

# The role of the P-53 gene and the P-53 protein in non-Hodgkin malignant lymphomas

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<b>Registration date</b> 12/11/2022	<b>Overall study status</b> Completed	<input type="checkbox"/> Statistical analysis plan <input checked="" type="checkbox"/> Results
<b>Last Edited</b> 03/05/2023	<b>Condition category</b> Cancer	<input type="checkbox"/> Individual participant data

## Plain English summary of protocol

### Background and study aims

P-53 gene mutations are the most common genetic abnormalities of cancer. They have been extensively studied in various mature B-cell malignancies, including chronic lymphocytic leukemia (CLL). 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 p53 gene, with progression to Richter Syndrome. Identifying different p53 gene mutations is very important because these mutations have an impact on the patient's clinical course in CLL.

### Who can participate?

Patients with CLL-B who were hospitalized in the Hematology departments of the Targu Mures Oncology Institute and Cluj-Napoca between November 2016 and September 2019

### What does the study involve?

Participants undergo a complete physical examination and laboratory blood tests. p53 protein levels are measured at a single timepoint.

### What are the possible benefits and risks of participating?

Not provided at time of registration

### Where is the study run from?

Titu Maiorescu University (Romania)

### When is the study starting and how long is it expected to run for?

October 2016 to January 2020

### Who is funding the study?

Investigator initiated and funded

### Who is the main contact?

Dr Aurelian Udristioiu, aurelianu2007@yahoo.com

# Contact information

## Type(s)

Principal investigator

## Contact name

Dr Aurelian Udristioiu

## ORCID ID

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## Contact details

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# Additional identifiers

## Clinical Trials Information System (CTIS)

Nil known

## Protocol serial number

UEFISCDI ID (UEF-iD): U-1900-063Y4656

# Study information

## Scientific Title

The role of the P-53 gene and the P-53 Protein in the oncogenesis of non-Hodgkin malignant lymphomas

## Study objectives

p53 gene mutations are the most common genetic abnormalities of cancer. They have been extensively studied in various mature B-cell malignancies, including chronic lymphocytic leukemia (CLL). 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 p53 gene, with progression to Richter Syndrome. Identifying different p53 gene mutations is very important because these mutations have an impact on patients' clinical course in CLL with the p53 protein mutant isoform.

## Ethics approval required

Old ethics approval format

## Ethics approval(s)

Ethics approval not required

## **Study design**

Observational cohort study

## **Primary study design**

Observational

## **Study type(s)**

Diagnostic

## **Health condition(s) or problem(s) studied**

Non-Hodgkin malignant lymphomas

## **Interventions**

**Complete physical examination:** In patients diagnosed with CLL-B, symptoms such as frequent cough, night sweats, and retrosternal pain were evaluated. Clinical examination and ultrasound revealed lymphadenopathy and/or splenomegaly, with an enlarged spleen.

**Laboratory examinations:** Hemoleukogram with 5 Diff and cytological examination of the blood smear on the peripheral blood in the May Grunwald-Geimsa staining, and bone marrow puncture, BM with medullary forcing. The cases were classified as CLL with >5000 lymphocytes in absolute value, present at the cytological examination of the blood smear, from the peripheral blood or LLC with less than 10% prolymphocytes based on the peripheral blood smears May-Grunwald Giemsa, stained.

**Immunophenotyping:** The diagnosis of CLL was confirmed by immune phenotyping. All samples that entered the study were lymphocytes with positive CD19, CD20, CD5 and CD23 cell receptors. The CD38+ receptor was considered positive if the distinct lymphocytes of the population showed a higher intensity of staining than the granulocytes in the sample and was associated with the presence of protein ZAP-70.

A sandwich ELISA colorimetric quantitative method was used for direct detection of the p53 isoform protein, the 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 1620 has been reported to be specific for the conformation of the normal p-53 protein, and PAb 240 antibodies bind specifically to denatured p-53 protein. Compatible sample types: cell culture supernatants, plasma, serum; solid support: 96-well microplate; firm: Ray Biotech Life, Inc.

Plasma is collected from patient samples using vacutainers with EDTA or heparin as an anticoagulant by centrifuging the samples for 15 minutes at 4500 rpm (280 G). After the blood sample has been centrifuged and its plasma has been separated from red blood cells, the plasma is fractionated into four distinct fractions placed on a layer of white blood cells (lymphocytes).

