

RESEARCH PROGRESS IN CHRONIC LYMPHOCYTIC LEUKEMIA

Iftikhar Ali¹, Yanting Jiang¹, Wu fan¹ and Shangqin Liu^{1*}¹Department of Hematology, Zhongnan Hospital, Wuhan University, Wuhan, China.***Corresponding Author: Shangqin Liu**

Department of Hematology, Zhongnan Hospital, Wuhan University, Wuhan, China.

Article Received on 13/04/2021

Article Revised on 04/05/2021

Article Accepted on 25/05/2021

ABSTRACT

Cancer is an uncontrolled division of cell occurs due to genetic alterations and mutation. Chronic lymphocytic leukemia is the heterogeneous lymphocytic malignancy worldwide that leads to death. The advancement of genetic analysis techniques and the identification of suitable biomarkers show significant differences in the treatment period. In this paper will discuss the microRNA that is small Non-coding RNAs and is involved in CLL beginning, development, and resistance to therapy and also will explain the stages of CLL. Moreover, we will also discuss the fluorescent in-situ hybridization FISH-based prognostic factors common genetic aberrations or mutations in CLL. In this paper we will also discuss about the methods for the treatment of lymphomas.

KEYWORDS: Lymphocytic leukemia, miRNA, Biomarkers, Genetic mutations, Treatment methods.**INTRODUCTION**

Lymphocytic leukemia is a chronic lymphoproliferative disorder.^[1,2] CLL is of the primary heterogeneous lymphocytic malignancies which happen by aggregation of natural B lymphocytes.^[1,3] CLL begins with the lymphocytes in the bone marrow through overexpression of white blood cells.^[4] It is one of the common hematological malignancies which is still a public health problem worldwide.^[1] We haven't been able to spot this deadly disease in the past. CLL patients will now be treated by identifying the symptoms of the disorder and using current analysis techniques.

The key dynamics in CLL are V-gene mutation, (IGHV)^[5], CD38, ZAP-70, deletion of chromosomes^[5], Rai and Binet staging systems, LDT.^[6,7] Early diagnostic approaches are needed to diagnose a specific disease. Finding various biomarkers at different stages of disease can provide better treatment of specific diseases.^[8] In terms of survival prediction, two well-known staging systems Rai and Binet staging systems^[2,9,10] are used. These staging systems are easy to use and predicts the endurance and response to treatment.^[5,11]

Furthermore, Rai and staging system does not show the high variability in CLL.^[12] They derived from the original Rai and Binet staging system having different subgroups. Low-risk CLL patients have chronic inflammation, intermediate-risk CLL patients have a palpable lymph node, and high-risk CLL patients have anaemia (Hemoglobin (hb 110g/l) or thrombocytopenia.^[3,13] Cancer causing gene is TCL1 that plays a significant role in the initial phases of the

syndrome.^[13-15] The expression of various miRNAs were assessed in different types of CLL such as Aggressive CLL, Indolent CLL, Aggressive CLL with 11q deletion.^[15,16] In indolent and aggressive CLL, when it is compared to a central group miR-29 is up-regulated. Lower expression was expressed in MicroRNA-29 in aggressive than it was in the indolent CLL type^[15] (Figure 1).

