Collaborative ROMD research on diagnosis, prognosis and treatment strategies for patients with HIV(+) DLBCL

Contract no.31ROMD/2024

Project Director Lector Ciprian Tomuleasa MD

Title: Collaborative ROMD research on diagnosis, prognosis and treatment strategies for patients with HIV(+) DLBCL

Budget: 710.520 lei

Implementation period: 20.05.2024-19.05.2026

Parteners:

- "Nicolae Testemiteanu" University of Medicine and Pharmacy, Chisinau, Republic of Moldova
- Oncology Institute, Chisinau, Republic of Moldova

The main aim of this joint research and development project proposal is to leverage the potential of excellence of researchers from the Iuliu Haţieganu University of Medicine and Pharmacy in Cluj Napoca, Romania (Coordinator), the Nicolae Testemitanu State University in Chișinău, Moldova (Partner P1), and the Institute of Oncology in Chișinău, Moldova (Partner P2), by providing access to the existing research infrastructure in Romania, in order to create a lymphoma-targeted genetic panel by using next-generation sequencing (NGS) to serve HIV(+) DLBCL as well as HIV(-) DLBCL patients, allowing a comprehensive assessment of the genetic landscape of DLBCL in both patient populations, facilitating a better understanding of the molecular mechanisms underlying the disease. This can lead to improved diagnosis, prognosis and personalized treatment strategies adapted to each patient. The project will contribute to the expansion of the Romanian research team by creating a full-time equivalent position. In addition, it will facilitate the mobility of researchers from both countries, promoting the exchange of good practices and knowledge transfer, will strengthen and advance the careers of 6 young researchers in Moldovan research institutions, improving their professional status through a set of educational visits to Romania, allowing them to fully and completely understand the scientific context behind the pathogenesis of HIV-associated lymphomas.

Team members:

"Iuliu Hatieganu" University of Medicine and Pharmacy Cluj Napoca

- Tomuleasa Ciprian-Project Director

- Adrian Bogdan Tigu-Member- Postdoctoral researcher
- Diana Cenariu- Member- Researcher
- Sabina Iluta- Member- PhD
- Minodora Silvia Desmirean- Member- Postdoctoral researcher
- Madalina Nistor-Member- Postdoctoral researcher

"Nicolae Testemiteanu" University of Medicine and Pharmacy, Chisinau, Republic of Moldova

- Maria Robu- Responsible for Partner 1 (P1)
- Daniela Galea-Abdusa-Member- Postdoctoral researcher
- Ivan Negara- Member- PhD
- Victor Tomacinschii- Member- PhD

Oncology Institute, Chisinau, Republic of Moldova

- Oleg Arnaut- Responsible for Partner 2 (P2)
- Ina Sclifos- -Member- Postdoctoral researcher
- Dumitru Brinza- Member- PhD
- Cristina Dudnic- Member- PhD

Intermediary results:

Development of the nucleic acid extraction protocol for the evaluation of gene expression from paraffin-embedded tissue samples
Isolation of RNA and DNA from paraffin blocks, sections of 20 microns from the samples of interest will be made. Thus, each sample consists of 4-8 paraffin sections from the lymph node.

a. The paraffin-embedded tissue is stored in sterile plastic tubes, with a volume of 1.5 mL and a conical bottom.
b. On top of each group of 4x20 um or 8x20 um sections, 1mL of 100% Xylene - analytical purity is added.
c. The tubes are centrifuged and the sediment is then incubated at 50 degrees Celsius for 3 minutes, followed by centrifugation at maximum speed, at room temperature.

d. The samples are washed with pure ethanol, by centrifugation. Then they are left for 15-40 minutes in a sterile laminar flow hood to evaporate the ethanol residue.
   Further processing is performed by using the Kit produced by Ambion/ThermoScientific – Recover All Total Nucleic Acid Isolation Kit AM1975.

e. Each 40 µm is immersed in 100 µL of Digestion Buffer and 4 µL of protease is added for protein degradation. The sample is mixed gently, without vortexing!

f. For RNA isolation – incubate for 15 minutes at 50°C followed immediately by 15 minutes at 80°C, without shaking.

g. Then digestion buffer (prepared according to the kit) is added and mixed by pippeting.

h. The samples are transferred to filter columns and washed by centrifugation with Wash 1 and Wash 2/3 (according to the kit protocol).
   For RNA isolation, follow the steps in the kit.

i. Take an amount of 60 uL of DNAse mix and incubate it at room temperature for 30 minutes.

j. The samples are washed with Wash 1 and Wash 2/3, according to the protocol, and finally the filter columns are moved to a sterile collection tube and 60 uL of ultrapure water, free of nucleases and pyrogens, are pipetted over the filter.

k. Incubate for 1-2 minutes at room temperature – to allow the water to detach the RNA from the filter.
   Sample evaluation and storage

l. RNA is measured at Nanodrop following the absorption ratios at 260/280 nm and 260/230 nm so that it complies with the quality requirements for RNA.

m. Storage is carried out at -80°C in sterile tubes, in ultrafreezers.