

STEPUPIORS

Twinning for a European Consortium of Rectal Cancer Research Institutions through Stepping up Scientific, Technological and Innovation Excellence of IORS



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1. Description of the report

WP2 was focused on determining the molecular profile associated with response to neoadjuvant chemoradiotherapy (CRT) in patients with locally advanced rectal cancer (LARC). The main tool in achieving this goal are genomic, transcriptomic, radiomic and proteomic analysis of tumor samples and liquid biopsies to identify differentially expressed entities associated with response to CRT. *In silico* methods and wet-lab analyses from tissue and liquid biopsy were used for uncovering potential prognostic and predictive markers. Accordingly, one of the main goals of WP2 that was incorporated into D2.1 was building and improvement of professional capacities at Institute of Oncology and Radiology of Serbia (IORS) for “omics” analyses.

In this sense several activities conducted during the STEPUPIORS project were related to the deliverable D2.1:

1. expert visits conducted by IORS project participants
2. courses and schools organized by the project participants
3. courses and schools attended by the project participants
4. scientific papers that resulted from improvement of professional capacities in “omics”

2. Training visits to partner institutions

In the period from 05-07. December 2022, partner institution BRFAA offered training in proteomics analysis, including total proteome profiling and tumor immune cell profiling by CyTOF. STEPUPIORS project participants Aleksandra Stanojevic, MSc and Mladen Marinkovic, MD, went on a training visit to the BRFAA to gain knowledge in proteomics and transcriptomics. During the training, the Cytometry by time of flight (CyTOF) method, which enables simultaneous quantification of multiple cellular components using a detector based on mass spectrometry with inductively coupled plasma, was theoretically and experimentally presented by Dr Nikolaos Paschalidis.

STEPUPIORS project participants Aleksandra Stanojević, MSc, Dr Snežana Bjelogrić and Mladen Marinković, MD, went on a training visit to the partner institution IDIBAPS in Barcelona, Spain, to gain knowledge in genomics, in the period from 10-12.01.2023.

STEPUPIORS project participants Dr Milena Čavić, Dr Suzana Stojanović-Rundić, Dr Radmila Janković, Dr Miljana Tanić, Dr Marija Đorđić Crnogorac, Dr Ana Đurić and Dr Marko Radulović went on a training visit to the partner institution NKI AVL in Amsterdam, Netherlands, to get knowledge in genomics and radiomics. The visit took place on February 14-15, 2023.

On December 4, 2023, Dr Ana Đurić from IORS visited the Core Facility for Proteomics and Metabolomics at the Faculty of Biology, the National and Kapodistrian University of Athens (NKUA) where the BRFAA PI Prof. Ieronymos Zoidakis has set up a new proteomics facility. During this training visit, collaborative knowledge exchange with Professor Evangelos Gikas and Dr. Anthi Panara from the Metabolomics Laboratory took place. The research group of Professor Gikas agreed on a collaboration and a metabolomic study on LARC samples within the STEPUPIORS project expanding the scope of the project. During this visit, the meeting with Dr. Julie Courraud, co-director of the Proteomics facility at NKUA took place to discuss the use of advanced mass spectrometers (Bruker TIMS-TOF Flex, Bruker TIMS-TOF Pro) in collaborative activities.

3. Training activities organized by project participants

For the purpose of improving skills and widening knowledge about “omics” techniques one training was organized at IORS while one workshop and one summer school related to omics analyses were organized at NKUA.

3.1 ELISA training

STEPUPIORS participants, Dr Ana Vuletić and Dr Katarina Mirjačić Martinović, organized a hands-on two-day ELISA Training on March 22-23, 2023 for IORS project participants and other interested IORS colleagues. During the course, participants gained knowledge on principles and applications of ELISA method and also had an opportunity to see demonstration of this method step-by-step. Documents relevant to the training: [Report](#).

3.2 Omics data generation and integration with clinical information

The two-day interactive workshop "Omics data generation and integration with clinical information" was held on June 01-02, 2023, in Athens, Greece. During the workshop, 17 physicians and researchers of IORS and 43 course participants from 4 partner institutions gained knowledge principles of proteomics, transcriptomics, metabolomics and genomics along with computational tools for integrating omics datasets and establishing connections with clinical information. This workshop also highlighted the statistical and bioinformatics tools available for the analysis of omics and clinical data.