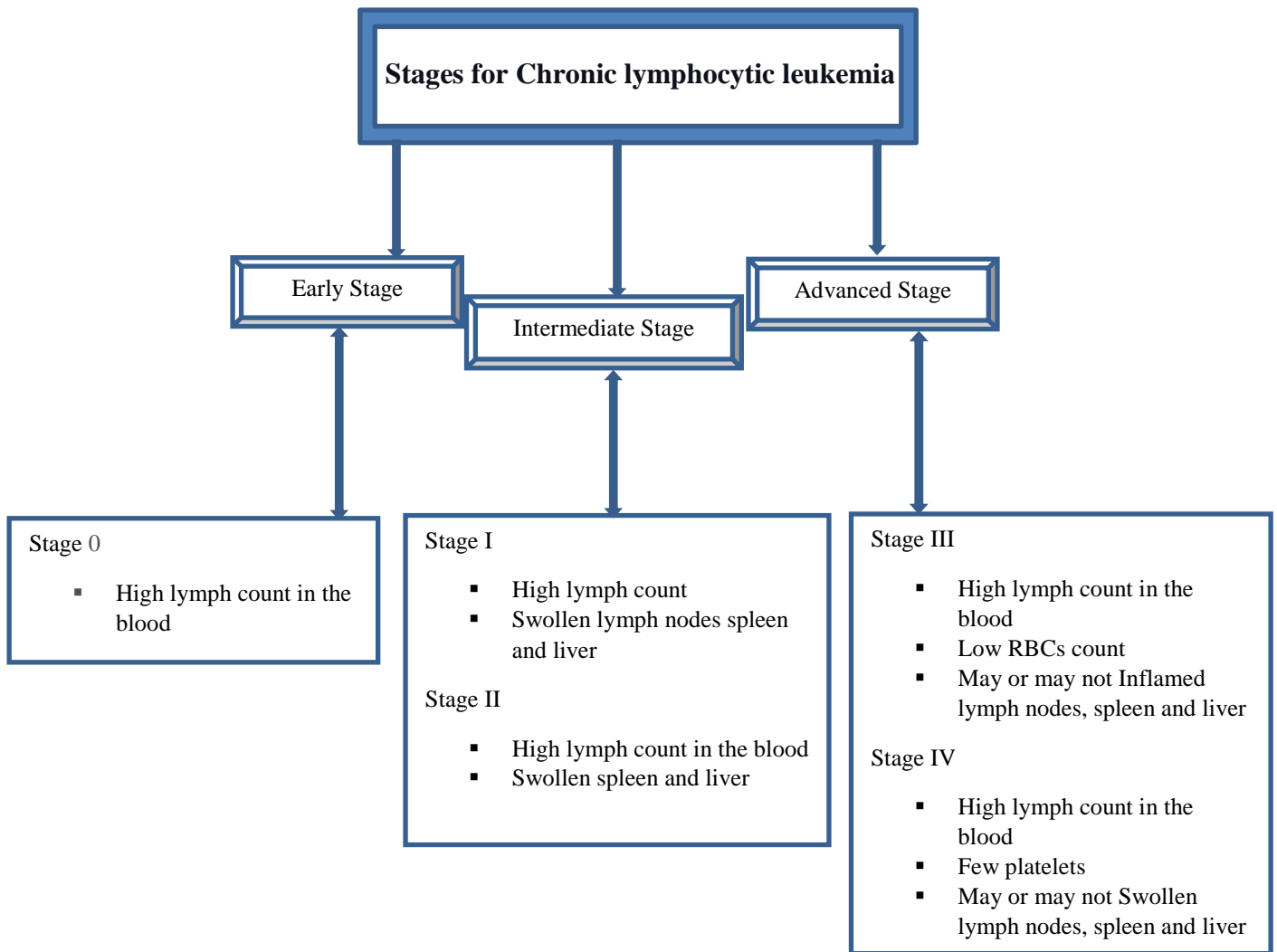


Figure 1: Stages of chronic lymphocytic leukemia that show how serious and advance is the stage of disease. By observing these stages physician prescribe the diagnostic procedures to deal with the CLL.

MicroRNA as a Diagnostic Marker

MicroRNAs is a non-coding RNAs function in various cellular and molecular processes and because of their profile expression its show a vital role in the beginning and development of cancers.^[5,17,18] MicroRNAs as a biomarker in different cancers stages such as CLL.^[17] Therefore, MicroRNA are presented to be the potential biomarkers in multiple type of cancer for evaluation of the response for treatment in CLL patients.^[19]

