Current Science
Current Science
CS 3 (6), 486– 495 (2023)
Received 25 September 2023 | Revised 28 October 2023 | Accepted 15 November 2023 | Online Available 05 December 2023
https://doi.org/10.52845/CS/2023-3-6-7

OPEN ACCESS
ISSN(O)2795-8639

ORIGINAL ARTICLE

Isoform P53 Protein, a Marker Unfavorable Prognostic in Chronic Lymphocytic Leukemia

Delia Nica-Badea[1*], Aurelian Udristioiua[2]

[1] Faculty of Medical and Behavioral Sciences, Constantin Brâncuși University, Târgu - Jiu, Romania

[2]Medicine Faculty, Titu Maiorescu University, Bucharest, Romania

Corresponding Author: Delia Nica- Badea

Abstract

The paper highlights the stages of Chronic Lymphocytic Leukemia B type, (CLL-B), which did not meet the standard treatment criteria for malignant hematological diseases due to P53 gene mutations, with progression to diffuse large lymphoma.

The mutated P53 gene is the most common genetic abnormality in cancer and has been studied in various mature B-cell malignancies, including chronic lymphocytic leukemia (CLL). The identification of the mutated P53 gene is very important because this mutation has an impact on the clinical course of patients in CLL with the p53 protein isoform.

The frequency of p53 protein expression in 85 patients diagnosed with CLL was analyzed by the ELISA (Enzyme Linked Immune-Absorbent Assay) technique, investigating the relationship of this protein with the stage of the disease and the impact on treatment response and survival. Cell extracts $10^3 \times 10^3$/L in 100 µl lysis buffer were applied to ELISA plates coated with PAb 240 antibodies, specific antibodies for isoform p53 protein. The average concentrations of p53 proteins in 17 of the 20 cases were found to be 16.76 µg / dl, with CV = 0.5% and the probability index p = 0.034. The study was done from sample blood patients included in screening analyses from a diagnosis of leukemia, as a clinical trial of phase I.

ELISA method has proven to be a useful prognostic tool for CLL for the application of personalized treatment in cases diagnosed with CLL resistance to the specific treatment applied by the first line with standard onco-immune therapy.

Kay words: **Chronic Lymphocytic Leukaemia; P53 gene, Intrinsic apoptosis, CD-5 receptor, Non-Hodgkin lymphoma.**

Introduction

This paper aimed to highlight the cases of chronic lymphocytic leukemia type B, (CLL-B), which did not meet the standard treatment criteria for malignant hematologic diseases due to mutations in the P53 gene, with progression forward to Diffuse Large Lymphoma. Identifying different P53 gene mutations is very important because these mutations have an impact on the patient's clinical course in CLL with the p53 protein mutant isoform.

The most common type of leukemia found in adults and the elderly is chronic B lymphocytic leukemia (CLL-B). It is characterized by different clinical expressions depending on the age or sex of the patient. Among the factors that control and regulate the apoptosis process, progression of the disease in the patients, p53 protein and p21 protein are considered to be of major importance. Over the last decade, several paraclinical investigation methods have been identified to predict the progression of the disease [1].

In the last years, was discovered that, the

production of some percentage of isoform p53 protein, with the increased stability in type B lymphocytes, leads to the carcinogenesis process. This discovery led to the identification and quantification of the p53 protein by different methods such as immunohistochemistry (IHC), polymerase chain reaction (PCR), single-stranded peptide microarray, (SSPMa), next-generation sequencing (NGS), and the sandwich enzyme-linked immunosorbent assay (sandwich ELISA) [2].

P53 gene mutation is a very common event in human neoplasia and a single allele is responsible for hereditary cancer susceptibility syndrome (Li Fraumeni). This variant encodes distinct isoforms of the p53 protein, which may disrupt its transcriptional activity (Fig. 1) [3, 4, 5].