The course was led by 4 main researchers from the partner institutions of this project, Prof. Ieronymos Zoidakis from BRFAA. Dr. Sergi Castellvi-Bel from FRCB-IDIBAPS, Dr. Remond Fijneman from NKI-AVL and Dr. Milena Čavić.

The topics that were presented to the participants by experienced lecturers included the application of clinical proteomics by Tonia Vlahou (BRFAA), clinical transcriptomics - Soufyan Lakbir (NKI-AVL), epigenomics – Miljana Tanić (IORS), metabolomics - Ana Đurić (IORS), clinical applications of mass spectrometry – Nikos Paschalidis (BRFAA), HAP-1 cell models - Gemma Llargués (IDIBAPS), gene editing – Xavier Dominguez-Rovira (IDIBAPS), organoids - Laia Bonjoch (IDIBAPS) and fragmentomics – Alessandro Leal (Delfi Diagnostics) in oncology research.

Furthermore, topics related to the application of circulating tumor DNA in oncological diagnostics - Remond Fijneman (NKI-AVL), the discovery of frequent and rare genetic variants in colorectal cancer - Sergi Castellví-Bel (IDIBAPS), the application of omics techniques in predicting the response to chemoradiotherapy in rectal cancer - Mladen Marinković, Aleksandra Stanojević, Milena Čavić (IORS), spatial transcriptomics - Emmanouil Athanasiadis (UniWA), metabolomics in screening - Julie Courraud (NKUA), chemoproteomics in the identification of oncological therapeutic targets - Guillaume Médard (NKUA), multiplex ELISA in validation and clinical implementation of biomarkers - Leonidas Alexopoulos (PROTAVIO), as well as the erroneous application of markers from urine - Ieronymos Zoidakis (BRFAA).

The training agenda and included topic are shown in documents relevant to the workshop:

- [Agenda](#)
- Attendance list [Day 1](#), [Day 2](#)
- [Report](#)

3.3 Summer school *Liquid biopsy technologies*

In the period from September 30 to October 4, 2024, 16 clinicians and researchers from IORS attended the four-day summer school "Liquid Biopsy Technologies" that was held at the Analysis of Circulating Tumor Cells Laboratory (ACTC) lab, NKUA, Athens, Greece hosted by Prof. Evi Lianidou (NKUA), Dr Remond Fijneman (NKI AVL) and Prof. Ieronymos Zoidakis (BRFAA & NKUA).

The 3-day-course on liquid biopsies was organized in the form of lectures that were held on the first day of the course and "hands-on" demonstrations of liquid biopsy techniques that were held in laboratory. The participants gained theoretical and practical knowledge of the applications of liquid biopsies in oncology, isolation of circulating tumor DNA (ctDNA) and circulating tumor cell (CTC), characterization and enumeration of CTCs, detection of mutations in ctDNA, ctDNA methylation analyses, digital droplet PCR and preanalytical aspects and quality control in liquid biopsies. The lectures and practical part of the course were led by Prof. Evi Lianidou, Ass. Prof. Athina Markou, Dr Aliko Ntzifa, Post-Doctoral Research Associate, Stavroula Smilkou, MSc; Elena Thanou, MSc, and Elina Lagopodi, MSc, from the ACTC lab, Dept of Chemistry, NKUA, Dr Miljana Tanic, Senior Research Associate, IORS, Dr Remond Fijneman and Dr Paul Van der Leest, from NKI AVL.

The summer school included topics:

1. Liquid Biopsy: an introduction | E. Lianidou
2. Liquid biopsy: an overview in Serbia - M. Tanic
3. ctDNA applications in colorectal cancer - R. Fijneman
4. CTC: an overview of enrichment technologies - A. Markou
5. CTC: enumeration and phenotypic characterization - S. Smilkou
6. CTC: molecular characterization - A. Markou
7. ctDNA: mutation detection / PCR, NGS - A. Markou
8. ctDNA: methylation analysis /MSP, cfMeDIP - A. Ntzifa
9. The technical possibilities of droplet digital PCR analyses - P. Van der Leest
10. Pre-analytical aspects and Quality Control in liquid biopsy applications: How important is it? - A. Ntzifa

Teaching material and the agenda of the course from summer school Liquid Biopsy Technologies is available at the project website [Training section](#).