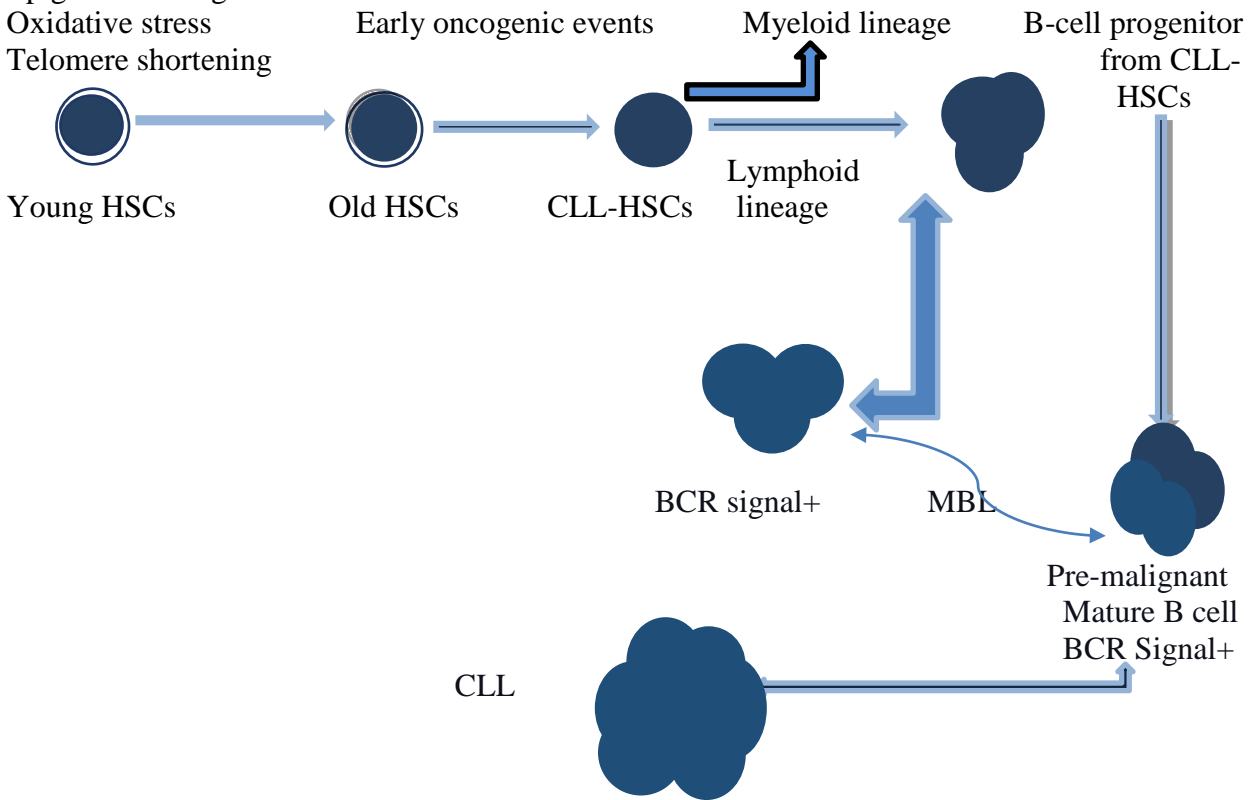
Fathullahzadeh *et al.*, 2016 “shows the expression of the circulating miR-192 in the peripheral blood mononuclear cell (PBMCS) of 20 patients by applying real-time PCR.^[4,5] Their findings indicate that miR-192 was down-regulated (2.5folds) in the patient group than the healthy group.^[4,16] Based on the silicon analysis, they found that CDKNLA/P21 could be one of the main targets miR-192 in CLL cells.

An additional study, Li Moffett *et al.* 2011 “evaluating microRNAs profiles in 38 CLL patients and nine regular

donors. Gene set enrichment analysis (GSEA) shows that there is an association between the expression of a variety of MicroRNAs in the standard and CLL group samples. They showed that some MicroRNAs, including miR-34a, mir-155, miR-342-3p, were up-regulated, and miR-103, miR-181a, and miR-181b were down-regulated in CLL patients when their expression was compared in a regular group. Moreover, the regulation of the miR-29 and the miR-223 might be associated with the level of ZAP71 (+) and IGV (H).^[20] These finding shows that microRNAs can be used as a diagnostic biomarker for a patient with CLL.

Schematic representation of CLL

Epigenetic changes
Oxidative stress
Telomere shortening



Oncogenic processes

Figure 2: Diagrammatic representation of development of CLL by Hematopoietic stem cells.

Treatment algorithm for CLL patients

Treatment landscape has been changed over the period of time by the discovery of different way of treatment such as chemotherapy, immunotherapy, stem cell transplant

and targeted drug therapy.^[21] All these ways of treatment and staging systems help to cure this genetic disease and increase the rate of survival of the patient.

