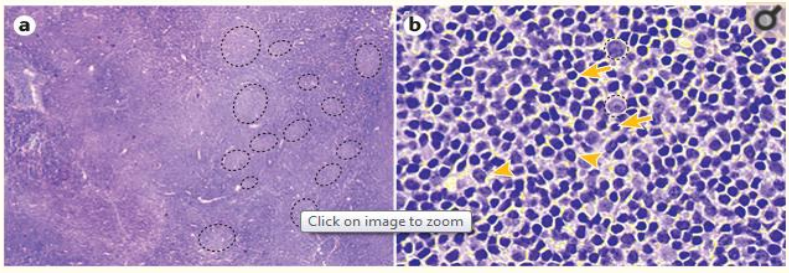


Marrow biopsies from patients with CLL

Tissue sections of a marrow biopsy specimen stained with haemotoxylin and eosin showing interstitial (I) or nodular (N) chronic lymphocytic leukaemia (CLL) cell involvement (part **a**) or diffuse CLL cell marrow involvement (part **b**), which is typically associated with advanced-stage disease (original magnification ×100).

Figure (2) :marrow biopsies from CLL patient *(Kipps et al., 2017)*

A lymph node biopsy might be performed in a patient with an enlarged lymph node as part of a diagnostic evaluation for suspected lymphoma. Excised lymph nodes typically have a diffuse infiltration of well-differentiated small lymphocytes, often obliterating the normal nodal architecture, and scattered, vaguely nodular, pale haematoxylin and eosin-staining areas, appearing as pseudo-follicles , which are enriched with prolymphocytes and paraimmunoblasts; these areas comprise the proliferation centres. The pseudofollicles or proliferation centres are hallmark features in the lymph nodes of patients with CLL or small lymphocytic lymphoma, as they are not observed in other types of lymphomas *(Kipps et al., 2017)*.



Lymph node of patients with CLL

a | Tissue sections of a lymph node stained with haemotoxylin and eosin showing numerous pale-staining pseudofollicles, which are circled (original magnification ×20). **b** | Higher (×400) magnification of a proliferation centre. Representative lymphocytes (arrows), prolymphocytes (arrowheads) or paraimmunoblasts (circles) in a proliferation centre are shown.

Figure (3) :LN biopsies from CLL patient *(Kipps et al., 2017)*

Immunological Findings:

Protein electrophoresis:

Hypogammaglobulinemia and agammaglobulinemia are often observed in CLL patients. The degree of hypogammaglobulinemia correlates with the clinical stage. Reduction in the serum level of IgM precedes that of IgG and IgA. However, 5% of patients have a serum monoclonal immunoglobulin paraprotein. Some cases may show defective and/or unbalanced immunoglobulin chain synthesis by the leukemic B cell clone, resulting in μ heavy chain disease and/or immunoglobulin light chain proteinuria, which can be detected on urine immunoelectrophoresis ***(Kipps et al., 2006b).***

Immunophenotyping:

CLL B cells typically express CD5, CD19 and CD23 (also known as low-affinity immunoglobulin-ε Fc receptor), and have low levels of CD20, but lack expression of CD10 and stain poorly, if at all, with the FMC7 monoclonal antibody, which recognizes an epitope of CD20. CLL cells also express CD200 (also known as OX-2 membrane glycoprotein), which can help to distinguish CLL from mantle cell lymphoma. In addition, the CLL cells of >95% of patients express the onco-embryonic surface antigen ROR1 ***(Kipps et al., 2017).***

Immunophenotyping of the lymphocytes in B-CLL shows them to be B cells CD19 positive also CD23 +ve but sIg, CD22, CD79b and FMC 7 negative or weak. They are CD5 +ve and CD10 negative. All leukemic cells are monoclonal, showing evidence of light chain restriction (either kappa or lambda) ***(Delgado et al., 2003).***

The scoring system for diagnosis of CLL:

