

## **Abstract**

A deeper understanding of the molecular background of colorectal cancer (CRC) can help explain the development of the disease and its resistance to treatment, predict disease progression, and improve treatment prognosis. Some minimal molecular testing has been incorporated into standard clinical management to determine if a particular patient will benefit from a particular therapy, but more and more new genetic alterations are being discovered that appear to be associated with the development of resistance. Tissue biopsy of the tumor is the gold standard in terms of molecular testing, but there is an increasing demand for more non-invasive methods such as liquid biopsy. Using targeted next-generation sequencing, we analyzed sequence variants present in primary tumor, metastases, and cell-free tumor DNA - ctDNA of patients with metastatic CRC. The objectives were to analyze sequence variants of the primary tumor and identify possible pathogenic variants, to analyze differences between DNA of the primary tumor and metastases, to evaluate the use of ctDNA as a diagnostic tool, and to identify potential tumor-specific markers in ctDNA that can be used to monitor disease progression. Our results suggest the feasibility of using ctDNA for diagnostic purposes or even to monitor disease progression, but at the same time we found that this method needs to be optimized and have its limits due to sensitivity issues.

**Keywords:** sequence variants; mutation; colorectal cancer; circulating free tumor DNA; next generation sequencing