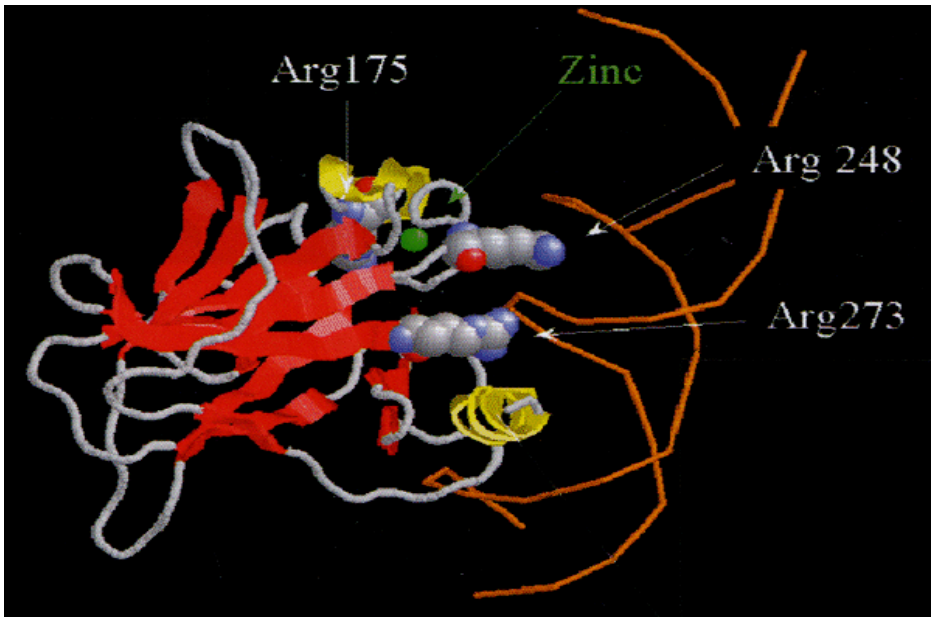

Figure 1. Three dimensional shapes of the p-53 x ray protein structure in isomorphic form.

Five genes P53 mutations were identified in hereditary cancer clinics. A targeted mutation screen of cancer cases identified an Arginin-282-Triptophan mutation carrier

2. Role of the pair of antibodies for the detection of p53 by the ELISA method

In the present study was used Human p53 ELISA Kit for cell culture supernatants, plasma, and serum samples with the specificity for antibody pair detect human p53. This sandwich ELISA is a quantitative method, for direct detection of p53 isoform protein, product of Gene P53**:** Specificity: human p53 protein (aa20-25); Format: Purified product: Monoclonal antibody clone: Isotype DO-1: IgG2a. The antibody is suitable for the techniques: ICC / IF and ELISA. The research antibody; PAb 240 antibodies bind specifically to denatured p53 protein**.** Sample Types: Cell Culture Supernatants, Plasma, Serum; Solid Support**:** 96-well Microplate; Firm: Ray Biotech Life, Inc.

The monoclonal antibody PAb 240 recognizes an epitope that is structurally hidden in the wild-type conformation of p53 and becomes exposed by denaturing the p53 protein or the isoform conformations of p-53, where point mutations in the P53 gene alter the terminal structure of the p53 protein.

Using the ELISA technique, the frequency of p53 protein expression in 20 representative patients diagnosed with CLL-B was analyzed in order to investigate the relationship of the p53 protein at the different stages of the disease and the impact on patient survival. ELISA Kit Component: Coated 96-well, Strip Plate 1, Standard (Lyophilized) 2 vials Assay, Diluent (5x) 1 vial x 15 ml, Biotinylated Detection Antibody 2 vials, HRP-Streptavidin Conjugate (800x) 1 vial x 200 µl, Wash Buffer (20x) 1 vial x 25 ml, TMB Substrate 1 vial x 12 ml, Stop Solution sulfuric acid (H_2SO_4) 1 vial x 8 ml, Plate Sealers 4.

Other Materials Required; Distilled or deionized water; Precision pipettes to deliver 2 µl to 1 µl

volumes; Adjustable 1-25 µl pipettes for reagent preparation; 100 µl and liter graduated cylinders; Tubes to prepare standard and sample dilutions; Absorbent paper; Microplate reader capable of measuring absorbance at 450nm; Log-log graph paper or computer and software for ELISA data analysis. This ELISA kit is recommended for use in serum, plasma or tissue homogenates. The sample collection protocols below were adapted from the references, (Ray Biotech's Speed ELISA platform).

The data of diagnosis were acquired from current clinician doctors of government hospitals which collected blood samples from its patients with the diagnosis of chronic lymphocytic leukemia and sent them the blood samples to Stefan Nicolau Institute, National Institute of Researches, City Bucharest. The peripheral blood samples were collected from the patients with the diagnosis of chronic lymphocytic leukemia with a poor response to conventional chemotherapy and radiotherapy after more cycles of oncologic treatments.

The diagnosis of chronic lymphocytic leukemia, clinical staging and response to specific therapy, were based on the criteria recommended by the International Workshop on CLL [6]. The patients underwent the evaluation in a complete physical exam for the diagnosis of chronic lymphocytic leukemia-type B, (CLL-B), which presented symptoms such as frequent cough, night sweats and retrosternal pain. Also, the same patients were undergoing an ultrasound and tomography CT/PET which revealed lymphadenopathy and/or splenomegaly, with an enlarged spleen in the context of the hematologic disease.

After analyzing the 85 samples with the diagnosis of CLL, in different stages of evolution, disease, starting with stage zero (stay and watch) and up to stage IV, 20 patients were selected, eligible for this study, to be investigated for the detection of p53 protein isoforms responsible for resistance to conventional oncological treatments of the disease with Rituximab, Cyclophosphamide, Doxorubicin hydrochloride, Vincristine sulfate (Oncovin), and Prednisone, (R-CHOP), after 2 cycles of relapses, representing a group of 16 men and 4 women aged 39-85 years.