The immunophenotype of typical B cell CLL includes the coexpression of weak monotypic surface immunoglobulin, CD5, CD19, CD23 and weak or absent CD79b, CD22 and FMC7 ***(Oscier et al., 2004).*** The expression of these surface markers may vary. Hence an immunophenotypic scoring system is used for the diagnosis of CLL and to differentiate CLL from other B cell malignancies ***(Yee and O'Brien, 2006).***

Scores in CLL are usually >3, in other B-cell malignancies the scores are usually <3 ***(Oscier et al., 2004).***

Table (1): *Scoring system for diagnosis of CLL.*

Points		*Marker*
0	*1*	
Negative	Positive	*CD5*
Negative	Positive	*CD23*
Positive	Negative	*FMC7*
Medium/high	Low	*sIg*
Medium/high	Low/negative	*CD22/CD79b*

(Matutes et al., 2010)

Biochemical Findings:

- Beta 2 micorglobulin (β2-MG):

It has been suggested that serum β2-MG is the most powerful prognostic factor in patients with CLL. High β2-MG has been associated with significantly shorter survival for both untreated and previously treated patients ***(Abbott, 2006).***

- Lactate dehydrogenase (LDH):

The LDH is generally elevated in patients with aggressive disease and in nearly all patients with Richter's transformation ***(Abbott, 2006).***

- Serum calcium:

Hypercalcemia is rare in patients with CLL and may indicate Richter's transformation ***(Vlasveld et al., 1999).*** Other laboratory tests (creatinine, urea, electrolytes, bilirubin, transaminases, Coombs' test, protein electrophoresis and urine status) are usually recommended to assess the extent of the disease ***(Barbara and Michael, 2007).***

Molecular Finding:

Genomic aberrations:

CLL cells carry several recurrent genomic aberrations, though none can be considered specific of the disease. In contrast to other mature lymphoproliferative disorders, translocations are rarely observed, while the most typical aberrations lead to loss or gain of genetic material. Most aberrations appear during the course of the disease whereas none can be considered an early transforming event.

In contrast, the development of molecular cytogenetic techniques as Fluorescent In Situ Hybridization (FISH) enables the detection and direct visualization of chromosomal abnormalities in up to 80% CLL cases ***(Dohner et al., 2000)***.

The most frequent aberrations are:

- Deletion of 11q
- Deletion of 13q
- Deletion of 17p and TP53 mutations
- Trisomy 12 **(Cimmino et al., 2005)**

The advent of next-generation sequencing (NGS) techniques has helped uncover genetic mutations in, for example,

- ❖ Neurogenic locus notch homolog protein 1 (NOTCH1) mutations
- ❖ Splicing factor 3B sub-unit 1 (SF3B1) mutations
- ❖ Baculoviral inhibitor of apoptosis repeat– containing 3 protein (BIRC3) mutations ***(Fabbri et al., 2011 and Wang et al., 2011)***

More than 50% of the cases show only a single abnormality, 20% carries two and about 10% more than two aberrations. Multivariate analysis showed that genomic aberrations are independent prognostic factors in CLL with 13q deletions being associated with a favorable prognostic effect whereas the remaining abnormalities are associated with a progressively poorer prognosis ***(Oscier et al., 2002)***.

Genomic aberrations, detected by FISH, should be evaluated at least once during the course of the disease, and in particular before initiating therapy, because cytogenetic abnormalities (17p–, 11q–) have apparent prognostic value and may influence therapeutic decisions ***(Barbara and Michael, 2007)***.

CLL can be diagnosed when the following conditions exist:

1. A lymphocyte count > $5x10^3$/cmm of small mature lymphocytes in the peripheral blood.
2. The immunophenotype as detected by flow cytometry with the following features:
    a. Co-expression of CD19 and CD5 together with CD23 expression;
    b. Low-expression of surface immunoglobulin (sIg) and, accordingly, absence or low expression of CD79b.
    c. Light chain restriction.

