Nat. Volatiles & Essent. Oils, 2021; 8(6): 500-510

NVEO
Natural Volatiles &
Essential Oils

Chromosomal changes of chronic lymphocytic leukemia in Al Kut

Haider Mohammad Abed Al-Aziz , Dr. Sada Jasim Abd Al-Ameer and Dr. Tariq Dawood Salman

College of Education for Pure Sciences, University of Wasit, Iraq

Abstract

Background and Objectives: Analyze the chromosomes of patients with chronic lymphocytic leukemia (CLL) in Kut to identify chromosomal abnormalities and study the chromosomal changes in patients.

Patients and Methods: Clinical notes and cytogenetic analysis were studied for all patients. Cases were collected from oncology unit at Al Karama Hospital in Al Kut.

Results: Chromosomal analysis of all patients showed chromosomal aberrations, It was found that most of the chromosomal changes are structural changes. The abnormalities of the patients' chromosomes were divided according to their karyotype into 3 groups: chromosomal bridge (36.8) , complex chromosomes (31.6) and normal (31.6) .

Conclusion: Chromosomal abnormalities, especially structural abnormalities are responsible for the occurrence and development of chronic lymphocytic leukemia and the high complexity of karyotyping of patients.

Keywords: Chronic lymphocytic leukemia in adult patients and chromosomal structural changes.

Introduction

The term "chronic" in chronic lymphocytic leukemia (CLL) usually derives from the fact that the disease progresses more slowly than other types of leukemia. The term "lymphocyte" in chronic lymphocytic leukemia comes from cells affected by the disease, a group of white blood cells called lymphocytes. Lymphocytes are cells that help the body fight infections. In addition it is common in the elderly. There are treatment options that can help control the disease (1) . CLL is a blood and bone marrow malignancy, which affects the spongy area inside the bone where blood cells are produced (2). In patients with lymphocytic leukemia, proliferating B lymphocytes accumulate abnormally in blood, bone marrow, or lymphoid tissue (3) . Also it is the disease with the highest global prevalence and it affects about 500 people per year worldwide. Beginning in the year 2000 CLL prevalence jumped from 33/100000 to 52/100000 people in 2015. This rise was attributed to an increase in CLL patients' survival

rates. Men suffer more than women (2: 1). Around the world, the average age at diagnosis is 71 years old (4).

The aims of the study is performing chromosomal analysis of patients with chronic lymphocytic leukemia and studying the chromosomal changes of the patients. Structural chromosomal changes are the most common in chronic lymphocytic leukemia compared to other leukemia, so they are more dangerous and complex even in most of these changes are represented by translocations, deletions, replacements, or the presence of more than one chromosomal change for the same cell and this is called the complex karyotypes and this is very dangerous and sometimes kills the patient. Immune phenotypic data is frequently included to improve diagnostic specificity. Most recent studies indicate a difference in the genetic material of chronic lymphocytic leukemia cells, and most of those studies are at the cellular level by using cytogenetic techniques to study the chromosomal structure or by using the method of fluorescence hybridization (FISH) and most studies indicated chronic lymphocytic leukemia (CLL) chromosomal abnormalities are detected in up to 80% of patients. Between them, deletions of 17p, 11q, 13q, and trisomy 12 have known prognostic value, play an important role in the pathogenesis and development of the disease , and also determine patient results and treatment strategies (5).

Patients and Methods

This study was conducted during a period of one year from March 2020 to March 2021 on patients attending Hematology Center at Al-Karama Teaching Hospital in Al-Kut who was diagnosed with chronic lymphocytic leukemia. The study included 32 patients, 20 males (62.5%) and 12 females (37.5%). Complete clinical data and blood samples were taken from the patients. Cytogenetic analysis of peripheral blood samples was performed using traditional cytogenetic methods according (short time culture), and the use of culture media (Chromosome medium P) and colchicine solution, reagents, and stains (KCL, PBS, fixative solution, trypsin solution) many laboratory equipment and tools. Cellular proliferation and chromosomal analyses were performed according to (6,7) with some modifications.

