1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:

Identification of low-molecular-weight metabolites of the gut microbiome as biomarkers of colorectal cancer – A novel approach to the analysis of biological samples

1.1. Project goals

Main Project Objective:

The primary aim of the project is to develop and apply advanced analytical techniques for the qualitative and quantitative determination of low-molecular-weight microbiota-derived metabolites in biological samples, and to correlate these findings with gut microbiota profiles (metagenomics) in order to identify risk biomarkers for colorectal adenocarcinoma.

This overarching goal will be achieved through:

- developing and validating analytical procedures for the isolation and quantitative analysis of selected low-molecular-weight metabolites from biological material using chromatographic techniques coupled with mass spectrometry (LC-MS/MS, GC-MS) and MALDI;
- dentification of a set of metabolites differentiating patients with colorectal adenocarcinoma from the control group (colonoscopy) with the aim of discovering potentially predictive or diagnostic biomarkers.;
- developing time-series and predictive regression models to quantitatively describe metabolic changes over time and their associations with gut microbiota composition, the patient's clinical status, and surgical intervention.

1.2. Outline

In recent years, there has been growing interest in the role of the gut microbiome in the pathogenesis of various chronic diseases, including colorectal cancer (CRC). Colorectal adenocarcinoma is the most common form of this malignancy, and its development is driven by complex interactions between genetic and environmental factors—as increasingly confirmed by research—as well as the composition and activity of the gut microbiota. A growing body of evidence suggests that the intestinal microbiota plays a crucial role in the initiation, progression, and therapeutic response in colorectal cancer, particularly in populations with obesity, which itself is a known risk factor for cancer development.

In this context, low-molecular-weight metabolites—products of fermentation and biochemical transformations involving gut bacteria—are of particular significance. Compounds such as short-chain fatty acids (SCFAs), biogenic amines, tryptophan metabolites, and trimethylamine N-oxide (TMAO) may influence inflammatory processes, trigger mutagenic changes, and modulate signaling pathways involved in tumorigenesis. Detailed analysis of these metabolites may serve as the basis for identifying non-invasive diagnostic biomarkers useful in the early detection of colorectal cancer.

This project proposes a modern analytical approach that integrates advanced instrumental techniques (LC-MS, GC-MS, MALDI) with innovative methods for the preparation of biological samples. Special emphasis will be placed on optimizing the extraction and purification of metabolites from complex biological matrices, including techniques such as solid-phase microextraction (SPME), dispersive solid-phase extraction (dSPE), and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), which—despite limited use in microbiome analysis to date—show significant research potential.

At a later stage, bioinformatic and statistical tools will be used for data integration and the identification of discriminative biomarkers. The resulting findings may form the foundation for developing a diagnostic panel with potential clinical applications.

The project is interdisciplinary in nature—combining analytical chemistry, microbiology, medicine, and informatics. Its implementation may contribute not only to improved early detection of colorectal cancer but also to a deeper understanding of the role of the microbiome in its development and treatment response.

1.3. Work plan

Stage I – Literature Review and Research Strategy

Analysis of current studies on the microbiome, metabolites, and colorectal cancer. Development of the research concept, including selection of patient groups and sample types. Preparation of bioethical documentation and coordination of collaboration with clinical units.

Stage II – Analytical Methods and Validation

Development and optimization of extraction procedures and the determination of low-molecular-weight metabolites using LC-MS/MS and GC-MS. Validation of methods in terms of sensitivity, selectivity, and reproducibility.

Stage III – Sample Collection and Biological Analysis

Recruitment of patients and collection of biological material. Execution of metabolomic analyses required to determine the microbiome profile.

Stage IV – Data Interpretation and Statistical Analysis

Compilation and analysis of microbiological and chemical data. Application of statistical and chemometric methods to identify discriminating factors and propose regression models describing metabolic changes.

Stage V – Conclusions, Publications, and Dissertation Defense

Interpretation of results, preparation of scientific publications, and completion of the doctoral dissertation. Presentation of results at conferences and finalization of the project.

1.4. Literature *(max. 7 listed, as a suggestion for a PhD candidate preliminary study)*

- G.D. Christian, et al., *Analytical chemistry, Wiley, 2014*
- K. Robards, D, Ryan, *Principles and Practice of Modern Chromatographic Methods,* Elsevier, 2022
- J. Pawliszyn, H. L. Lord *Handbook of Sample Preparation,* Wiley & Sons, 2010
- B. Buszewski, I. Baranowska, *Handbook of Bioanalytics,* Springer 2022
- Y. Jianing, et al. *Metabolic interactions of host-gut microbiota: New possibilities for the precise diagnosis and therapeutic discovery of gastrointestinal cancer in the future—A review,* Critical Reviews in Oncology/Hematology, 203, 2024, 104480

1.5. Required initial knowledge and skills of the PhD candidate

Theoretical knowledge:

- Understanding of the fundamentals of analytical chemistry, including separation techniques (particularly LC-MS, GC-MS, etc.) and sample preparation methods.
- Knowledge of metabolomics and biomarkers.
- General understanding of human biology and the basics of gastrointestinal physiology.

Practical skills:

- Experience in laboratory work – particularly in the preparation of biological samples.
- Ability to operate analytical instrumentation.

Soft skills:

- Ability to work in a team.
- Independence, good organizational skills, research initiative.
- Willingness to work with clinical material and potential collaboration with medical professionals.

1.6. Expected development of the PhD candidate's knowledge and skills

The implementation of the research project will enable the doctoral candidate to acquire specialized, interdisciplinary knowledge and practical skills at the intersection of analytical chemistry, microbiology, biomedical sciences, and bioinformatics. Throughout the project, the candidate will develop competencies in designing and conducting scientific research, integrating analytical and clinical data, and drawing conclusions relevant to translational medicine.

In the area of analytical chemistry, the doctoral candidate will learn to design and validate advanced methods for determining low-molecular-weight metabolites (such as short-chain fatty acids, biogenic amines, indoles, choline derivatives), using gas and liquid chromatography coupled with mass spectrometry. They will also gain practical expertise in modern strategies for preparing biological samples.

Given the clinical relevance of the research, the doctoral candidate will also acquire knowledge of human biology, gut microbiology, the mechanisms of colorectal cancer development, and the relationship between the microbiome and obesity. Thanks to collaboration with a co-supervisor who is a medical doctor, and with other specialists, the candidate will have the opportunity to participate directly in the planning of the clinical part of the project and in the interpretation of data from a medical perspective.

Another important aspect of development will be the acquisition of skills in multivariate data analysis and the integration of information from various sources (metabolomics, metagenomics, clinical data) using statistical, chemometric, and bioinformatics tools. This will enable the candidate to independently build predictive models and identify potential biomarkers for gastrointestinal diseases.

Moreover, the project will contribute to the development of scientific competencies, including preparing publications, presenting results at national and international conferences, working in a research team, and conducting studies in accordance with good scientific practice and ethical standards.