3. Bone Marrow aspirates showing greater than 30% lymphocytes.*(Hallek et al., 2008)*

References:

[1] Dreger P, Schetelig J, Andersen N et al. (2014): Managing high-risk CLL during transition to a new treatment era: stem cell transplantation or novel agents?. Blood. 2014; 124: 3841–3849
[2] Dutta-Simmons J, Zhang Y, Gorgun G, et al., (2009): Aurora kinase A is a target of Wnt/beta-catenin involved in multiple myeloma disease progression. Blood. 2009; 114:2699–2708.
[3] Dühren-von Minden M, Übelhart R, Schneider D et al., (2012): Chronic lymphocytic leukaemia is driven by antigen-independent cell-autonomous signalling. Nature; 489:309–312.
[4] EL-SHARNOUBY, J. A. H., EL-KHAMESSY, G. A., & TAMER, A. E. H. E. (2018). The Role of Expression of Lymphoid Enhancer-Binding Factor-1 (LEF-1) in Patients with Chronic Lymphocytic Leukemia. The Medical Journal of Cairo University, 86(September), 2931-2937.
[5] ElSourdy, M. A., Ayad, M. W., Fayad, A. I., & Youssef, S. M. (2019). Lymphoid enhancer factor 1 gene expression in comparison to other prognostic markers in adult B-acute lymphoblastic leukemia. The Egyptian Journal of Haematology, 44(1), 40.
[6] Erdfelder, F., Hertweck, M., Filipovich, A., Uhrmacher, S., & Kreuzer, K. A. (2010). High lymphoid enhancer-binding factor-1 expression is associated with disease progression and poor prognosis in chronic lymphocytic leukemia. Hematology reports, 2(1).
[7] Fabbri G, Rasi S, Rossi D, et al., (2011): Analysis of the chronic lymphocytic leukemia coding genome: role of NOTCH1 mutational activation. J. Exp. Med; 208:1389– 1401.
[8] Fabbri M, Bottoni A, Shimizu M et al., (2011): Association of a microRNA/TP53 feedback circuitry with pathogenesis and outcome of B-cell chronic lymphocytic leukemia. JAMA. 2011; 305:59–67.
[9] Fazi C, Dagklis A, Cottini F et al., (2010): Monoclonal B cell lymphocytosis in hepatitis C virus infected individuals. Cytometry B Clin Cytom. 2010; 78: S61–S68.
[10] Feder, K. (2019). Dissecting the role of lymphoid enhancer factor 1 (LEF1) in acute myeloid leukemia (Doctoral dissertation, Universität Ulm).
[11] Fetisov, T. I., Lesovaya, E. A., Yakubovskaya, M. G., Kirsanov, K. I., & Belitsky, G. A. (2018). Alterations in WNT signaling in leukemias. Biochemistry (Moscow), 83(12-13), 1448-1458.
[12] Fink A, Bahlo J, Robrecht S et al., (2017): Lenalidomide maintenance after first-line therapy for high-risk chronic lymphocytic leukaemia (CLLM1): final results from a randomised, double-blind, phase 3 study. Lancet Haematol. 2017; 4: e475–e486
[13] Fischer, K., Bahlo, J., Fink, A.M. et al., (2016): Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. Blood. 2016; 127: 208–215
[14] Fischer, K., Cramer, P., Busch, R., et al., (2012). 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. Journal of Clinical Oncology, 30(26), 3209-3216.
[15] Foà R, Del Giudice I, Cuneo A et al., (2014): Chlorambucil plus rituximab with or without maintenance rituximab as first-line treatment for elderly chronic lymphocytic leukemia patients. Am J Hematol. 2014; 89: 480–486
[16] Gao X, Mi Y, Ma Y et al., (2014): LEF1 regulates glioblastoma cell proliferation, migration, invasion, and cancer stem-like cell self-renewal. Tumour Biol. 2014;35:11505–11511.
[17] Getahun A, Beavers N, Larson S et al., (2016): Continuous inhibitory signaling by both SHP-1 and SHIP-1 pathways is required to maintain unresponsiveness of anergic B cells. J. Exp. Med. 2016; 213:751–769.
[18] Ghia P, Chiorazzi N and Stamatopoulos K (2008): Microenvironmental influences in chronic lymphocytic leukaemia: the role of antigen stimulation. J Intern Med. 2008; 264: 549–562.
[19] Giambra V, Jenkins C, Lam S et al., (2015): Leukemia stem cells in T-ALL require active Hif1α and Wnt signaling. Blood 2015; 125: 3917–3927