Statistical Analysis

The tables and data were analyzed by test (Chi square test), average and percentage. Statistical analysis was performed using SPSS v.26 (Statistical Package for Social Science, Chicago, IL, USA).

Results

The results of the group of chronic lymphocytic leukemia patients showed that the minimum age to start the research was from the age of 47 years, while the maximum recorded age was 76 years. A total of 32 CLL cases were recorded. Their mean age was ± SD of (60.81 ± 7.56). This included 12 (37.5%) female and 20 (62.5%) male, and the male to female ratio was (2:1.2). As shown in figure (1) and the table (1) in this study 32 cases of stage CLL under chemotherapy 3 cases (9.4%) showed no cell or metaphase, 6 cases (18.7%) clumpy metaphases as in Figure (2a) , 4 cases (12.5%) short chromosomes which It could be counted but not analyzed as in Figure (2b) and only 19 cases (59.4%) had good growth and good metaphase.

Table (1): The number of patients with chronic lymphocytic leukemia and their percentages according to their age and gender groups

Age group (Year)	Gender				Total	
	Male		Female			
	N	%	N	%	N	%
40 – 49	2	10.0	1	8.3	3	9.4
50 – 59	7	35.0	3	25.0	10	31.3
60 – 69	8	40.0	6	50.0	14	43.8
70 – 79	3	15.0	2	16.7	**5**	13.6
Total	20	100	12	100	32	100
Chi-Square Tests			P-value		0.930 $^{N.S}$	

Figure (1) Distribution of patients age and gender CLL

in this study 32 cases of stage CLL under chemotherapy 3 cases (9.4%) showed no cell or metaphase, 6 cases (18.7%) clumpy metaphases as in Figure (2a) , 4 cases (12.5%) short chromosomes which It could be counted but not analyzed as in Figure (2b) and only 19 cases (59.4%) had good growth and good metaphase.

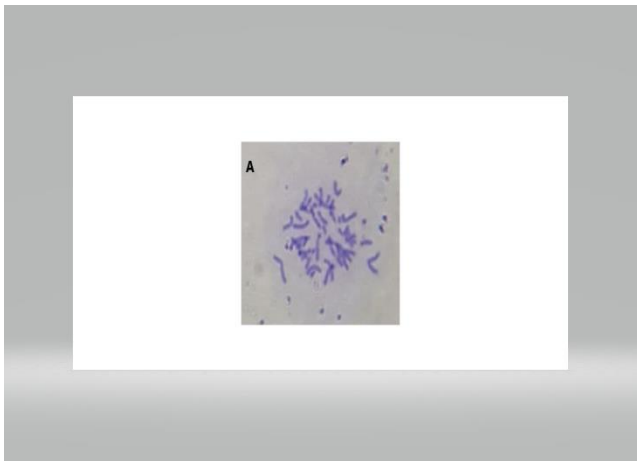

Figure (2) A-clumped metaphase of patient No.(10) B- Metaphase with short chromosomes of patient No.(22)

Table (2): Summary of chromosomal abnormalities in chronic lymphocytic leukemia in 19 patients

No. of patient	Age	Gender	Chromosomal numerical aberration	duration of treatment
1	**55**	**M**	**46,XY, normal chromosomes**	**5 year duration so far**
2	**59**	**M**	**chromosomal bridges**	**1 year duration so far**
3	**65**	**M**	**46,XY,normal chromosomes**	**10 year duration so far**
4	**55**	**M**	**chromosomal bridges**	**2 year duration so far**
5	**70**	**M**	**chromosomal bridges**	**3 year duration – died**
6	**53**	**M**	**46, XY,del(13q), bridge chromosome.**	**3 year duration so far**
12	**66**	**M**	**chromosomal bridges**	**2 year duration so far**
14	**65**	**F**	**46,XX,del (13q), translocation, fragile Sites**	**1 year duration – died**
15	**67**	**M**	**46, XY,del(13q),ring chromosome, bridge chromosome.**	**7 year duration so far**
16	**70**	**M**	**chromosomal bridges**	**5 year duration so far**
17	**54**	**M**	**chromosomal bridges,**	**8 year duration so far**