Front line treatment

Table 1: Frontline treatment strategies to start diagnosis.

| Phase | Ability to intake the dose | Treatment |
|--|----------------------------|---|
| Binet staging system (A-b) | Inapplicable | Not required |
| Rai staging system | | |
| 0-II | Go | FCR and BR above 60 years |
| Inactive | | |
| Active disease Binet Stage (c) and Rai staging III-IV | Go | Apply one of the following way of treatment. Ibrutinib, Idelalisib, Rituximab, Allogeneic SCT |
| | Slow go | Chkorambucil+ Obinutuzumab or + Rituximab or +Ofatumumab or Ibrutinib |
| | Slow go | Ibrutinib, Alemtuzumab,HD, Rituximab or Ofatumumab |

Second line treatment

Table 2: Second line treatment strategies to start diagnosis.

| Response to treatment | Ability to intake the dose | Treatment |
|-----------------------|----------------------------|---|
| Growth within 3 years | Go | FA,FCR after BR, Venetoclax,A Dex, Lenalidomide(+R) |
| Growth within 3 years | Slow go | Change the therapy from one of the following Ibrutinib, idelalisib+R Venetoclax A FCR lite BR Lenalidomide+R Ofatumumab, HD R Discuss consolidation with Allogeneic SCT |
| Progress after 3 year | All | Repeat front line therapy |

Parameters to treat CLL

Few parameters that physician must keep in mind while treating the CLL patient.

Parameters to treat CLL

- 1) Symptoms of the patient
- 2) Disease stage
- 3) Genetic risk leukemia
- 4) Patients fitness

Prognostic and diagnostic biomarkers in CLL

Advancement of genetic analysis technique like next-generation sequencing^[3] and the whole genome sequencing helps to discover the genetic mutations in

CLL.^[3,22] Fluorescent in-situ hybridization (FISH) helps to detect the specific sequences of DNA on chromosome.^[23] Some mutations offered new prognostic information to cure patients with CLL such as NOTCH1, (MYD88), (SF3B1) and (ATM).^[1,3,24,25]

A classified model for the genomic alterations in CLL was developed in downcast order.^[3] Kiefer *et al* “showed that trisomy 12, deletion of 17p, 11q, and 13q are common chromosomal abnormalities in CLL patients. These abnormalities affect various targets such as miR-15, miR-16, Tp53, and ataxia-telangiectasia mutated ATM. That’s why, these chromosomal abnormalities and their targets could be used as a potential biomarker in CLL.”^[25]

Table 3: Commonly used prognostic markers and their median of survival.

| Commonly used Prognostic markers | Median of survival years |
|----------------------------------|--------------------------|
| Deletion 13q | 17years |
| Deletion 11q | 6-8years |
| Trisomy 12 | 9-11years |
| Deletion 17P | 2-3years |
| IGHV mutated | 24years |
| IGHV un-mutated | 8Years |
| CD38>30% | <10years |
| CD38<30% | >15years |
| ZAP-70>20% | 6-10years |
| ZAP-70<20% | >15years |
| Normal Cytogenetic | 9-11years |

Deletion 13q-11q may cause the variability in the clinical course of the disease of that specific type of lymphoma.^[3] The most common genomic abnormalities in CLL is deletion of 13q14 band. Those patients who carry deletion 13q may have variability in their clinical course.^[25,26] Thus, based on the percentage of CLL with del(13q) two prognostic groups are established.^[3,12,27] Deletion 11q in CLL affects the region that quays the ATM gene.^[10,25,26] Advanced stage disease identifies the deletion of 11q. In different analysis, deletion of 11q region are associated with the autonomous factor of poor prognosis.^[28]

Deletion of 17P13 and TP53 mutation deletion (17p) usually includes most of the short arm of

chromosome.^[29] The deletion 17p13 band harboring TP53.^[25,30,31] In most of the cases with the del(17p) display damage of one copy and alteration of the remaining copy.^[29] Screening for del(17p) by using FISH techniques is recommended for all CLL patients before the start of the treatment.^[6,11] Identification of cytogenetic abnormalities by using FISH must be performed before therapy and at disease relapse.^[3,25]

Trisomy 12 is the 3rd most common abnormality in CLL identified by fluorescent In-situ hybridization.^[3,28] Trisomy 12 is linked with analytical morphology and immune-phenotype. These aberrations are related with early development of cancer.^[26] Recent analysis suggest

that development-free survival (PFS) may be shorter OS is promising.^[5,9,26]