3. Cytologic examination for the diagnosis of CLL

The diagnosis of CLL was prior established by the cytologic exam of blood smear from peripheral blood, in microscopy exam, with> 5000 lymphocytes in absolute value and less than 10% prolymphocytes in the hemogram with 5 Differential count, (CBC) (Fig. 2).

Laboratory hematological diagnosis of B-CLL was confirmed with the immunophenotyping using monoclonal antibodies, (Flow Cytometry), analyzing the monoclonal antibodies in $CD5^+$, $CD19^+$, $CD20^{+-}$, $CD23^+$, $CD28^+$ receptors, and with B lymphocytes expressing IgM or IgG heavy chains with kappa or lambda light chains, [7, 8].

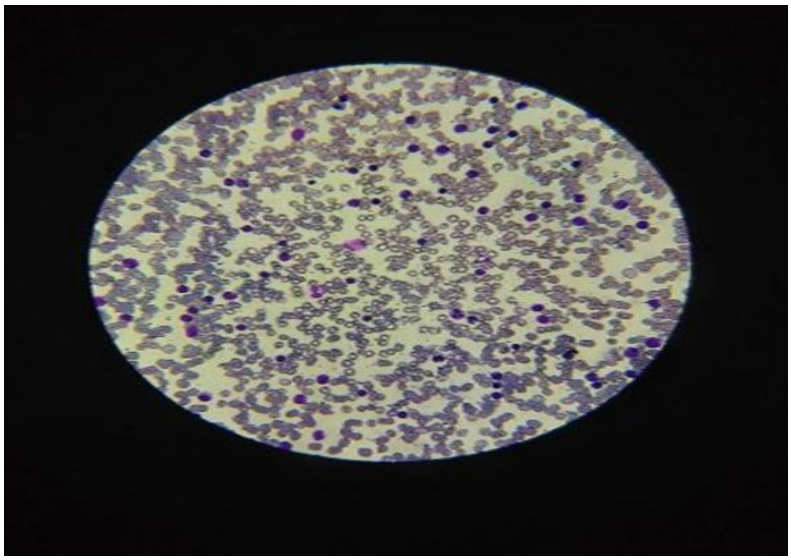

Figure 2. Image of the microscopic smear in CLL: numerous lymphocytes presenting a nucleus with an irregular contour, arranged in isolation on the peripheral blood slide and relative frequency of nuclear shadows Gumprecht

4. Results obtained by the ELISA method.

This analysis is based on the sandwich ELISA principle. Each well of the microtiter plate was pre-coated with a specific target capture antibody. Standards or samples are added to the wells and the target antigen, in this case the p-53 protein, binds to the capture antibody. Prepared a standard curve from the serial dilution data with concentration on the x-axis (logarithmic scale) from the absorption on the Y-axis (linear), was made in conformity of references protocol (Fig. 3).

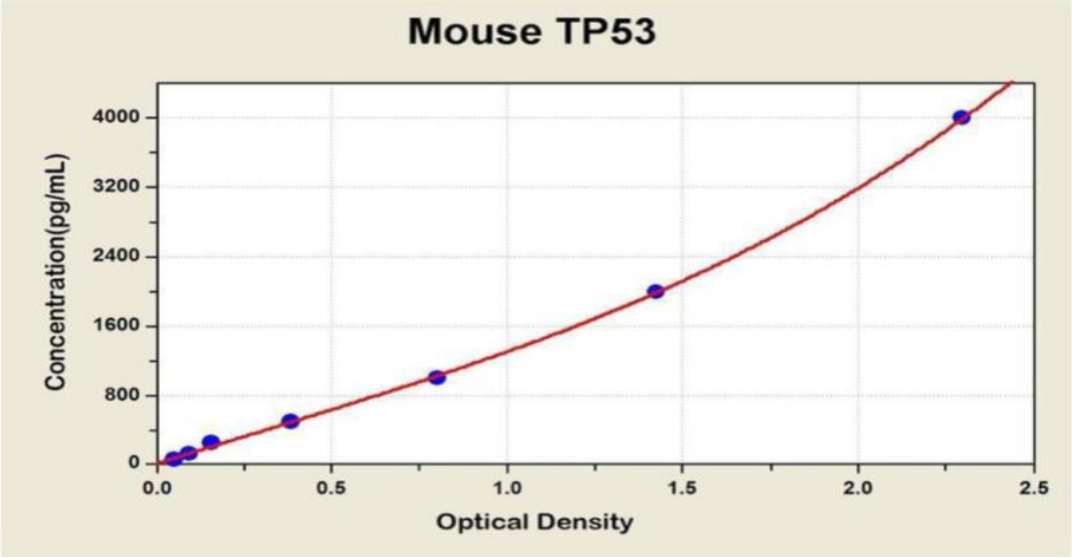

Figure 3. Standard control dilution series. Results of eligible patients: The average concentrations of p53 proteins in 17 of the 20 cases were found to be 16.76 µg / dl, with CV = 0.5% and the probability index p = 0.034.