18	**49**	**M**	**46, XY,del(13q), bridge chromosome.**	**2 year duration so far**
19	**65**	**M**	**46,XY, normal chromosome**	**9 year duration so far**
20	**76**	**F**	**46,XX,Normal chromosome**	**5 year duration so far**
21	**62**	**M**	**46,XY,Normal chromosome**	**7 year duration so far**
23	**61**	**F**	**46,XX, t(21q:1q),acentric ,bridge chromosome**	**without treatment**
25	**55**	**F**	**46,XX, dicentric chromosomes**	**2 year duration so far**
26	**71**	**F**	**chromosomal bridges**	**3 year duration**
28	**52**	**F**	**46,XX,normal chromosomes**	**20 year duration so far**

Discussion

The group of chronic lymphocytic leukemia patients showed that the minimum age to start the research was from the age of 47 years, while the maximum recorded age was 76 years. A total of 32 CLL cases were recorded.

Cases diagnosed with chronic lymphocytic leukemia in a range: 40-84 years, median 64 years. Another study found that the median" age at diagnosis is around 70, which is uncommon (8) . But in" a local study in Baghdad of a total 30 registered CLL cases were found. Their age range (30-67) years in median (56.17 ± 8.45) is 57 years. They included 21 (70%) men and 9 (30%) women (9). In another study, the total number of CLL patients registered consisted of 40 males (72.27%) and 15 females (27.27%), with a higher male-to-female ratio (ratio of 2.6:1) (10,11) also reported that the highest ratio of males and females (2.1:1) also occurred most frequently in middle age. In addition, (12) report that the incidence of CLL increases with age, with a mean age at onset (65-70) years. They also reported a 2:1 male: female ratio. Another study reported that CLL is a male-dominated disease, but the reason for this difference is unknown (13).

The incidence of leukemia in men compared to women may be due to differences in the nature of the effects of work on genetic differences related to hormonal effects and gender (14). In the current study, consistent with local studies, the average age at diagnosis of CLL patients in Erbil, Iraq was similar to the age reported in neighboring countries such as Iran and Turkey (65 vs. 60.73, respectively). The median age at diagnosis in Western countries is much higher, ranging from 67 to 72 years (15).

However, this increase is not as significant as the disease (P> 0.05), but it is not well known by gender. It has been reported that the incidence of CLL is highest in the age group of 60 to 69 years. This discrepancy may be due to the possibility of taking samples from the medical center at the same time.

In other studies, approximately 10% of patients with CLL report a family history of CLL or related lymphoproliferative disorders, and genetic predisposition is the best-understood risk factor for CLL

(16,17). Researchers have found that the increased risk of chronic lymphocytic leukemia (CLL) depends on the age of the affected relatives and the number of first-degree relatives. For non-Hodgkin's lymphoma, HL and CLL, individuals with siblings of the disease were at higher risk (18).
Cancer is a disease with multiple causes. In other words, it is not necessarily hereditary but it is one of the causes (19,20).
According to the above table (2), 19 patients with chronic lymphocytic leukemia were studied. It was found that most of the chromosomal changes are structural changes. The abnormalities of the patients' chromosomes were divided according to their karyotype into 3 groups:
Chromosomal bridge, complex or cyclic chromosomes and phenotype. The normal chromosomes are as shown in Table (3).

