

The unfavourable prognosis of patients with colorectal cancer (CRC) is usually associated with the development of local recurrence and/or distant metastases. The proposed clinicopathological factors for predicting recurrence are inconsistent and lose prognostic significance over time. Regional lymph node metastasis (RLNM) is an important prognostic factor throughout the disease course and affects both overall survival and disease-free survival. However, there are few data on risk factors for lymph node metastasis in RLNM that can be determined before surgery. Therefore, it is necessary to search for molecular predictive factors of RLNM that can be assessed prior to treatment in order to create an appropriate predictive scale to select optimal personalised treatments for patients with CRC.

The main objective of the proposed project is to verify the hypothesis that the presence and levels of specific proteomic components in colon and rectal primary tumours and serum-derived small extracellular vesicles (sEVs) are specifically associated with disease invasiveness, progression and metastasis risk in CRC. In the proposed study, we will use state-of-the-art targeted proteomic approaches based on mass spectrometry techniques and tissue matrix analysis based on immunohistochemistry, as well as flow cytometry methods, to evaluate proposed multiplex proteomic panels of metastases, including metachronous distant metastases of colon cancer and regional lymph node metastases in colon and rectal cancer. A special effort will be made to characterise (identify VCAN protein isoforms) and evaluate VCAN and related matrisome proteins (THBS1, THBS2, ANXA2) as signatures of RLNM in rectal cancer in the primary tumour and proximal margin, as well as serum-derived sEVs. We will address the hypothesis that VCAN accumulation is associated with tumour metastasis and may serve as a strong prognostic and predictive biomarker in CRC.

Among the ongoing efforts to identify molecular predictors of CRC progression, mass spectrometry-based translational proteomics represents a unique tool for the global and in-depth search for protein biomarkers, as well as validation of potential tumour signatures and therapeutic targets, which is essential for their successful implementation in clinical practice. Verification of specifically elevated VCAN levels in patients with positive metastatic status, both in sEV and tumour tissues, could help to develop a potential non-invasive tool as a 'liquid biopsy' and/or 'tissue biopsy' (the gold standard in clinical practice) to determine tumour progression and metastatic risk. This type of comprehensive, systematic and multilevel validation study has not been presented in the literature to date.

The project will be conducted in two arms, comprising a retrospective (FFPE and fresh frozen primary tumour tissues) and a prospective (serum-derived sEV) arm of the study. Linking VCAN protein levels and other potential proteomic signatures present in sEV and primary tumour tissues to disease invasiveness, progression and metastasis status will enable clinically useful prognostic and predictive signatures to be proposed.

An innovative and measurable outcome of the project will be the development of a multi-centre platform for the validation of potential cancer biomarkers from a very small amount of clinical material. This is important as Poland lacks research units for comprehensive and reliable validation of test results based on mass spectrometry.