Other documents relevant to the training:

- [Agenda](#)
- [Attendance list](#)
- [Report](#)

4. Courses and webinars

During the course of STEPUPIORS project, researchers attended online “omics” webinars and workshops that contributed to their continuous education in “omics”:

1. EASI-Genomics and BBMRI Workshop on Implementing Genomic Research Projects, Feb 1, 2023 to Feb 2, 2023, attended by Aleksandra Stanojević, Miljana Tanić
2. Precision Oncology: Genomics Guided Care – Update of the Recommendations for the Use of Next-Generation Sequencing (NGS) for Patients with Metastatic Cancer. 22.11.2023., attended by Milena Čavić
3. ESMO Deep Dive Webinar Series on Lower - Digestive Cancers - A Deep Dive into molecular selection & monitoring of adjuvant strategies for patients with CRC – 08 March 2023 at 18:30 CET, attended by Mladen Marinković
4. X-omics festival 2023 “The future of multi-omics research is now!”, virtual event, 17.04.2023, attended by Milena Čavić, Ana Đurić
5. X-omics training school - online workshop cBioPortal by Mariska Bierkens and Iris Huitink of the Netherlands Cancer Institute, July 4, 2024, attended by Isidora Pantović
6. ELBS Workshop on Data Integration of Methylation and Fragmentomics in cfDNA, March 26, 2025, attended by Milena Čavić

Furthermore, STEPUPIORS researchers attended onsite courses and trainings that would aid them in performing “omics” analyses. The courses were on the subjects of advanced programming in R, metabolomics analysis, biochemical methodological approach in biomedicine research, excellence in qPCR and dPCR, Bulk transcriptomics”, and application of artificial intelligence in imaging analysis.

1. Advanced Programming in R, Royal Statistical Society, 7 - 8 December 2022, attended by Miljana Tanić
2. Biochemistry in the service of health: modern methodological approaches in biomedical research, Biochemical Society of Serbia, Belgrade and Novi Sad 15-20 May 2023, Milena Čavić (lecturer), attended by Aleksandra Stanojević, Ana Đurić
3. Taq Academy the qPCR and dPCR excellence Training- Academic & Biopharma Session 26 September 2023, attended by Aleksandra Stanojević
4. Bulk transcriptomics” during the training workshop entitled "MedILS School in Bioinformatics (Part II)" held at Mediterranean Institute for Life Sciences, Split, Croatia, from 16 to 20 October 2023., attended by Aleksandra Stanojević
5. EUCanImage Workshop: Trustworthy Artificial Intelligence in Multi-Country Cancer Imaging Rotterdam, Netherlands: 10 June 2024, attended by Ana Đurić
6. Training on Targeted and Non-targeted MS-data Analysis in Lipidomics, organized by the STRIMHealth team from the University of Belgrade – Institute for Biological Research “Siniša Stanković”, Belgrade, Serbia, 11 – 12 March, 2025, attended by Ana Đurić

5. Scientific papers resulting from improved professional capacities in “omics”

Improvement of professional capacities pertaining to the “omics” technics that participants of the project gained during collaboration with the partner institutions, and by attending trainings, courses, summer schools and workshops resulted in *in extenso* [publications](#), presentations of the results and conclusions of the research at conferences and publications of datasets. These results were achieved by joint work and collaboration of researchers from all 4 partner institutions.

5.1 *In extenso* publications

In extenso publications published during STEPUPIORS project include original research papers. The research paper by Marinković et al. presented genetic markers along with the hematological predictors of response to neoadjuvant CRT in LARK while the research paper by Stanojevic A et al. evaluated polymorphic variants of methylenetetrahydrofolate reductase in response to neoadjuvant CRT in Slavic population of rectal cancer patients.

Marinkovic M, Stojanovic-Rundic S, Stanojevic A, Ostojic M, Gavrilovic D, Jankovic R, Maksimovic N, Strogilos R, Zoidakis J, Castellví-Bel S, Fijneman RJA, and Cavic M. Exploring novel genetic and hematological predictors of response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer. *Front. Genet.* 2023, 14:1245594. doi: 10.3389/fgene.2023.1245594. link: <https://www.stepupiors.eu/wp-content/uploads/2023/09/2023-Marinkovic-et-al>.

Stanojevic A, Spasic J, Marinkovic M, Stojanovic-Sundic S, Jankovic R, Djuric A, Zoidakis J, Fijneman RJA, Castellvi-Bel S, Cavic M. Methylenetetrahydrofolate reductase polymorphic variants C677T and A1298C in rectal cancer in Slavic population: significance for cancer risk and response to chemoradiotherapy. *Frontiers in Genetics.* 2023; 14. <https://doi.org/10.3389/fgene.2023.1299599>.)