IGHV gene mutation to predict the clinical prognosis at diagnosis significantly, IGHV gene mutation was the 1st biological marker. Mutated IGHV genes correlates with the longer times to clinical progression, treatment, and prolonged median survival time.^[3,6] IGHV mutational the most considerable predictive factors currently used to help in the treatment. The detection of IGHV mutation status is costly and requires expertise in the analysis. Therefore, researcher tried to find the alternate marker of IGHV.^[3,5]

Expression of CD38 is glycoprotein controlled by the cancer microenvironment.^[3] Expression of CD38 work as a surrogate marker for IGHV mutation status because of CLL with unmutated IGHV genes expressed a high level of CD38.^[3] Its levels can fluctuate over the course of the disease. The T-cell receptor complex signalling protein ZAP-70 is expressed in CLL but not in normal B lymphocytes.^[14] ZAP-70 expression seems to be constant over the progression of CLL and may predict the survival rate.^[10] The expression of CD38 and ZAP-70 has been linked to a better prognosis.^[3]

NOTCH1 mutation NOTCH1 is a trans-membrane protein encoded through the NOTCH1 gene.^[3,32] That are involved in regulating the hematopoietic CLL development.^[28,33,34] NOTCH1 mutation is strongly associated with the marker of poor prognosis. Studies shows that NOTCH1 mutation eliminating the PEST domain, Glutamic acid (E), serine (S), and threonine (T) have NOTCH1 mutations influence survival.^[29,30,35]

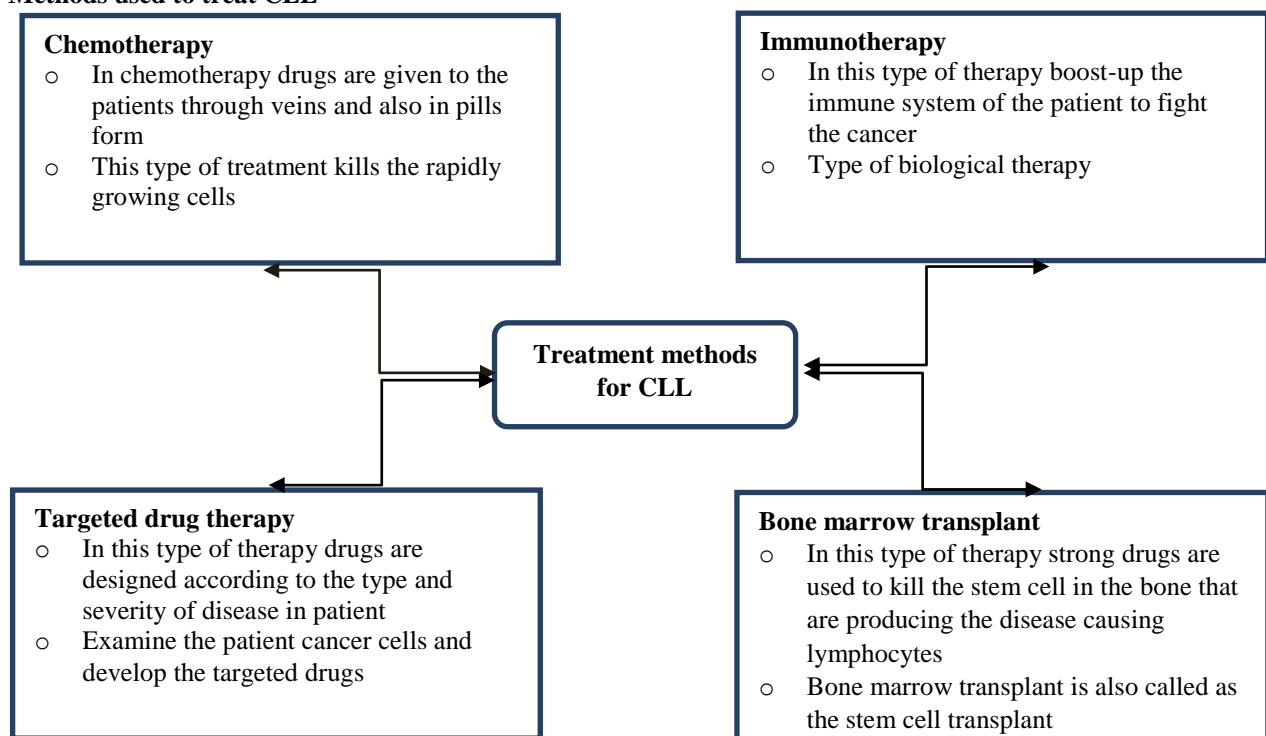
whereas prognostic value as a novel risk marker in CLL.^[36,37]