Male results: Protein concentration in p-53 / µg / dL: 20, 15, 18, 40, 10, 12, 14, 60, 30, 10, 13, 15, 5, 10, 15, 12. *Women's results*; Protein concentration p-53 / µg / dL: 140, 30, 13, 10. Normal values of normal cell lines on equipment: ELISA = 0.25-0.5 µg / dL, or 2.5- 5ng/mL. Very high pathological values in the 3 cases of isoform p53 protein were calculated in 2 men, (PM) in the value of 60 µg / dL, respectively at 40 µg / dL and in the case of females, PW), it calculated in the amount of 140 µg / dL, with the transformation into Diffuse Large Lymphoma, (DLL) (Fig. 4).

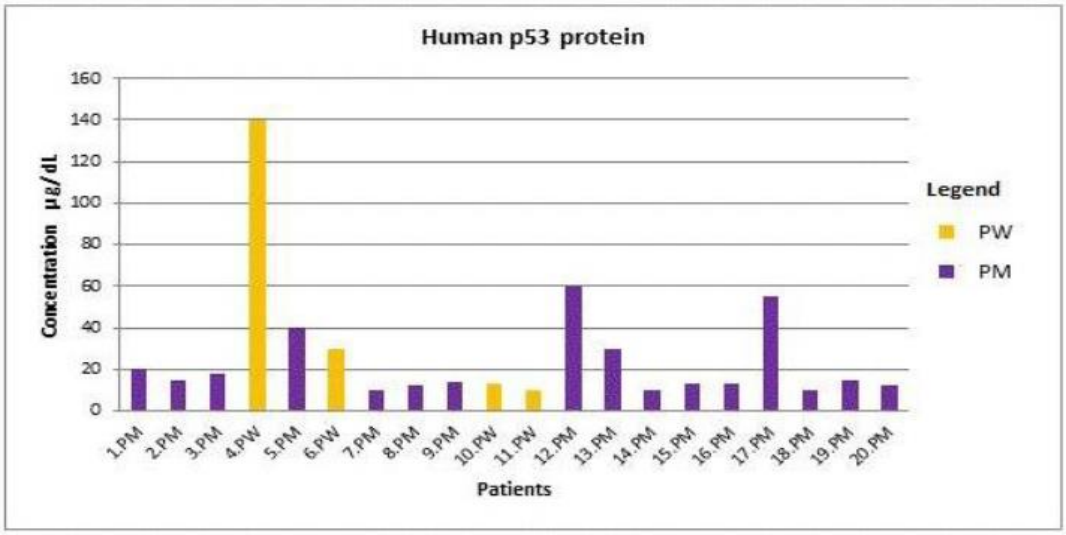

Figure 4. Values of p53 protein concentration, analyzes performed on the ELISA line. Research on the ELISA system was carried out in the Research Laboratory of the Immunology department, within the Stefan Nicolau Institute, Bucharest, in a 2019 retrospective study.

5. Multivariate analyzes (MA)

The concentration of the p53 isoform protein representing the P53 mutant gene in 17 cases, after excluding the 3 out-line cases present in the study, was calculated at the mean value of 14.8 µg / dL, with Standard Deviation, STDEV = 6.46, CV = 0.4% and the probability index (NORMDIST), "p" was calculated in the value of p = 0.079. Multivariate (MA) analyses were performed for OS on any significant variables at the level of P <0.20 at univariate analysis, with the gradual elimination of insignificant variables.

The presence of protein isoforms in the studied group was calculated as a percentage of 17%, and the unfavorable evolution and transformation of Chronic Lymphocytic Leukemia in the stages studied in Diffuse Large Lymphoma were calculated to be in a percentage of 3.5% of the studied cases, as in the meta-analyzes from the specialized literature, Diffuse Large lymphoma being considered a rare disease. The comparison between the categorical variables and the numerical variables was performed using the exact Fisher test.

5.1. Fisher test

Number of patients in the study group are 85; eligible patients for this study=20, (a + b = 3 + 17=20); ineligible patients for this study=65, (c + d = 5 + 60=65); total sum , a + c b + d a + b + c + d = 85; calculation of p value = {a + b} \ {a} x {c + d} \ {c}: [{n} \ {a + c}] = 20 / 3x65 / 5 / 8,125, = 1.02, indicate that all statements are true, (a + b)! (c + d)! (a + c)! (b + d)!} {a! b! c! d! n!}, in the calculations made by the statistical software, where the symbol "!" show the factorial number, (Fig. 5).