[20] Goede V, Fischer K, Engelke A et al.,(2015): . Obinutuzumab as frontline treatment of chronic lymphocytic leukemia: updated results of the CLL11 study. Leukemia. 2015; 29: 1602–1604
[21] Goldman J and Mughal T (2005): Chronic lymphcytic leukemia and other B-cell disorders in: Postgraduate Haematology, 5th edition. Hoffbrand A, Catovsky D and Tuddenham E (eds).Blackwell publishing Ltd; P: 619.
[22] Grainger, S., Traver, D., & Willert, K. (2018). Wnt signaling in hematological malignancies. In Progress in molecular biology and translational science (Vol. 153, pp. 321-341). Academic Press.
[23] Greil R, Obrtlíková P, Smolej L et al., (2016): Rituximab maintenance versus observation alone in patients with chronic lymphocytic leukaemia who respond to first-line or second-line rituximab-containing chemoimmunotherapy: final results of the AGMT CLL-8a Mabtenance randomised trial.Lancet Haematol. 2016; 3: e317–329
[24] Gunnarsson, R., Mansouri, L., Isaksson, et al., (2011). Array-based genomic screening at diagnosis and during follow-up in chronic lymphocytic leukemia. Haematologica, 96(8), 1161.
[25] Guo X, Zhang R, Liu J, et al., (2015): Characterization of LEF1 high expression and novel mutations in adult acute lymphoblastic leukemia. PLoS One;10:e0125429.
[26] Gutierrez A J, Tschumper R, Wu X et al,. (2010): LEF-1 is a prosurvival factor in chronic lymphocytic leukemia and is expressed in the preleukemic state of monoclonal B-cell lymphocytosis. Blood 116: 2975–2983.
[27] Gutierrez Jr, A., Tschumper, R. C., et al., (2010). LEF-1 is a prosurvival factor in chronic lymphocytic leukemia and is expressed in the preleukemic state of monoclonal B-cell lymphocytosis. Blood, The Journal of the American Society of Hematology, 116(16), 2975-2983.
[28] Haferlach C, Dicker F, Schnittger S, et al., (2007): Comprehensive genetic characterization of CLL: a study on 506 cases analysed with chromosome banding analysis, interphase FISH, IgV (H) status and immunophenotyping. Leukemia; 21: 2442– 2451.
[29] Hall A, Vickers M, McLeod E, et al., (2005): Rh autoantigen presentation to helper T cells in chronic lymphocytic leukemia by malignant B cells. Blood; 105:2007–2015.
[30] Hallek M, Cheson B, Catovsky D et al., (2008): Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: A report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood; 111: 5446–5456.
[31] Hallek M, Fischer K, Fingerle-Rowson G et al., (2010): Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial . Lancet; 376: 1164 – 1174.
[32] Hallek, M. (2019). Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. American journal of hematology, 94(11), 1266-1287.
[33] Hamblin T (2006): Autoimmune complications of chronic lymphocytic leukemia. SeminOncol; 33:230–239.
[34] Hamblin T, Davis Z, Gardiner A et al., (2000): Unmutated IgVH genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood; 94: 1848.
[35] Hamblin, T. J., Orchard, J. A., Ibbotson et al., (2002). CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. Blood, The Journal of the American Society of Hematology, 99(3), 1023-1029.
[36] Hanahan D and Weinberg R (2011): Hallmarks of cancer: the next generation. Cell; 144:646-674.
[37] Hao, Y. H., Lafita-Navarro, M. C., Zacharias et al., (2019). Induction of LEF1 by MYC activates the WNT pathway and maintains cell proliferation. Cell Communication and Signaling, 17(1), 1-16.
[38] Herishanu Y, Pérez-Galán P, Liu D, et al., (2011): The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. Blood. 2011; 117:563–574.
[39] Huang, P., Yan, R., Zhang, et al., (2019). Activating Wnt/β-catenin signaling pathway for disease therapy: challenges and opportunities. Pharmacology & Therapeutics, 196, 79-90.
[40] International C. L. L. I. P. I. working group. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol.2016;17:779–790.

