Reviewer's evaluation of the doctoral Thesis presented by Sona Vodenkova.

The PhD Thesis named "Molecular biomarkers related to DNA damage and repair: their role in carcinogenesis, patients' treatment and monitoring" on its 259 pages contains both relevant publications co-authored by Sona Vodenkova (7 primary papers, one manuscript under the review and 4 review articles) as well as comprehensive and informative overview of Thesis-relevant literature together with to the attached primary papers relevant methods, summary of the results and justified conclusions. In the theoretical introduction Sona Vodenkova presents cancer etiology and overview of cellular, biochemical or molecular biomarkers further divided into 1) Biomarkers of exposure/internal dose, 2) Biomarkers of early biological effect, 3) Biomarkers of susceptibility and 4) Biomarkers of the disease (Cancer biomarkers). In the last part of the literature overview are introduced various causes or modes of the chromosomal DNA damage together with relevant DNA repair pathways and mechanisms.

Sona Vodenkova's Thesis is linked to three main stated aims focused on the genomic analysis (chromosomal aberrations, length of telomeres, etc.), DNA repair capacity and analysis of SNPs in peripheral blood leukocytes from cancer patients and deals with them also in her primary publications. In the Material & methods part is presented concise overview of used methodical approaches and applied statistical analysis. The following Results and Discussion part comments on and discusses attached primary papers and is wrapped up by 322 references to used literature. The next part of the Thesis, page-wise constituting its 2/3, is composed of attached primary papers, a manuscript and the review articles. Moreover, to already 12 papers linked to the Thesis, Sona Vodenkova is co-author of additional 6 publications/manuscripts, which still do have some either topical or methodical overlap with the Thesis, mounting together remarkable 18 publications with cumulative IF over 85 (not counting also noted, published meeting abstracts).

In general, this robust Thesis provides well-written literal introduction on cancer biomarkers linked to DNA damage and its repair mechanisms, overview of publications-related methodics and a guide through 7 published primary papers and one under-review manuscript. Out of the 8 presented papers, Sona Vodenkova is a first author on 3 of them and thus I assume that her contribution towards these 3 papers was a major one (just a note - in such robust Thesis linked to a number of papers would be informative to specify role and contribution of the Thesis defendant). Therefore my questions, comments or inquisitive notes will be reflecting mainly the content and conclusions out of these three reports.

<u>Questions & comments:</u>

1. The authors of e.g. yet unpublished report II claim that chromosomal aberrations (CAs) - and telomeres length-aimed analyses of cultured human blood or better PBLs (at least cultured in report II) could serve as predictive biomarkers for various cancers. However, the tests were run using blood of already diagnosed patients with breast, lung, CRC with lesser than 2-fold increase of CAs mainly in lung and breast cancer patients. So how predictive factor for early diagnosis of tumorigenesis i.e. in yet non-diagnosed individuals this approach might be?
2. As I followed the protocol CAs and TL analyses were run using "cultured blood" i.e. likely PHA-activated T cells. By a chance did not you check if e.g. these CAs are also present in B cells/monocytes? Are these nuclear aberrations in activated T cells result of a tumor environment or do they reflect also other factors (environment, life style, etc.)?
3. Cancer, at least solid cancer is also considered as unhealed wound or persistent inflammation, which likely affects surrounding environment. Thus is it possible that some of chromosomal

aberrations uncovered in activated lymphocytes of cancer patients are affected or caused by this pro-inflammatory environment? Along with this question surfaces another one – are these chromosomal abnormalities also found in normal lymphocytes of patients with hematopoietic malignancies (leukemia, lymphoma)?

4. In the report VIII Sona Vodenkova and co-authors document that at least to the level of BER in CRC patients with more efficient BER in tumor surrounding mucosa show higher 5-year overall survival than those with less effective BER. The experiments relied on comet assay using Ro 19-8022-damaged substrate DNA and formamidopyrimidine DNA glycosylase (FPG)-containing protein extracts from CRC tumors and surrounding tissue obtained from frozen samples. Thus the essential condition for this assay is stability and activity of substrate-cleaving FPG enzyme. Did you analyze its levels in protein samples – e.g. by western blotting and/or its activity by some other mean? Did you have a chance to quantify BER initial activity (FPG-mediated cleavage) also in normal intestinal tissue from healthy individuals and compare it to BER activity in non-malignant tissue from cancer patients?
5. Just a note – in the theoretical introduction is not mentioned p53 as possible cancer biomarker. P53 is mutated in about 50% of human tumors and thus is being considered as a biomarker for cancer susceptibility (Li-Fraumeni syndrome) as well as a diagnostic/prognostic biomarker. Moreover, p53 also participates in various modes of DNA repair and its effect on DNA repair pathways might be one its crucial tumor suppressing functions (see e.g. Nat Med. 2018 Jul;24(7):947-953).

In conclusion Sona Vodenkova's PhD Thesis represents huge though focused piece of work, supported by 8 primary publications (on 3 of them as first author), 4 reviews and six additional still project-related papers. Her 3 main publications deal both with the analysis of chromosomal aberrations in cancer patients and efficacy of BER in colorectal tumors and even these 3 papers would be sufficient for successful defense of this Thesis. Thus I do recommend Sona Vodenkova's Thesis for I believe flawless defense and upon its completion awarding her with a PhD title.

.....

In Vestec, 21.5.2020

RNDr. Ladislav Andera, CSc.