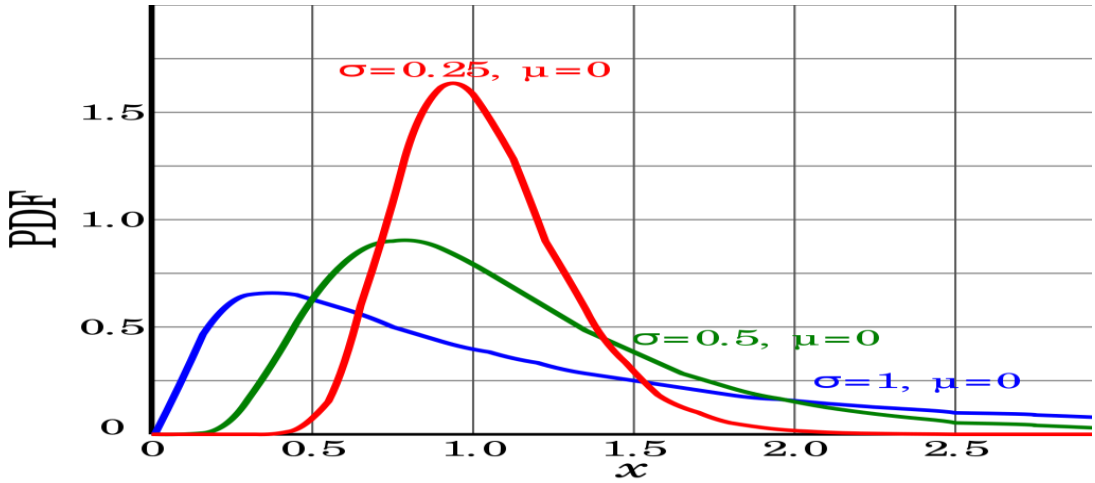

Figure 5. All expressions in the sign of the logarithm 1 are positive and these indicate a linearity relationship between

Frequency of p-53 isoform protein in cohort of study was evaluated with ANOVA test (Table 1).

Table 1. Calculation of frequency of isomorph p-53 protein by ANOVA test.

The interval of isomorph p-53 protein (i)	The middle classes average (m)	Frequency of concentration isomorph p-53 protein (f)
30-32	31	1
27-29	28	0
24-26	25	0
21-23	22	0
18-20	19	2
15-17	16	2
12-14	13	8
9-11	10	3
6-8	7	0
3-5	4	1

The overall frequency of positivity of the p53 protein, in the increased number of CLL cases studied, was 15% (3 out of 20 cases). The expression of the high-concentration p53 proteins in stage 2/3 of the disease was associated with a significantly weaker response to chemotherapy (p = 0.034) (Table 2).

Table 2. Expression of hemogram parameters and p-53 protein concentration in different stages of CLL-B

CLL – Age patients	CLL stage I/II, (n=17 patients) P-53 protein concentration in reactive limfocytes B	CLL stage III/IV (n = 3 patients] Percentage of p53 izoform proteins	p value
The age of patients with LLC, ranging from 39 to 85 years.	*The average p-53 protein concentration in CLL, 16.76 µg / dL*	*P-5 izofoorm proteins with elevated values was present in 15% (3 of 20 cases* *2 Men = 50µg / dL and 60µg / dL, respectively* *1 Female = 140µg / dL*	*p value 0.034*
Hematological parameters in peripheral blood	*Mean values of haemogram:* *No. Leukocytes = 35-50 x 10³/µL* *Hb = 11.8g / dL;* *Platelet = 140 x 10³/µL* *Lymphocytes = 65-80%*	*No. Leucocytes = 250-500 x 10³/µL* *Hb = 8.6g / dL* *Thrombocytosis = 45x10³/µL* *Limphocytes = 85-90%*	*p value 0.05*

6. Immunological characteristics of patients with CLL

In the 3 cases, the number of lymphocytes at presentation was 5000 /, uL or more, the highest being 30,000 /. Lymphocytes with incised nucleus. In May-Grunwald-Giemsa smears (Fig. 1), the incised nucleus cells are on average slightly larger than small lymphocytes and have very little pale blue cytoplasm, often apparently limited to one-third to two-thirds of the cell circumference. The nucleoli are not visible under an optical microscope. The association of lymphocytosis and lymphocytic infiltration of the bone marrow with morphology, poorly differentiated lymphocyte, or "histiocyte" in the ganglion sections is in marked contrast to the common finding in classical lymphocytic leukemia. For example, at some patients with a history of Chronic Lymphocytic Leukemia, treated with chemotherapy, was discovered a Diffuse Non-Hodgkin's Lymphoma, with Richter Syndrome.

The destruction of the normal lymph node architecture and the invasion of the capsule and the adjacent fatty tissue by the characteristic neoplastic cells, constituted the histological criteria for PET / CT imaging analysis. Histopathological examination of lymph node was performed after surgical excision of whole lymph node, [9].

