Colorectal cancer (CRC) is a leading cause of death worldwide. Surgical intervention is a successful treatment for stage I patients, whereas other more advanced cases may require adjuvant chemotherapy. The selection of effective adjuvant treatments remains, however, challenging. Accurate patient stratification is necessary for the identification of the subset of patients likely responding to treatment, while sparing others from pernicious treatment. Targeted sequencing approaches may help in this regard, enabling rapid genetic investigation, and at the same time easily applicable in routine diagnosis.

We propose a set of guidelines for the identification, including variant calling and filtering, of somatic mutations driving tumorigenesis in the absence of matched healthy tissue. We also discuss the inclusion criteria for the generation of our gene panel. Furthermore, we evaluate the prognostic impact of individual genes, using Cox regression models in the context of overall survival and disease-free survival. These analyses confirmed the role of commonly used biomarkers, and shed light on controversial genes such as CYP2C8.

Applying those guidelines, we created a novel gene panel to investigate the onset and progression of CRC in 273 patients. Our comprehensive biomarker set includes 266 genes that may play a role in the progression through the different stages of the disease. Tracing the developmental state of the tumour, and its resistances, is instrumental in patient stratification and reliable decision making in precision clinical practice.