Table (3) Summary of Cytogenetic analysis of patients' groups of CLL

Groups	Number of patients	Frequency%	Sex M/F	Median age (year)
Chromosomal bridges	7	36.8	1:6	66
Complex chromosomes	6	31.6	3:3	58
Normal(46)	6	31.6	4:2	63.5

Through the chromosomal structural aberration were found in most of the samples of the patients of this study, they can be explained according to what was stated in the previous researches or studies. If the broken piece is lost, the cellular damage left by the presence of a chromosome with a deletion depends on the size of the broken chromosome piece, where the broken is fatal in the case of losing a large portion of the chromosome, or cellular damage left by the loss of small parts depending on the importance of the missing genes (21,22). There are possibilities for chromosomal damage. The chromosomes may be broken and linked to each other, leading to the emergence of overlapping chromosomes containing (dicentromeric chromosomes). Different types of cells generate a chromosomal bridge that prevents the two new cells from separating and in the best case leads to their random rupture, leaving many various additional chromosomal damages, as shown in Figure (3).
The other possibility may be occur chromosomal translocations were the adhesion of a chromosome part between the arm of another chromosome that has been broken or the exchange of internal pieces called this translocation (insertion translocation) Or those in which more than two chromosomes are involved, called cyclic or complex translocation. These translocation chromosomes are mostly lethal, as in the cases in figure (4). In addition to the above structural chromosomal damages, there are other mechanisms for this damage. So, the study of chromosomes at the cellular and molecular level showed that human chromosomes carry fragile and easy to break sites called (fragile sites), (23,24), and we also noticed such a case in the samples included in our research as in Figure (4). There are another chromosomal damages that may result from a break in the two arms of the chromosome, followed by

the fusion of its ends and the loss of the final parts of it, that is, the loss of the final genetic material of the chromosome, which was maintaining the stability of the chromosome, and this condition occurs by a complex mechanism that leads to the formation of a ring chromosome (25,26) such as shown in figure (5).

Through the previous damages, we note that these changes cause many mutations that are related to chronic lymphocytic leukemia, and according to what has been proven by many researches, including A study by (27), he proposed an incorporated model of cytogenetic. Mutations analysis classifies newly diagnosed CLL patients into four subgroups at risk of death. The high-risk group includes patients with the 17pdel / TP53 mutation and / or the BIRC3del / BIRC3 mutation, and the intermediate group includes patients with NOTCH1. It contained mutations and / or SF3B1 mutations and / or 11qdel. Patients in the low risk group include patients with trisomy 12 who are cytogenetic normal and have no mutations, and the last subgroup is a very low-risk group , with only one patient having a genetic abnormality. Includes del13q as. The result of(28). But there are cases in which the chromosomal set is also normal, and we found it in our research. It can be interpreted as being normal at the cellular level, but it is highly likely that it has damages at the molecular level. But in this study we used cytological methods to diagnose and examine chromosomes. The method of G-Banding is better. A cellular method for examining normal and abnormal chromosomes with significant structural damage. But there are methods at the molecular level that detect very small or minute damage, such as (Fluorescence in Situ Hybridization FISH) or the (Primed in Situ Labeling) method (29,30).Most studies agree with what we obtained about chromosomal changes and genome instability in patients with chronic lymphocytic leukemia (31).

Genome instability was found to be a hallmark of most cancers and was mainly observed in CLL patients associated with short telomere.

General genetic instability of cells, kinetochore loops can cause the formation of anaphase bridges that create new fusions by breaking their breakpoint (“Break-Fusion-Bridge Cycle”), and thus new chromosomal rearrangements (32).

In another study, chromosomal bridges occur more frequently in mitosis there is also evidence that capturing telomere sequences from other chromosomes can cause meiosis to loop into large linear marker chromosomes. Therefore, BFB events are not likely to negatively affect cell proliferation as with non-neoplastic cells. All of these tumors have a slow growth pattern, but they are still able to invade surrounding tissues and often reach a large size before clinical symptoms appear (33). The telomere system plays an important role in the specific stages of CLL pathogenesis and development.