In some international studies, the immunological characteristics of patients with CLL having p-53 protein positive, were measured by immunohistochemistry, (IHC). In the second part of the study CLL on the stage I/II, (n=47 patients), the P-53 protein isoform concentration in reactive Lymphocyte B, the average p53 protein concentration in CLL was on average 47 U/m with 16.7% samples studied, (7 out of 42 cases), [10].

Hematological parameters in peripheral blood, Leucocyte number = 35x10³/µL, Hb = 12,2g /dL, Thrombocyte = 140 x10³/µL, Lymphocytes in peripheral blood = 75-80%. The average p-53 protein concentration in CLL was on average 47 U/m, in CLL on the stage III/IV (n = 140 patients] and percentage of p53 isoform varied from 7-32%.; Hematological parameters in peripheral blood were changed as Leukocytes number = 350 x10³/µL, Hb = 10,8g / dL, Thrombocytes = 80 x 10³/µL, Lymphocytes in peripheral blood = 80-90%; Percent p-53 positive was in percent of 15% (Table 3) [10].

Table 3 Immunological characteristics of patients with CLL having p-53 protein positive measured by IHC

CLL – Age patients	CLL stage I/II, n=47 patients) P-53 protein concentration in reactive Lymphocyte B	CLL stage III/IV (n = 140 patients] Percentage of p53 izoform proteins	*p value*
The age of patients with LLC, ranging from 52 to 84 years, with average 62 years.	*In the samples studied at CLL it was 16.7% (7 out of 42 cases).*	*The percentage of positive p53 cells varied from 7-32%.*	*0.398*
Hematological parameters in peripheral blood	*No. Leucocyte = 35x x 10³/µL Hb = 12,2g / dL Thrombocyte = 140 x 10³/µL Lymphocytes = 75-80%*	*No. Leukocytes = 350 x10³/µL Hb = 10,8g / dL Thrombocytes = 80 x 10³/µL Lymphocytes = 80-90%*	*0.398*
p53 (+ve) n=7/42	*4/30*	*3/12*	*0.398*
The mean p-53 protein concentration: 27.9 U / ml in healthy people	*The average p-53 protein concentration in CLL was on average 47 U/m*	*Percent p-53 positive = 15%.*	*0.398*

In another international study**,** 103 LLC cases were investigated for the impact of p53 protein expression with its β and γ isoforms. Interestingly, the relative levels of expression between the p53 protein full length and its β and γ isoforms were significantly altered in LLC even without 17-β13 deletion compared to normal B cells (p = 0.005.), (Table 4) [11]**.**

Table 4. Differential expression of p53 isoforms could disrupt p53 response and may contribute to pathogenesis LLC.

LLC - Patients	CLL stage I / II, n = 47 patients) P-53 protein concentration in reactive B LymphocyteS	*CLL stadiul III/IV (n = 140 de pacienți] Procent de proteine izoforme p53*	*p value*
The age of CLL patients ranged from 52 to 84 years, with a mean age of 62 years.	*In the samples studied at CLL it was 16.7% (7 out of 42 cases).*	*The percentage of p53 positive cells ranged from 7-32%.*	*0.398*

Hematological parameters in peripheral blood	*No. Leukocyte = 35x10³ / dl Hb = 12.2g / dL Thrombocytes = 140 x 10³/µL Lymphocytes = 75-80%*	*Nr. Leukocytes = 350 x10³/µL Hb = 10.8g / dL Thrombocytes = 80 x 10³/µL Lymphocytes = 80-90%*	*0.398*
P53 proteins form, (positive) n = 7/42	*15%*	*25%*	*0.398*

Differential expression of p53 isoforms could disrupt p53 response and may contribute to LLC pathogenesis. Between the two methods, ELISA, ICC, the CC, Pearson (r) Correlation Coefficient was calculated according to the following formula:

$$r = \frac{\sum(x - M_x)(y - M_y)}{n * Sx * Sy}$$

where: x, y is the sum of the products between the two variables = 567 and 1313.3, My and My are the averages of the two variables, 38,35 and 27,94, n = the number of subjects in the sample = 20 + 47 = 67, Sx and Sy are the standard deviations of the two variables, 8.25 and 17.35. (CC = (567-16.7) x (1313.3-27.9) / 67 x 8.25 x 17.35; CC = 550.3 x 12854 / 9.590.2125 = 7.073.556.2 / 9.590.2125 = 0.74).

It was observed that "r", CC obtained (0.74), is also significant at a level of significance higher than p <0.001, respectively 0.034. The (r) Pearson index can be significant, with "r" values being compared between 0.74 and 0.80, (> 0.50). When the percentage of p-53 positivity was correlated with the clinical stage of the disease, the proportion of positive p53 cases increased significantly from stage A Binet, (7.4%) stage B (24.4%) and stage C, (29.2%) (p = .002). The results of this study indicated that in CLL, p53 protein expression analyzed by an immunocytochemical method is strongly associated with p-53 gene mutations and a variant morphological analysis of p53.