With a pipette, a quantity of 100 µl is extracted from the lymphocyte ring. The extracted lymphocytes are introduced into 25 ml cuvettes with a 3 ml wash buffer medium for washing the lymphocytes. Washing is done three times, once after 10 minutes at 1500 revolutions/minute and twice for 10 minutes at 1000 revolutions/minute. Lysis of washed lymphocytes is done with a Mini Wave Smart Laboratory microwave. In the case of small-volume samples, a preliminary step dilution, such as 1: 5 or 1:10, can be performed using PBS buffer (0.02 mol / L pH 7.0-7.2) as the diluent. The final dilution should always be done using the same buffer used to dilute the Standards.

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 p53 protein, binds to the capture antibody.

Summary of test procedure:

1. Prepare all reagents, samples and standards: add 100 µl of sample, standard or blank to each well and incubate for 2.5 hours at room temperature or overnight at 4 °C
2. Aspirate the volume of liquid initially added and wash three times
3. Add 100 µl of biotinylated detection antibody (biotin detection antibody) and incubate for 1 hour at room temperature
4. Vacuum and wash three times
5. Add 100 µl of HRP-streptavidin conjugate and incubate for 45 minutes at room temperature
6. Add 100 µl of TMB substrate and incubate for 30 minutes at 37 °C
7. Add 50 µl of stop solution
8. Read immediately at 450 nm wavelength

A series of dilutions of the positive control standard must be performed in duplicate or triplicate, the last well in each series being the negative control sign. The tests should also be performed in duplicate or in triplicate. Unknown samples should function as dilution series to identify the optimal dilution that produces an OD value in the OD range of the standard control dilution series.

Data analysis: Prepare a standard curve from the serial dilution data with concentration on the x-axis (logarithmic scale) from the absorption on the Y-axis (linear). Peroxidase (HRP) and alkaline phosphatase (ALP) are the two most widely used enzymes for detection in ELISA tests. Measure the yellow color of nitrophenol at 405 nm after 15-30 minutes of incubation at room temperature and stop the reaction by adding an equal volume of 0.75 M NaOH.

## **Intervention Type**

Genetic

## **Primary outcome(s)**

P-53 isoform protein concentration measured using sandwich ELISA colorimetric quantitative method at a single timepoint

## **Key secondary outcome(s)**

There are no secondary outcome measures

## **Completion date**

15/01/2020

## **Eligibility**

### **Key inclusion criteria**

1. Patients diagnosed with CLL-B who were hospitalized in the Hematology departments of the Targu Mures Oncology Institute and Cluj-Napoca between November 2016 and September 2019
2. CLL with > 5000 lymphocytes in absolute value, present at the cytological examination of the blood smear, from the peripheral blood or LLC with less than 10% prolymphocytes based on the peripheral blood smears May-Grunwald Giemsa, stained

### **Participant type(s)**

Patient

**Healthy volunteers allowed**

No

**Age group**

Senior

**Sex**

All

**Total final enrolment**

20

**Key exclusion criteria**

Does not meet inclusion criteria

**Date of first enrolment**

01/11/2016

**Date of final enrolment**

01/09/2019

## **Locations**

**Countries of recruitment**

Romania

**Study participating centre**

**Titu Maiorescu University of Bucharest**

Faculty of Medicine

Damboviciului Street, NoL 22

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## **Sponsor information**

**Organisation**

Titu Maiorescu University

**ROR**

<https://ror.org/0367qb939>

# Funder(s)

## Funder type

Other

## Funder Name

Investigator initiated and funded

# Results and Publications

## Individual participant data (IPD) sharing plan

The datasets generated and/or analysed during the current study will be published as a supplement to the results publication

## IPD sharing plan summary

Published as a supplement to the results publication

## Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
<a href="#">Results article</a>			03/05/2023	Yes	No
<a href="#">Other publications</a>	Review	01/10/2018	03/05/2023	Yes	No
<a href="#">Other unpublished results</a>			03/05/2023	No	No
<a href="#">Study website</a>	Study website	11/11/2025	11/11/2025	No	Yes