[41] Jain N and O'Brien S (2012): Chronic lymphocytic leukemia with deletion 17p: emerging treatment options. Oncology; 26(11): 1067–1070.
[42] Jain N and O'Brien S (2015): nitial treatment of CLL: integrating biology and functional status. Blood; 126: 463–470
[43] Janovská P and Bryja V (2017): Wnt signalling pathways in chronic lymphocytic leukaemia and B-cell lymphomas. British journal of pharmacology, 174(24), 4701-4715.
[44] Jeromin S, Weissmann S, Haferlach C, et al., (2014): SF3B1 mutations correlated to cytogenetics and mutations in NOTCH1, FBXW7, MYD88, XPO1 and TP53 in 1160 untreated CLL patients. Leukemia; 28(1): 108–117.
[45] Jia M, Zhao H, Shen H et al., (2015): Overexpression of lymphoid enhancer-binding factor-1 (LEF1) is a novel favorable prognostic factor in childhood acute lymphoblastic leukemia. Int J Lab Hematol. 2015;37:631–640.
[46] Jia, M., Zhao, H. Z., et al., (2015). Overexpression of lymphoid enhancer-binding factor-1 (LEF 1) is a novel favorable prognostic factor in childhood acute lymphoblastic leukemia. International journal of laboratory hematology, 37(5), 631-640.
[47] Johnston J (2004). Chronic lymphcytic leukemia in: Wintrobe's Clinical Hematology, 11th edition. Greer J, Foesrster J, Lukens J, Rodgers G, Paraskevas F. and Glader B. (eds). Lippincott Williams and Wilkins publishers; P: 1971.
[48] Joshi, A. A., Nerurkar, A. V., & Shirsat, N. V. (2018). Hsa-miR-106b-5p Negatively Regulates LEF1. Journal. Vol, 6(4), 112-117.
[49] Kasar S and Brown J (2016): Mutational landscape and underlying mutational processes in chronic lymphocytic leukemia. Mol Cell Oncol. 2016; 3: e1157667
[50] Kasar S, Kim J, Improgo R et al., (2015): Whole-genome sequencing reveals activation-induced cytidine deaminase signatures during indolent chronic lymphocytic leukaemia evolution. Nat Commun. 2015; 6: 8866.
[51] Kaucka M, Plevova K, Pavlova S, et al,. (2013): The planar cell polarity pathway drives pathogenesis of chronic lymphocytic leukemia by the regulation of B-lymphocyte migration. Cancer Res 73: 1491–1501.
[52] Ke, X., Do, D. C., et al,. (2019). Ras homolog family member A/Rho-associated protein kinase 1 signaling modulates lineage commitment of mesenchymal stem cells in asthmatic patients through lymphoid enhancer–binding factor 1. Journal of Allergy and Clinical Immunology, 143(4), 1560-1574.
[53] Kipps T (2001): Chronic lymphocytic leukemia and related diseases. In Williams's Hematology, 6th edition. Beutler E, Lichtman M, Coller B, and Kipps T. (eds).Health Professions Division; p: 261.
[54] Kipps T (2006): Chronic lymphocytic leukemia and related diseases. In Williams's Hematology, 7th edition. Beutler E, Lichtman M, Coller B, and Kipps T. (eds).MC Graw-Hill Inc; p:1343.
[55] Kipps T, Stevenson F, Wu C et al., (2017): Chronic lymphocytic leukaemia. Nat Rev Dis Primers.2017; 3:16096
[56] Klein U, Lia M, Crespo M et al., (2010): The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. Cancer Cell; 17(1):28–40.
[57] Kobayashi W and Ozawa M (2013): The transcription factor LEF-1 induces an epithelial-mesenchymal transition in MDCK cells independent of β-catenin. Biochem Biophys Res Commun; 442:133–138.
[58] Kralik, P., & Ricchi, M. (2017). A basic guide to real time PCR in microbial diagnostics: definitions, parameters, and everything. Frontiers in microbiology, 8, 108.
[59] Landau D, Tausch E, Taylor-Weiner A et al., (2015): Mutations driving CLL and their evolution in progression and relapse. Nature. 2015; 526:525–530.
[60] Landgren O, Rapkin J, Caporaso N et al., (2007): Respiratory tract infections and subsequent risk of chronic lymphocytic leukemia. Blood. 2007; 109: 2198–2201.
[61] Lane S, Wang Y, Lo Celso C et al., (2011): Differential niche and Wnt requirements during acute myeloid leukemia progression. Blood 2011; 118: 2849–2856.
[62] Lento W, Congdon K, Voermans C, et al., (2013): Wnt signaling in normal and malignant hematopoiesis. Cold Spring Harb Perspect Biol e-pub ahead of print 1 February 2013 doi:10.1101/cshperspect.a008011.
[63] Leotard S, Chastang C, Travade P et al., (2000): Prognostic relevance of a scoring system based on clinical and biological parameters in early chronic lymphocytic leukemia. Hematol J; 1(5):301-6.