P53 genes have been shown to promote hematopoietic stem cell stagnation (HSCs) and self-renewal. And recent studies have shown that p-53 protein deficiency promotes CLL with Richter Syndrome and Non-Hodgkin's Lymphoma, Diffuse Large Lymphoma (DLL), by eliminating its ability to limit aberrant self-renewal in hematopoietic progenitors, [12].

Research has also shown that acetylation, deacetylation and phosphorylation of non-mutant p-53 protein can repair DNA damage in cancer cells but different isoforms of p53 protein have different cellular mechanisms in cancer. Altered activity of the p53 protein in the isoform state affects DNA damage and even accelerates forms of cancer from the mild form to the form severe (Fig. 6).

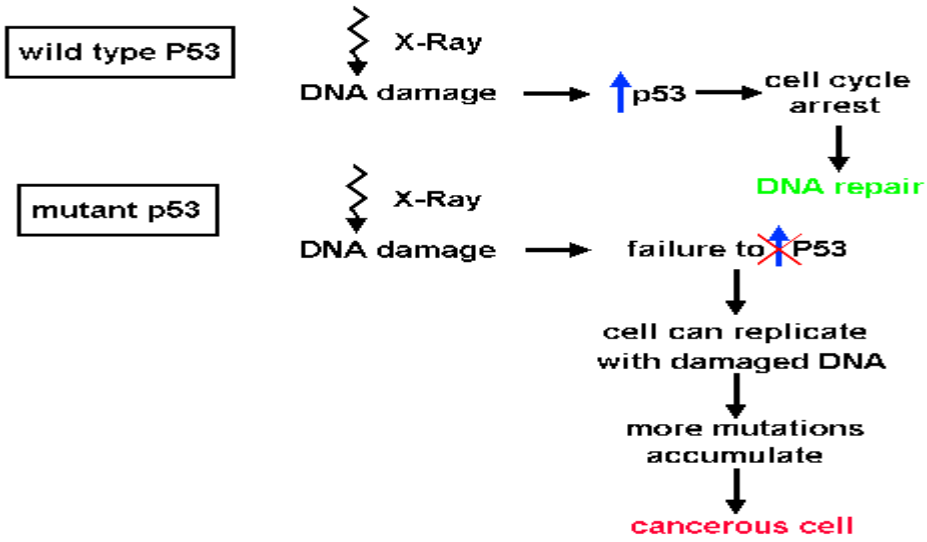

Figure 6. Alteration of p53 protein function can induce cancer in cells

Replacing the amino acid serine in the p53 protein with the amino acid alanine resulted in preventing the p53 protein from inhibiting the cell cycle. Research has shown that restoring the function of the isomorphic p53 protein could lead to the regression of certain cancer cells without damaging other cells in the process, [13].

Nutlin-3, a cis-imidazole analog, was found to strongly induce apoptosis in cell lines derived from hematologic malignancies and B cell translocation LLC with frequent translocation 14q32-17p, with a good therapeutic response. Other studies have suggested that a high concentration of adenosine triphosphate (ATP) in malignant B-cell lymphocytes in CLL affected the P53 gene to induce cell apoptosis, [14].

It is recommended that before initiating treatment in CLL, ATP levels related to the presence or absence of P-53 gene mutations should be measured to adjust doses of cytostatic treatments that have been resistant, causing the disease to relapse. In order to avoid very high doses of cytostatic, which can induce side effects, in relapses from CLL it is also recommended the administration of drugs such as AZD2014, (Vistusertib), ATP-competitive or ATP inhibitory analogues, e.x. isopentenyl ester of ATP (AppI), which is a triphosphoric acid ester and inhibits the signals of the uncontrolled cell division of the cell cycle. The respective researches are in stage I clinical trials (Clinical Trials) [15].

Consistent with this role in the autophagy process, p53 activity is compromised in a large proportion of all cancers, either by mutation of the P53 gene (p53 coding) or by altering the status of p-53 modulators. The contextual role of autophagy in cancer, which could be changed by p-53 status, is expected to be developed in a new anti-cancer therapeutic approach, [16, 17].

7. Conclusion

P-53 Gene mutation is the most common genetic abnormalities of cancer. In last years, have been extensively studies in various mature B cell malignancies, including Chronic Lymphocytic Leukemia, (CLL) with isoform p-53 protein, products of this gene, in malignant diseases.

In recent years, more attention has been paid to the importance of the p53 expressed protein in CLL, and a combination with low survival and non-response to classical conventional chemotherapy, due to mutations in the P-53 gene, with progression to Diffuse Large Lymphoma. Identifying different P-53 gene mutations is very important because these mutations have an impact on patients' clinical course in CLL with the p-53 protein mutant isoform.

Declaration of interest The authors declare that they have no conflict of interest.