As a result of improved professional capacities in proteomics one original research paper based on characterization of response to neoadjuvant CRT by the spectrometric analysis of rectal cancer samples was published in *International Journal of Molecular Sciences* in 2023 by Stanojevic A et al. (listed below). More importantly, this publication contains proteomic data set obtained in this study (<https://www.mdpi.com/article/10.3390/ijms242015412/s1>).

Stanojevic A, Samiotaki M, Lygirou V, Marinkovic M, Nikolic V, Stojanovic-Rundic S, Jankovic R, Vlahou A, Panayotou G, Fijneman RJA, Castellví-Bel S, Zoidakis J, and Cavic M. Data-Independent Acquisition Mass Spectrometry Analysis of FFPE Rectal Cancer Samples Offers In-Depth Proteomics Characterization of the Response to Neoadjuvant Chemoradiotherapy. *International Journal of Molecular Sciences.* 2023; 24(20):15412. <https://doi.org/10.3390/ijms242015412>. <https://www.mdpi.com/1422-0067/24/20/15412>

The article by Cavic M et al. published in *Biomedicines* journal in 2024. gave an overview on applications of “omics” technique through the presentation of the experience of personalized medicine in treatment of colorectal cancer in Institute for Oncology and Radiology of Serbia.

Cavic M, Nikolic N, Marinkovic M, Damjanovic A, Krivokuca A, Tanic M, Radulovic M, Stanojevic A, Pejnovic L, Djordjic Crnogorac M, Djuric A, Vukovic M, Stevanovic V, Kijac J, Karadzic V, Nikolic S, Stojanovic-Rundic S, Jankovic R, Spasic J. Two Decades of Progress in Personalized Medicine of Colorectal Cancer in Serbia—Insights from the Institute for Oncology and Radiology of Serbia. *Biomedicines*. 2024; 12(10):2278. <https://doi.org/10.3390/biomedicines12102278>
<https://www.mdpi.com/2227-9059/12/10/2278>

Research achievements in radiomics resulted in one original research paper by Marinkovic M et al., which appeared in *Journal of Clinical Medicine* in 2024.

Marinkovic M, Stojanovic-Rundic S, Stanojevic A, Tomasevic A, Jankovic R, Zoidakis J, Castellví-Bel S, Fijneman RJA, Cavic M, Radulovic M. Performance and Dimensionality of Pretreatment MRI Radiomics in Rectal Carcinoma Chemoradiotherapy Prediction. *Journal of Clinical Medicine*. 2024; 13(2):421. <https://doi.org/10.3390/jcm13020421>
<https://www.mdpi.com/2077-0383/13/2/421>

Building of professional capacities in “omics” based approach during the STEPUPIORS project contributed to the creation of several review articles.

Vuletić A, Mirjačić Martinović K, Spasić J. Role of Histone Deacetylase 6 and Histone Deacetylase 6 Inhibition in Colorectal Cancer. *Pharmaceutics*. 2024; 16(1):54. <https://doi.org/10.3390/pharmaceutics16010054>. Link: <https://www.mdpi.com/1999-4923/16/1/54>

Kijac J, Mirjagic Martinovic K, Cavic M. The significance of circulating cytokines in cancer development, prognosis and response to anticancer therapy. *Chemical Review of the Serbian Chemical Society*. 2024, 65(4): 81-90. ISSN 04406826.

Tanić M, Ecker S, Lyskjær I. Editorial: Epigenetic biomarkers for cancer risk stratification and patient management. *Front Genet*. 2024 May 8;15:1421500. doi: 10.3389/fgene.2024.142150

Review by Vuletić A et al. published in *Pharmaceutics* journal in 2024. presents the role of histone deacetylase 6 in maintenance of malignant phenotype and invasiveness of colorectal cancer and possibilities for its therapeutic inhibition in this malignancy. Furthermore, Kijac J et al. in review published in *Chemical Review of the Serbian Chemical Society* in 2024 gave insights on the significance of circulating cytokines in pathogenesis, prognosis and response to anticancer drugs in various malignancies including colorectal cancer.

Open Science Datasets derived from these studies are freely available online: [STEPUPIORS Datasets](#).

