Supervisor's review of the doctoral thesis of Jana-Aletta Thiele

„Morphological and genomic profiling of circulating tumor cells in metastatic colorectal cancer"

Presented thesis is focused on the detection and analysis of circulating tumor cells (CTCs) in advanced colorectal cancer (CRC) patients. The author was using unique method for direct detection of CTCs called High Definition – Single Cell Analysis (HD-SCA). Over 220 pages are divided onto chapters typical for doctoral thesis and are well documenting the work that has been done.

After the well-organized list of abbreviations, the thesis is presenting theory around CRC epidemiology, development, diagnostics, therapy and biomarkers. It is summarizing current knowledge with emphasis on importance of further research in search for novel biomarkers. As one of the possible future markers, Jana-Aletta is discussing CTCs. These cells can serve as fluid surrogates of solid tissues and their analysis may provide crucial information about the tumor. Jana-Aletta is providing information about methods used for their detection and current clinical utility of CTCs.

Experimental part of the thesis is divided onto two major topics – enumeration of CTCs and genome wide copy number variation analysis of individual selected cells. In total, there are four hypotheses and all of them are discussed, accepted or disproved further in the text.

A chapter describing methods used during the project is providing all of the information necessary for understanding individual steps of the HD-SCA workflow. It is preparing a reader for the result chapter that is the longest part of the thesis. The chapter is subdivided onto multiple subchapters based on the goal of the experiment and/or analysis. In enumeration-based analysis of the CTCs, results of the experiments were unexpected, as only weak connections to clinical characteristics were found for the classical HD-CTCs, called here HD-CTCs. Instead of them, other categories like circulating tumor cell clusters or CTCs that are smaller than typical CTCs seem to have connection to clinical parameters of the tumor. Similarly, in the genomic analysis of HD-CTCs, most of them had normal diploid genome and only few of them had observable alterations. The author is explaining reasons of these observations together with other results in the discussion chapter. Very interesting is an option that the detected HD-CTCs might be actually endothelial cells that are released

from the tumor mass. Jana-Aletta is briefly presenting experiments that will address this question in the near future.

All of the most important results of the thesis are summarized in the conclusion chapter, including the future outline. Throughout the thesis, we can find 270 citations with a high percentage of recent articles, showing the importance of CTCs for CRC research in the scientific community. At the end of the work we can find publications supporting the Ph.D. defense – one review article in an excellent journal as a first author, one co-authored article in a journal awaiting impact factor in the next months and a first author chapter in a book listed in Scopus database. In addition, another article that Jana-Aletta wrote as a first author is actually submitted. As Jana's supervisor, I know that an article around thesis' topic is currently prepared and will be submitted before her Ph.D. defense.

Overall, after four years of cooperation with Jana Aletta, I would like to say that she is hardworking, friendly and is a great member of our laboratory. She is always available when needed and ready to help even with the projects outside her major topic. I am grateful for a chance to be her supervisor during the first parts of her scientific carrier and I hope that our collaboration will continue in the near as well as far future.

In conclusion, I would like to recommend Jana-Aletta Thiele to obtain her Ph.D. title after successful defense.

In Pilsen, 10th of July

Mgr. Pavel Pitule, Ph.D.