References

1. M. Fischer, Census and evaluation of p53 target genes, Oncogene 36 (2017), 3943–3956.
2. K. Sabapathy, D.P. Lane, DP, Understanding p53 functions through p53 antibodies, J. Mol. Cell Biol, 11 (2019), 1-35.
3. E. Campo, F. Cymbalista, P. Ghia, U. Jäger, S. Pospisilova, R. Rosenquist, A. Schuh, S. Stilgenbauer, TP53 aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics, Haematologica 103, (2018) 1956-1968. Doi: 10.3324/haematol.2018.187583.
4. D. Gonzalez, P. Martinez, R. Wade, S. Hockley, D. Oscier, E. Matutes, C.E. Dearden, S.M. Richards, D. Catovsky, G.J. Morgan, Mutational status of the TP53 gene as a predictor of response and survival in patients with chronic lymphocytic leukemia: results from the LRF CLL4 trial, J Clin Oncol 29, (20 11)2223-2229. Doi: 10.1200/JCO.2010.32.08 38.
5. I. Cordone, S. Masi, F.R. Mauro, S. Soddu, O. Morsilli, T. Valentini, M.L. Vegna, C. Guglielmi, F. Mancini, S. Giuliacci, A. Sacchi, F. Mandelli, R. Foa, p53 expression in B-cell chronic lymphocytic leukemia: a marker of disease progression and poor prognosis, Blood, 91, (1998) 4342-4349.
6. M. Hallek, B.D. Cheson, D. Catovsky, F. Caligaris-Cappio, G. Dighiero, H. Döhner, P. Hillmen, M. Keating, E. Montserrat, N. Chiorazzi, S. Stilgenbauer, K.R. Rai, J.C. Byrd, B. Eichhorst, S. O'Brien, T. Robak, J.F. Seymour, T.J.. Kipps, TJ iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive manage ment of CLL, Blood, 21 (2018) 2745-2 760. Doi: 10.1182/blood-2017-09-806398.
7. S. C.ordone Masi, F.R. Mauro, S. Soddu, O. Morsilli p53 Expression in B-Cell Chronic

Lymphocytic Leukemia: A Marker of Disease Progression and Poor Prognosis, Blood 91, (2019) 4342-4349.
8. F. Bosch, R. Dalla-Favera, Chronic lymphocytic leukaemia: from genetics to treatment. Nature review, Clinical Oncology; (2022) www.nature.com/nrclinonc, Accessed in March
9. S. Spiro, D.A.G. Galton, E. Wiltshaw, R.C. Lohmman., Folicular Lymphoma: A survey of 75 cases with special references to the Syndrome resembling Chronic Lymphocitic Leukemia, Br. J. Cancer, Suppl. II, (1975) 60.
10. B. Murnyák, T. Hortobágyi, Immunohisto chemical correlates of TP53 somatic mutations in cancer. Oncotarget 40 (2016) 649 10-64920.
11. L. Sellmann, A. Carpinteiro, H. Nückel, R. Scholtysik, D. Siemer, p53 protein expression in chronic lymphocytic leukemia, Leuk Lymphoma, 53 (2012) 1282-1298.
12. N. Allende-Vega, E. Krzywinska, S. Orecchioni, N. Lopez-Royuela, F. Reggiani, G.Talarico, J.F. Rossi, R. Rossignol, Y. Hicheri, G. Cartron, F. Bertolini, M. Villalba, The presence of wild type p53 in hematological cancers improves the efficacy of combinational therapy targeting metabolism, Oncotarget, 22 (2015) 19228-192 45. Doi: 10.18632/oncotarget.4653.
13. M.J. Demma, S. Wong, E. Maxwell, Dasmahapatra (2004) 24–CP-31398 Restores DNA-binding Activity to Mutant p53 in Vitro but Does Not Affect p53 Homologs p63 and p73, The Journal of Biological Chemistry, 279 ,4,(2004) 45887–45896.
14. A. Udriștioiu, C. Florescu, A.M. Popescu, M. Cojocaru, High concentration of anaerobic ATP implicated in aborted apoptosis from CLL, Lab. Med 41 (2010) 203–208.
15. B. Apsel, J.A. Blair, B. Gonzalez, T. Nazif, M.E. Feldman, B. Aizenstein, R. Hoffman, K.M. Willi Kokat, Z.A. Knight, Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases, Nat Chem Biol, 4 (2008) 691–699.
16. S.K. Kondapuram, S. Sarvagalla, M. Coumar, Targeting autophagy with small molecules for cancer therapy, Journal of Cancer Metastasis and Treatment, 1 (2019) 23-25.
17. M. Shahjahani, J. Mohammadiasl, F. Noroozi, M. Seghatoleslami, S. Shahrabi. F. Saba, N. Saki, Molecular basis of chronic lymphocytic leukemia diagnosis and prognosis, Cell Oncol, 38 (2015) 93-109. Doi: 10.1007/s13402-014-0 215-3.