FACULTY OF MEDICINE IN PILSEN
CHARLES UNIVERSITY IN PRAGUE

BIOMEDICAL CENTER

Surveillance of cancer treatment efficiency by genomic analysis of individual circulating tumour cells

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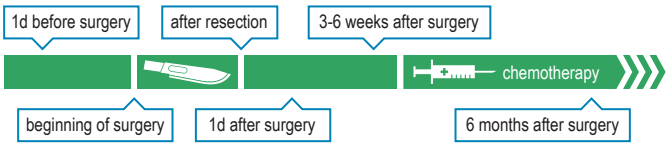
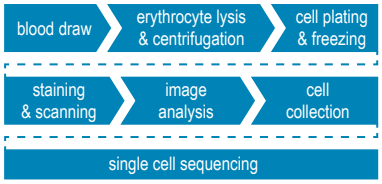
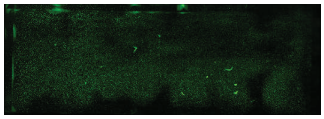
INTRODUCTION AND AIMS: A typical approach to the evaluation of treatment response in cancer patients is based on regular examination with imaging techniques like computer tomography, magnetic resonance and their variations. Some of these methods can bring additional burden to patients, which is why they cannot be repeated too often. Also, their ability to detect micrometastases is limited. Novel methods of fluid biopsy can overcome these limitations as they are based on normal blood draw and subsequent analysis of the sample. The goal of the presented study is to combine detection of circulating tumour cells (CTCs) in peripheral blood of advanced colorectal cancer patients with their genomic profiling. CTC characterization would employ copy number variation analysis combined with targeted sequencing of suspicious loci, both assessed using single cells sequencing in order to monitor treatment efficiency and potential progression of the disease.

METHODS: Consecutive blood draws are taken from the patients who agreed to enter the study according to a specified time schedule. Detection of circulating tumour cells is done by HD-CTC method that is based on processing the whole portion of nuclear blood cells without any preselection typical of most other methods for CTCs analysis. The cells are than plated on microscopy slides and stained by a combination of antibodies to differentiate between white blood cells and cells of epithelial origin. Subsequent analysis of cell phenotype and morphology leads to detection of putative CTCs, which are picked by micromanipulation, lysed and their DNA is isolated and amplified. Single-cell sequencing libraries are prepared using New England Biolabs chemistry for the sequencing on Illumina Miseq instrument.



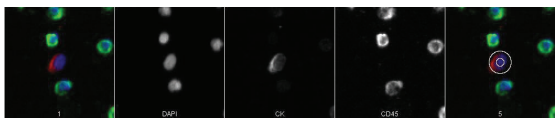


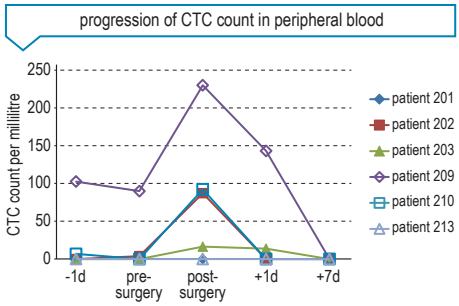
RESULTS: Twenty patients were already enrolled in the study. We have analysed blood for the presence of circulating tumour cells, characterized as CD45 negative, cytokeratin positive cells with the morphology corresponding to the previously described CTCs. Quantification of CTCs in individual time points of sample collection revealed an increase in their number in the first post-surgery sample (1 hour after the tumour resection), indicating an effect of physical manipulation with the tumour mass causing large amount of cells to be released into the bloodstream. Single-cell sequencing is now tested on the cell lines showing known chromosomal aberrations and mutations to validate the approach for the analysis of samples of unknown character. So far, our first results indicate that targeted sequencing panels offered by Roche tend to offer better performance in our experimental setup over those marketed by Illumina.



slide overview

snapshot from cell analysis (multiple markers)





SUMMARY: The fluid biopsy method combining quantification and genomic analysis of individual circulating tumour cells presents a promising and technologically feasible tool complementing present clinical techniques of cancer treatment efficiency assessment. Moreover, the method offers to provide valuable insights into the mechanisms underlying metastasis formation in cancerogenesis.

FUNDING AND SUPPORT: This study was supported by IGA MZ CR 13326 and 14329 and by the National Sustainability Program I (NPU I) Nr. LO1503 provided by the Ministry of Education Youth and Sports of the Czech Republic.