SF3B1 mutation was found in approximately 10% of CLL patients.^[38,39] Mutated SF3B1 associated to the adverse clinical outcomes protein of splicing factor 3b (Amaya-Chanaga & Rassenti, 2016; Shuai *et al.*, 2020). By the generation of alternative splicing transcripts contribute to CLL.^[40] SF3B1 mutations occurs due to missense mutation or frequently in-frame deletion. The most common mutation codons are Lys666, Lys700, and Gly742.^[3,38]

ATM mutations the gene that cause ATM mutation are found on the long arm of chromosome 11.^[3,9,10] This type of mutation may occurs with or without the deletion of 11q.^[3] This type of mutation is associated with the poor prognosis and genomic instability. Disruption and alteration of these genes cause the genetic instability.^[40] These insertions and deletion cause a lack of function of the ATM protein.^[38] ATM mutations are associated with the unmutated IGHV, ZAP-70 expression, and del(11q) and are found in the 25% of CLL patients.^[12]

MYD88 mutations are found in patients at low frequency at about 3-5% in comparison to patients with NOTCH1, TP53, ATM, And SF3B1.^[3,12] MYD88 mutations are called as driver mutation and develop in early CLL development.^[41,42] The gene that cause this mutation are present at the 3p22 position of the chromosome.^[10,43] MYD88 mutations occurs due to association with the IGHV, ZAP-70, CD38 expression and $\beta 2 M^3$. This type of mutation is found in younger patients.^[36,44]

Methods used to treat CLL



CONCLUSION

To summarize, from the last few years our understanding of the molecular pathogenesis of CLL expanded. It all started with the development of cytogenetic analysis, which is still useful for whole genome sequencing. As CLL is the primary death causing cancer over the globe, and treatment options for this deadly disease remain uncertain. However, as the world shifts towards modern sciences as a result, we are able to understand the genetic origin of the disease, which aids in the diagnosis and treatment of the disease. While the advancement of cytogenetic methods of treatment we are able to cure the disease at molecular level. But there, is a need to find more Researchers had already discovered new biological markers that assist in the identification of the genetics basis of the disease. But there is a need to develop the more diagnostic markers that help to cure this disease.