5.2 Abstracts presented at scientific meetings

The research results of STEPUPIORS project obtained using „omics“ techniques were also presented at several congresses and scientific meetings. In this sense, one abstract presented at EACR Congress in 2023. by Stefanovic A et al., was based on research results obtained by genomics and proteomics data regarding genetic and hematological predictors of response to neoadjuvant CRT. Furthermore, in-depth proteomic characterization of the response to neoadjuvant chemoradiotherapy in LARC using data-independent acquisition mass spectrometry was presented at the annual meeting of Hellenic Society of Biochemistry and Molecular Biology by Stefanović A et al. in 2023. Another genomics- based research on the detection of viral particles in specimens of LARC was presented at 6th Congress of Serbian Association for Cancer Research (SDIR): From Collaboration to Innovation in Cancer Research, in 2023. by Stefanović A et al. Furthermore, genomics based-research resulted in two congress presentations on hereditary colorectal cancer by Djordjic Crnogorac M et al. at 15th International Symposium of Variants in the Genome and 10th Biennial Meeting of the International Society for Gastrointestinal Hereditary Tumors in 2024. Radiomics-based research resulted in another presentation at SDIR 2023 congress by Dr Mladen Marinković. The management of data obtained by multiomics analyses was presented at the 5th Belgrade Bioinformatics Conference – BelBi2024 by Dr Miljana Tanić.

List of abstracts:

Stefanovic A, Lygirou V, Stojanovic-Rundic S, Jankovic R, Castellvi-Bel S, Fijneman R, Vlahou A, Zoidakis J, Samiotaki M, Cavic M. Correlation between genetic and proteomic expression of predictive biomarkers of response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer identified by DIA mass spectrometry. *Molecular Oncology*, Volume 17 (Suppl. 1):96-97. EACR 2023: Innovative Cancer Science, 12-15 June 2023, Torino, Italy. Abstract No. 1195.

Stefanović A, Lygirou V, Marinković M, Stojanović-Rundić S, Janković R, Vlahou A, Zoidakis J, Fijneman R., Castellvi-Bel S., Samiotaki M, Čavić M. In-depth proteomic characterization of the response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer using data-independent acquisition mass spectrometry (DIA-MS). Annual meeting of the Hellenic Society of Biochemistry and Molecular Biology. 2-4.12.2022. Patra, Greece, Abstract book, P3, P99.

Stanojević A, Samiotaki M, Lygirou V, Marinković M, Nikolić V, Stojanović-Rundić S, Janković R, Banko A, Miljanović D, Vlahou A, Panayotou G, J.A. Fijneman R, Castellvi-Bel S, Zoidakis J, Čavić M. Detection of viral proteins in locally advanced rectal cancer patient samples by mass spectrometry – predictive potential for response to neoadjuvant chemoradiotherapy. 6th Congress of Serbian Association for Cancer Research (SDIR): From Collaboration to Innovation in Cancer Research, October 02-04th 2023. Belgrade, Serbia. *Oncology Insight* ISSN 3009-3848 2023. 2023(1). Abstract book. p.77-78.

Marinković M, Stojanović-Rundić S, Stanojević A, Janković R, Zoidakis J, Castellvi-Bel S, J.A. Fijneman R, Čavić M, Radulovic M. Predicting response to chemoradiotherapy in locally advanced rectal cancer using MRI-based radiomics features. 6th Congress of Serbian Association for Cancer Research: From Collaboration to Innovation in Cancer Research, October 02-04th 2023. Belgrade, Serbia. *Oncology Insight* ISSN 3009-3848 2023. 2023(1). Abstract book. p.59.

Tanić M, Bierkens M, Radulović M, Stanojević A, Marinković M, Krivokuća A, Janković R, Đurić A, Castellvi-Bel S, Zoidakis S, Fijneman R, Čavić M. Navigating ELSI for FAIR multiomics data management within STEPUPIORS international rectal cancer project. 5 th Belgrade Bioinformatics Conference – BelBi2024, Belgrade, Serbia, June 17-20, 2024. Abstract book, p. 117-118.

Djordjic Crnogorac M, Karadzic V, Krivokuca A. Identification of two novel germline MLH1 mutations in colorectal carcinoma patients: Variant interpretation in practice. 15th International Symposium of Variants in the Genome. Porto, Portugal. Abstract book, p.79-80. POS39

Djordjic Crnogorac M, Krivokuca A, Karadzic V. Hereditary colorectal cancer – first report on mutational profile in Serbia. 10th Biennial Meeting of the International Society for Gastrointestinal Hereditary Tumors, June 19-22, 2024, Barcelona, Spain. InSight 2024 Abstract Book, p.433-433, P108.