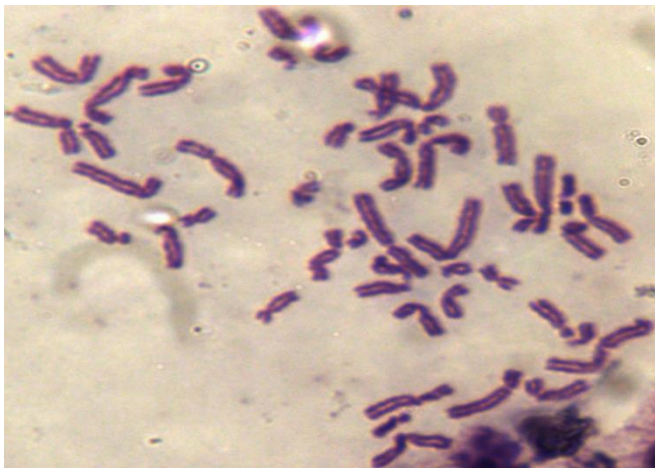

Figure (3) chromosomal aberration of case (25) 46, XX, dicentric chromosomes. (1000X).

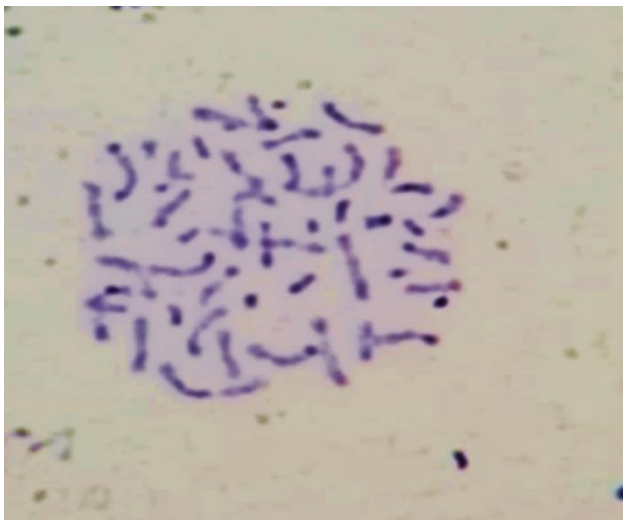

Figure (4) Chromosomal aberration of case (14) 46, XX, del. (13q), translocation, fragile Sites (1000X).

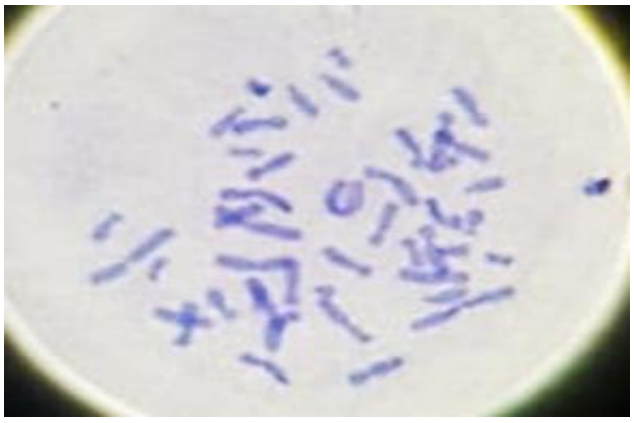

Figure (5) chromosomal aberration of case (15) 46, XY, del. (13), ring chromosome, bridge chromosome. (1000X)

Conclusion

This is the first study in Kut that has included the study of the chromosomes of patients among adults. The incidence is higher in this group and may be related to environmental factors, malnutrition and lack of awareness. The results of the chromosomal analysis were: Chromosomal bridges and Complex chromosomes, where patients undergo chemotherapy. This result may be useful in the diagnosis and progression of the disease.