REFERENCES

- Bosch F, Dalla-Favera R. Chronic lymphocytic leukaemia: from genetics to treatment. *Nat Rev Clin Oncol*, 2019; 16(11): 684-701. doi:10.1038/s41571-019-0239-8
- Ferrarini M, Cutrona G, Neri A, Morabito F. Prognostic factors in CLL. *Leuk*, 2012; 1(S2): S29-S30. doi:10.1038/leusup.2012.17
- Amaya-Chanaga CI, Rassenti LZ. Biomarkers in chronic lymphocytic leukemia: Clinical applications and prognostic markers. *Best Pract Res Clin Haematol*, 2016; 29(1): 79-89. doi:10.1016/j.beha.2016.08.005
- Fathollahzadeh S, Mirzaei H, Honardoost MA, Sahebkar A, Salehi M. Circulating microRNA-192 as a diagnostic biomarker in human chronic lymphocytic leukemia. *Cancer Gene Ther.*, 2016; 23(10): 327-332. doi:10.1038/cgt.2016.34
- Mirzaei H, Fathollahzadeh S, Khanmohammadi R, et al. State of the art in microRNA as diagnostic and therapeutic biomarkers in chronic lymphocytic leukemia. *J Cell Physiol*, 2018; 233(2): 888-900. doi:10.1002/jcp.25799
- Fernando TR, Rodriguez-Malave NI, Rao DS. MicroRNAs in B cell development and malignancy. *J Hematol Oncol*, 2012; 5: 1-10. doi:10.1186/1756-8722-5-7
- Smolewski P, Witkowska M, Korycka-Wołowiec A. New Insights into Biology, Prognostic Factors, and Current Therapeutic Strategies in Chronic Lymphocytic Leukemia. *ISRN Oncol*, 2013; 2013: 1-7. doi:10.1155/2013/740615
- Ahmed F, Muzaffar R, Fernandes H, Tu Y, Albaloooshi B, Osman MM. Skeletal metastasis as detected by 18F-FDG pet with negative ct of the PET/CT: Frequency and impact on cancer staging and/or management. *Front Oncol*, 2016; 6(OCT): 1-6. doi:10.3389/fonc.2016.00208
- Cramer P, Hallek M. Prognostic factors in chronic lymphocytic leukemia-what do we need to know? *Nat Rev Clin Oncol*, 2011; 8(1): 38-47. doi:10.1038/nrclinonc.2010.167
- Shuai W, Lin P, Strati P, et al. Clinicopathological characterization of chronic lymphocytic leukemia with MYD88 mutations: L265P and non-L265P mutations are associated with different features. *Blood Cancer J.*, 2020; 10(8). doi:10.1038/s41408-020-00351-w
- Delgado J, Doubek M, Baumann T, et al. Chronic lymphocytic leukemia: A prognostic model comprising only two biomarkers (IGHV mutational status and FISH cytogenetics) separates patients with different outcome and simplifies the CLL-IPI. *Am J Hematol*, 2017; 92(4): 375-380. doi:10.1002/ajh.24660
- Parikh SA, Shanafelt TD. Risk Factors for Richter Syndrome in Chronic Lymphocytic Leukemia. *Curr Hematol Malig Rep.*, 2014; 9(3): 294-299. doi:10.1007/s11899-014-0223-4
- Palamarchuk A, Yan PS, Zanasi N, et al. Tc11 protein functions as an inhibitor of de novo DNA methylation in B-cell chronic lymphocytic leukemia (CLL). *Proc Natl Acad Sci U S A.*, 2012; 109(7): 2555-2560. doi:10.1073/pnas.1200003109
- Balatti V, Rizzotto L, Miller C, et al. TCL1 targeting miR-3676 is codeleted with tumor protein p53 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A.*, 2015; 112(7): 2169-2174. doi:10.1073/pnas.1500010112
- Pekarsky Y, Santanam U, Cimmino A, et al. Tc11 expression in chronic lymphocytic leukemia is regulated by miR-29 and miR-181. *Cancer Res.*, 2006; 66(24): 11590-11593. doi:10.1158/0008-5472.CAN-06-3613
- Rodríguez AE, Hernández JÁ, Benito R, et al. Molecular Characterization of Chronic Lymphocytic Leukemia Patients with a High Number of Losses in 13q14. *PLoS One*, 2012; 7(11). doi:10.1371/journal.pone.0048485
- Mirzaei H, Naseri G, Rezaee R, et al. Curcumin: A new candidate for melanoma therapy? *Int J Cancer*, 2016; 139(8): 1683-1695. doi:10.1002/ijc.30224
- Saadatpour L, Fadaee E, Fadaei S, et al. Glioblastoma: Exosome and microRNA as novel diagnosis biomarkers. *Cancer Gene Ther.*, 2016; 23(12): 415-418. doi:10.1038/cgt.2016.48
- Balatti V, Pekarky Y, Croce CM. Role of microRNA in chronic lymphocytic leukemia onset and progression. *J Hematol Oncol*, 2015; 8(1): 8-13. doi:10.1186/s13045-015-0112-x
- Li S, Moffett HF, Lu J, et al. MicroRNA expression profiling identifies activated B cell status in chronic lymphocytic leukemia cells. *PLoS One*, 2011; 6(3). doi:10.1371/journal.pone.0016956
- Strati P, Jain N, O'Brien S. Chronic Lymphocytic Leukemia: Diagnosis and Treatment. *Mayo Clin Proc.*, 2018; 93(5): 651-664. doi:10.1016/j.mayocp.2018.03.002
- Rozovski U, Hazan-Halevy I, Calin G, et al. Describing a transcription factor dependent regulation of the micro RNA transcriptome. *J Vis Exp.*, 2016; 2016(112): 1-5. doi:10.3791/53300