[64] Li H, Zhang P, Sun X et al., (2015): MicroRNA-181a regulates epithelial-mesenchymal transition by targeting PTEN in drug-resistant lung adenocarcinoma cells. Int J Oncol; 47:1379–1392
[65] Liang J, Li X, Li Y et al., (2015): LEF1 targeting EMT in prostate cancer invasion is mediated by miR-181a. Am J Cancer Res. 2015; 5:1124–1132
[66] Lichtenstein P, Holm NV, Verkasalo PK et al., (2000): Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med. 2000 Jul 13; 343(2):78-85.
[67] Ling L, Nurcombe V and Cool S (2009): Wnt signaling controls the fate of mesenchymal stem cells. Gene; 433:1–7.
[68] Lu D, Zhao Y, Tawatao R et al,. (2004): Activation of the Wnt signaling pathway in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 101: 3118–3123.
[69] MacDonald B, Tamai K and He X (2009): Wnt/beta-catenin signaling: components, mechanisms, and diseases. Developmental cell, 17(1), 9-26.
[70] Mackus W, Frakking F, Grummels A et al., (2003): Expansion of CMV-specific CD8+CD45RA+ CD27- T cells in B-cell chronic lymphocytic leukemia. Blood; 102:1057–1063.
[71] Malavasi F, Deaglio S, Damle R et al., 2011): CD38 and chronic lymphocytic leukemia: adecade later. Blood; 118(13):3470-3478.
[72] Malavasi F, Deaglio S, Damle R, et al., (2011): CD38 and chronic lymphocytic leukemia: a decade later. Blood; 118:3470–3478.
[73] Malcikova J, Smardova J, Rocnova L et al., (2009): Monoallelic and biallelic inactivation of TP53 gene in chronic lymphocytic leukemia: selection, impact on survival, and response to DNA damage. Blood; 114: 5307 – 5314.
[74] Marasca R, Maffei R, Martinelli S et al., (2013): Clinical heterogeneity of de novo 11q deletion chronic lymphocytic leukaemia: prognostic relevance of extent of 11q deleted nuclei inside leukemic clone. Hematological Oncology; 31(2): 348–355.
[75] Marti, G. E., Rawstron, A. C., et al., (2005). Diagnostic criteria for monoclonal B-cell lymphocytosis. British journal of haematology, 130(3), 325-332.
[76] Martinez I, Gardiner A, Board K et al., (2008): Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells. Oncogene. 2008;27:2575–2582