References

1. Hallek M. Chronic lymphocytic leukemia: 2017 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2017;92(9):946–65.
2. Hallek, M., Shanafelt, T. D., & Eichhorst, B. (2018). Chronic lymphocytic leukaemia. The Lancet, 391(10129), 1524-1537.
3. Abdullah Hasan Jabbar et al., ECO-FRIENDLY SYNTHESIS OF SILVER NANOPARTICLES AGNPS AND ANTIBACTERIAL ACTIVITY, Turkish Journal of Physiotherapy and Rehabilitation; 32(3), p. 11225-11231
4. Li, Y., Wang, Y., Wang, Z., Yi, D., & Ma, S. (2015). Racial differences in three major NHL subtypes: descriptive epidemiology. Cancer epidemiology, 39(1), 8-13.
5. Döhner, H., Stilgenbauer, S., Benner, A., Leupolt, E., Kröber, A., Bullinger, L., ... & Lichter, P. (2000). Genomic aberrations and survival in chronic lymphocytic leukemia. New England Journal of Medicine, 343(26), 1910-1916.
6. Geleick, D., Müller, H., Matter, A., Torhorst, J., & Regenass, U. (1990). Cytogenetics of breast cancer. Cancer genetics and cytogenetics, 46(2), 217-229.
7. Yassen, N. Y. (1990). Cytogenetic study on human colorectal cancer cells (Doctoral dissertation, PhD. Thesis submitted University of Sheffield Medical School).
8. Willander, K. (2014). Molecular genetic studies on Chronic Lymphocytic Leukemia and Acute Myeloid Leukemia-with focus on prognostic markers (Doctoral dissertation, Linköping University Electronic Press).
9. Al-Thahir, S. K. (2021). Interphase Fluorescence in Situ hybridization Analysis of 17p13. 1 and 11q22. 3 Deletions among CLL Patients. Iraqi Postgraduate Medical Journal, 20(1), 39.
10. Haider Sadiq Abdul Hassan Al-Dahery(2016):thesis Alteration of Biochemical Immunological and Molecular Markers of Chronic Lymphocytic leukemia in Sample of Iraqi Patients.
11. Lamanna, N., Weiss, M., & Dunleavy, K. (2011). Chronic Lymphocytic Leukemia and Hairy-Cell Leukemia. Cancer Management.
12. Freireich EJ. Leukemias. In: The Merck Manual of Diagnosis and therapy. Edited by Porter RS; Kaplan TL (19 ed.). Mercck Sharp and Dohme Corp. NJ. Chpt . (2010), 117:pp:1010-1012.
13. Jemal, A., Center, M. M., DeSantis, C., & Ward, E. M. (2010). Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiology and Prevention Biomarkers, 19(8), 1893-1907.
14. Catovsky, D., Wade, R., & Else, M. (2014). The clinical significance of patients' sex in chronic lymphocytic leukemia. Haematologica, 99(6), 1088.