23. Hallek M, Fürstenau M. How to approach CLL in clinical practice. *Hematol Oncol*, 2019; 37: 38-42.
24. Rossi D, Rasi S, Fabbri G, et al. Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. *Blood*, 2012; 119(2): 521-529. doi:10.1182/blood-2011-09-379966
25. Kiefer Y, Schulte C, Tiemann M, Bullerdiek J. Chronic lymphocytic leukemia-associated chromosomal abnormalities and miRNA deregulation. *Appl Clin Genet*, 2012; 5: 21-28. doi:10.2147/TACG.S18669
26. Abruzzo L V., Herling CD, Calin GA, et al. Trisomy 12 chronic lymphocytic leukemia expresses a unique set of activated and targetable pathways. *Haematologica*, 2018; 103(12): 2069-2078. doi:10.3324/haematol.2018.190132
27. Oppezzo P, Dighiero G. Role of the B-cell receptor and the microenvironment in chronic lymphocytic leukemia. *Blood Cancer J.*, 2013; 3(9): 1-9. doi:10.1038/bcj.2013.45
28. 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
29. Gaidano G, Rossi D. The mutational landscape of chronic lymphocytic leukemia and its impact on prognosis and treatment. *Hematology*, 2017; 2017(1): 329-337. doi:10.1182/asheducation-2017.1.329
30. Bomben R, Gobessi S, Dal Bo M, et al. The miR-1792 family regulates the response to Toll-like receptor 9 triggering of CLL cells with unmutated IGHV genes. *Leukemia*, 2012; 26(7): 1584-1593. doi:10.1038/leu.2012.44
31. Lodé L, Cymbalista F, Soussi T. Genetic profiling of CLL: A “TP53 addict” perspective. *Cell Death Dis.*, 2016; 7: e2042. doi:10.1038/cddis.2015.415
32. Suresh S, Irvine AE. The NOTCH signaling pathway in normal and malignant blood cell production. *J Cell Commun Signal*, 2015; 9(1): 5-13. doi:10.1007/s12079-015-0271-0
33. Balatti V, Acunzo M, Pekarky Y, Croce CM. Novel mechanisms of regulation of miRNAs in CLL. *Trends in Cancer*, 2016; 2(3): 134-143. doi:10.1016/j.trecan.2016.02.005
34. Undi RB, Kandi R, Gutti RK. MicroRNAs as haematopoiesis regulators. *Adv Hematol*, 2013; 2013(2). doi:10.1155/2013/695754
35. Cahill N, Rosenquist R. Uncovering the DNA methylome in chronic lymphocytic leukemia. *Epigenetics.*, 2013; 8(2): 138-148. doi:10.4161/epi.23439
36. Van Roosbroeck K, Calin GA. MicroRNAs in chronic lymphocytic leukemia: MiRacle or miRage for prognosis and targeted therapies? *Semin Oncol*, 2016; 43(2): 209-214. doi:10.1053/j.seminoncol.2016.02.015
37. Willander K, Dutta RK, Ungerback J, et al. NOTCH1 mutations influence survival in chronic lymphocytic leukemia patients. *BMC Cancer*, 2013; 13: 1-6. doi:10.1186/1471-2407-13-274
38. Leeksa AC, Derks IAM, Kasem MH, et al. The Effect of SF3B1 Mutation on the DNA Damage Response and Nonsense-Mediated mRNA Decay in Cancer. *Front Oncol*, January, 2021; 10: 1-7. doi:10.3389/fonc.2020.609409
39. Tang AD, Soulette CM, van Baren MJ, et al. Full-length transcript characterization of SF3B1 mutation in chronic lymphocytic leukemia reveals downregulation of retained introns. *Nat Commun*, 2020; 11(1): 1-12. doi:10.1038/s41467-020-15171-6
40. Quesada V, Conde L, Villamor N, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nat Genet*, 2012; 44(1): 47.
41. Xia Y, Fan L, Wang L, et al. Frequencies of SF3B1, NOTCH1, MYD88, BIRC3 and IGHV mutations and TP53 disruptions in Chinese with chronic lymphocytic leukemia: disparities with Europeans. *Oncotarget*, 2015; 6(7): 5426.
42. Landau DA, Carter SL, Stojanov P, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell.*, 2013; 152(4): 714-726.
43. Cortese D, Sutton LA, Cahill N, et al. On the way towards a “CLL prognostic index”: Focus on TP53, BIRC3, SF3B1, NOTCH1 and MYD88 in a population-based cohort. *Leukemia*, 2014; 28(3): 710-713. doi:10.1038/leu.2013.333
44. Wang LQ, Chim CS. DNA methylation of tumor-suppressor miRNA genes in chronic lymphocytic leukemia. *Epigenomics*, 2015; 7(3): 461-473. doi:10.2217/epi.15.6