ISRCTN12539707 https://doi.org/10.1186/ISRCTN12539707

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

Submission date	Recruitment status	[_] Prospectively registered
08/11/2022	No longer recruiting	[_] Protocol
Registration date	**Overall study status**	[_] Statistical analysis plan
12/11/2022	Completed	[X] Results
Last Edited	**Condition category**	[_] Individual participant data
03/05/2023	Cancer	

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
https://orcid.org/0000-0001-8375-8287

Contact details
Tiru Maiorescu University of Bucharest
Faculty of Medicine, Molecular Biology
Bucharest
Romania
040051
+40 (0)723565637
aurelianu2007@yahoo.com

Additional identifiers

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
Dambovicilului Street, NoL 22
Bucharest
Romania
040051

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?
Results article			03/05/2023	Yes	No
Other publications	Review	01/10/2018	03/05/2023	Yes	No
Other unpublished results			03/05/2023	No	No
Study website	Study website	11/11/2025	11/11/2025	No	Yes