15. Hasan, K. M. (2018). Clinical Aspects, Immunophenotypic Analysis and Survival Rate of Chronic Lymphocytic Leukaemia Patients in Erbil City, Iraq. Sultan Qaboos University Medical Journal, 18(4), e461.
16. Brown, J. R. (2008). Inherited predisposition to chronic lymphocytic leukemia. Expert review of hematology, 1(1), 51-61.
17. Cerhan, J. R., & Slager, S. L. (2015). Familial predisposition and genetic risk factors for lymphoma. Blood, The Journal of the American Society of Hematology, 126(20), 2265-2273.
18. Ross, K. H., Gogineni, K., Subhedar, P. D., Lin, J. Y., & McCullough, L. E. (2019). Obesity and cancer treatment efficacy: Existing challenges and opportunities. Cancer, 125(10), 1588-1592.
19. Slager, S. L., Benavente, Y., Blair, A., Vermeulen, R., Cerhan, J. R., Costantini, A. S., ... & de Sanjosé, S. (2014). Medical history, lifestyle, family history, and occupational risk factors for chronic lymphocytic leukemia/small lymphocytic lymphoma: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. Journal of the National Cancer Institute Monographs, 2014(48), 41-51.
20. Strati, P., & Shanafelt, T. D. (2015). Monoclonal B-cell lymphocytosis and early-stage chronic lymphocytic leukemia: diagnosis, natural history, and risk stratification. Blood, The Journal of the American Society of Hematology, 126(4), 454-462.
21. Thompson, P. A., O'Brien, S. M., Wierda, W. G., Ferrajoli, A., Stingo, F., Smith, S. C., ... & Keating, M. J. (2015). Complex karyotype is a stronger predictor than del (17p) for an inferior outcome in relapsed or refractory chronic lymphocytic leukemia patients treated with ibrutinib-based regimens. Cancer, 121(20), 3612-3621.
22. Herling, C. D., Klaumuenzer, M., Rocha, C. K., Altmüller, J., Thiele, H., Bahlo, J., ... & Kreuzer, K. A. (2016). Complex karyotypes and KRAS and POT1 mutations impact outcome in CLL after chlorambucil-based chemotherapy or chemoimmunotherapy. Blood, The Journal of the American Society of Hematology, 128(3), 395-404.
23. Ruiz-Herrera, A., Garcia, F., Frönicke, L., Ponsà, M., Egozcue, J., Caldés, M. G., & Stanyon, R. (2004). Conservation of aphidicolin-induced fragile sites in Papionini (Primates) species and humans. Chromosome Research, 12(7), 683-690.
24. Glover, T. W., Arlt, M. F., Casper, A. M., & Durkin, S. G. (2005). Mechanisms of common fragile site instability. Human molecular genetics, 14(suppl_2), R197-R205.
25. Gebhart, E. (2008). Ring chromosomes in human neoplasias. Cytogenetic and genome research, 121(3-4), 149-173.
26. Halldórsdóttir, A. M., Sander, B., Göransson, H., Isaksson, A., Kimby, E., Mansouri, M., ... & Ehrencrona, H. (2011). High-resolution genomic screening in mantle cell lymphoma—specific changes correlate with genomic complexity, the proliferation signature and survival. Genes, Chromosomes and Cancer, 50(2), 113-121.
27. Rossi, D., Rasi, S., Spina, V., Bruscaggin, A., Monti, S., Ciardullo, C., ... & Gaidano, G. (2013). Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia. Blood, The Journal of the American Society of Hematology, 121(8), 1403-1412.

28. Rossi, M Dreger, P., Schnaiter, A., Zenz, T., Böttcher, S.,., Paschka, P., ... & Stilgenbauer, S. (2013). TP53, SF3B1, and NOTCH1 mutations and outcome of allotransplantation for chronic lymphocytic leukemia: six-year follow-up of the GCLLSG CLL3X trial. Blood, The Journal of the American Society of Hematology, 121(16), 3284-3288.
29. Döhner, H., Stilgenbauer, S., Benner, A., Leupolt, E., Kröber, A., Bullinger, L., ... & Lichter, P. (2000). Genomic aberrations and survival in chronic lymphocytic leukemia. New England Journal of Medicine, 343(26), 1910-1916.
30. Rawstron, A. C., Kreuzer, K. A., Soosapilla, A., Spacek, M., Stehlikova, O., Gambell, P., ... & Montserrat, E. (2018). 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 Part B: Clinical Cytometry, 94(1), 121-128.
31. Gebhart, E. (2008). Ring chromosomes in human neoplasias. Cytogenetic and genome research, 121(3-4), 149-173.
32. Chiarle, R., Zhang, Y., Frock, R. L., Lewis, S. M., Molinie, B., Ho, Y. J., ... & Alt, F. W. (2011). Genome-wide translocation sequencing reveals mechanisms of chromosome breaks and rearrangements in B cells. Cell, 147(1), 107-119.
33. Gisselsson, D. (2002). Ring chromosomes: vicious circles at the end and beginning of life. Atlas Genet Cytogenet Oncol Haematol, 6, 59-66.