Universitätsklinikum Essen
Anstalt des öffentlichen Rechts

Universitätsklinikum Essen, Hufelandstr. 55, D-45122 Essen
Klinik für Frauenheilkunde und Geburtshilfe

Daniela Vyzralova
Faculty of Medicine,
Charles University,
Department of Research and Science
Husova 3
30100 Pilsen
Czech Republic

Klinik für Frauenheilkunde und Geburtshilfe

Univ.-Prof. Dr. med. Rainer Kimmig
Direktor

Prof. Dr. rer. nat. Sabine Kasimir-Bauer

Telefon: +49-201/723-85292
Telefax: +49-201/723-3938
E-mail: sabine.kasimir-bauer@uk-essen.de

Ihr Zeichen/Tag | Unser Zeichen | Datum 24.07.2018

Dear members of the Faculty of Medicine in Pilsen,

Please find enclosed my review for the submitted PhD thesis of Jana-Aletta Thiele entitled "Morphological and genomic profiling of circulating tumor cells in metastatic colorectal cancer".

This PhD thesis describes the potential use of circulating tumor cells (CTCs) as a biomarker to monitor patients with colorectal cancer (CRC) using the High Definition Single Cell Analysis (HD-SCA) workflow enabling to analyze the entire spectrum of CTCs to categorize them as the regular CTCs (HD-CTC), CTCs with a smaller nuclear area (CTCSmall), CTCs with low expression of epithelial marker cytokeratin (CTC-LowCK), CTCs undergoing apoptosis and therefore, releasing cell free DNA (CTC-cfDNA producing) and CTC clusters (CTCCs). Besides the enumeration of CTCs and morphologic analysis, copy number variation (CNV) profiles on the single-cell level were performed.

The first part of the PhD thesis focuses on the enumeration of all sub-categories of CTCs and CTCCs detected in blood samples of 47 stage IV CRC patients before surgery and in a follow-up draw. The purpose of this analysis was to characterize and define a subset of CTCs associated with metastases or reduced survival. Whereas no association of the regular CTC (HD-CTCs) category with survival could be documented, the CTC-Small category in the follow-up draw was associated with overall survival (OS, p=0.040). Furthermore, the number of CTCCs per ml blood in the pre-resection draw was associated with shorter OS (p=0.021) and patients with CTCCs in the follow-up draw showed a significant shorter OS (p=0.033). In addition, an association of metastatic status at the time of CRC diagnosis (M1vs. M0) with higher average amount of cells per cluster (p=0.035) was found, indicating that larger CTCCs may be drivers for metastases.

When the obtained results were correlated with clinical characteristics, HD-CTC counts per ml blood in the pre-resection draw showed a significant difference in patients depending on the primary tumor locations (p=0.032) with highest amounts of HD-CTCs/ml observed in patients with transverse tumors. A difference was also observed between KRAS wildtype and KRAS mutated primary tumors and HD-CTC/ml in pre-resection (p=0.003).

When changes of HD-CTCs were associated with the resection type, HD-CTC counts dropped significantly after resection of the primary tumor compared to changes after resection of hepatic metastases (p=0.025). No significant drop in cell counts after a type of surgery was detected for the CTC subcategories or CTCCs. No association between cluster positivity in pre-resection draws, or number of cells in clusters with size or location of the primary tumor or size of hepatic metastases was observed. However, a significantly higher average amount of cells within detected clusters in patients with M1 status at time of diagnosis (p=0.035) was found. The amount of clusters/ml blood detected in the pre-resection draw has been significantly associated with the KRAS status of the patient's CRC (p=0.025) with higher numbers of CTCCs/ml in the pre-resection draws of KRAS mutated patients.

The second part of this thesis describes the analysis of single HD-CTCs from the pre-resection draws and single tumor cells from tumor tissue touch preparations using whole genome amplification (WGA) and Next-Generation-Sequencing (NGS) to compare copy number variation (CNV) profiles. The purpose of this analysis was to study clonality within CTCs and distinguish cancer promoting CTCs from less invasive and non-proliferating CTCs as well as the comparison of these profiles with CNV profiles obtained from tissue samples to gain insights into tumor evolution in CRC. 136 single HD-CTCs from 11 CRC patients could be analyzed for this purpose and specific alterations resulted in the detection of 21.3% (29 HD-CTCs) of the cells with partly deleted or amplified Chromosomes with no detection of any clonality (two or more single cells showing similar CNV changes). In contrast, analysis of single cells from CRC touch preparations revealed clonality in 83.3% of tested patients. Comparison between tissue cells from the primary tumor and the metastasis showed similar clonal profiles with minor adaptions in the hepatic metastasis.
In summary, four morphologically different groups of CTCs with high heterogeneity could be identified in stage IV CRC patients and these findings might help to characterize residual disease in CRC more precisely in future projects.

This PHD thesis by Jana-Aletta Thiele comprises 109 pages, structured into Abstract, Introduction, Goals and Hypothesis, Materials and Methods, Results, Discussion, Conclusions, References, Acknowledgements as well as an Appendix with own published data. These publications include one review article, one book chapter and one research article as a first

author as well as one coauthor for a research article. The thesis is well written and the methods used are very innovative and sophisticated. Especially the method used, HD-SCA, is very comprehensive including fluorescence staining, single cell extraction, WGA, CNV, library preparation, NGS as well as the evaluation of the obtained results. The topic of this thesis is highly interesting, the study has been designed correctly and the experimental work has been exposed concisely. Furthermore, these studies will give new insights into the field of CTC single cell analysis which is an upcoming topic.

I met Jana-Aletta Thiele personally when she was working for the company AdnaGen AG, one of our long time collaborators, and later during scientific meetings. She is very experienced in molecular methods and a very enthusiastic scientist. Thus, I fully recommend this very interesting thesis for the defense.

Essen, 24th of July 2018

Prof. Dr. rer.nat. Sabine Kasimir Bauer

Prof. Dr. Sabine Kasimir-Bauer
Laborleiterin
Klinik für Frauenheilkunde und Geburtshilfe
Direktor: Prof. Dr. med. Rainer Kimmig
Universitätsklinikum Essen
Hufelandstraße 55
D-45122 Essen
Tel. (Fax) 0201-723-2443 (3938)