

## Reviewer Evaluation of Master Thesis

Author Name: Eliška Šimičková

Thesis Title: 3 D culture of human cell lines for disease modeling and drug discovery

Reviewer Name: Ermin Schadich

Criteria n.	Evaluation Criteria	Points (0-5)
1	Completeness and timeliness of the literature search	3.5
2	Quality of the introductory part (number of used original articles, the suitability of literature selection)	3.5
3	Fulfilment of thesis goals	3.0
4	Logics of literature search or experimental work approaches	2.5
5	Completeness of methods and protocols description	2.5
6	Level of results presentation (suitable choice of graphs and tables etc.)	2.5
7	Adequate interpretation of obtained results and their discussion	3.0
8	Conciseness of thesis summary in English language	3.0
9	Graphical editing of text and images	3.0
10	Language and stylistic level, respect of valid nomenclature	3.5
11	Correctness and completeness of figure and table legends (intelligibility without regard to the main text, correct explanation of used symbols and units)	3.0
12	Correct usage of citation references (presence of non-cited data, compliance with a unified citation style, utilization of official journal names abbreviations)	3.5
<b>Total points</b>		<b>36.5</b>

max  
60

### Specific comments and questions to the thesis (please use additional sheets if necessary):

Please, for comments and question refer to additional sheets.

### Errors required to be corrected:

Please, for errors required to be corrected refer to additional sheets.

### Conclusion: I recommend / do not recommend this thesis to defence.

In Olomouc on:

Signature

Thesis evaluation according to total points:

- A- 56-60
- B- 51-55
- C- 46-50
- D- 41-45
- E- 36 -40
- F- 35 and less

### **Specific comments and questions to the thesis**

The presented thesis of Eliška Šimičková represents solid body of novel work. The studies presented add to the growing body of literature describing the development of 3D spheroid cultures for modelling tumor microenvironment. The study aims were established and methodology used to address them was appropriate. The data were obtained, and they indicate the novel findings that could be applicable for further modelling of human tumors. There two major findings of this thesis, one is that the tumor microenvironment can be assessed *in vitro* by using the 3 D spheroid cultures and the other is that lymphocytes can be conditioned by interleukin 2 (IL-2) and drug diethyl thiocarbonate (CeUT) to modify the cancer cell growth in 3 D spheroid cultures. In addition, in separate study, it was also found that among three tested drugs tested for anticancer activity, irinotecan, 5-floururacuil and cisplatin, two of them, irinotecan and 5-floururacuil have the synergistic effects with cisplatin against cancer cells in the 3 D spheroid cultures. However, there are the issues associated thesis. The main issues are related to the flaws in the results section. While the data were obtained during these studies their analyses were not complete. Because of these issues, the thesis has some parts that needs be corrected.

#### **There are the questions for candidate to be answered:**

1. Why did you chose CeUT and interleukin-2 in treatments of lymphocytes?
2. What is the IC<sub>50</sub> of CeUT against lymphocytes?
3. Coud the use of the counterstain calcein-AM improve the assessment of percentage of dead cell in propidium iodide stainings?
4. Accoding to your data, the KRAS protein could be implicated in resistance of the HCT-116 cells to the effects of lymphocytes treated by CeUT and (Figures 7. 10 and 19). Which pathway will be implicated in this resistance?
5. According you your data, the p53 could be implicated in this resistance to in resistance of the HCT-116 cells to the effects of lymphocytes treated by CeUT and (Figures 7. 10 and 19). Which pathway will be implicated in this resistance?
6. According you your data, the p53 could be implicated in this resistance to in resistance of the HCT-116 cells to the effects of lymphocytes treated by CeUT and (Figures 7. 10 and 19). Which pathway will be implicated in this resistance?
7. According to your data, irinotecan and 5-floururacuil have the synergistic effects with cisplatin in the 3 D spheroid cultures. In this part there are two questions. What are targets of these two drugs? What might be the possible mechanisam associated with synergy among these drugs in their anticancer activity against the HCT-116 cells.

#### **Errors required to be corrected:**

1. In all subsections of the results sections the presented data were not analysed by statistical tests. This would be easily corrected if the students provide the outputs from statistical test amend the entire section according to them.
2. Also one subsection on statistical analyses in the material and methods section is needed.
3. In the subsection of the Results section where the propidium iodide staining method was used visualise the damaged zones of spheroids after treatments, the images of

spheroids do not correspond to the presented corresponding graphs. It is necessary to state in the figure legends that the images were taken before removal of lymphocytes.

4. It is also important to adjust the parts of the discussion and conclusion section according to the changes in the results section.
5. In the abstract, it is important also to state that the spheroids from colorectal carcinoma cells were obtained and exposed to lymphocytes, CuET treated lymphocytes and IL-2 treated lymphocytes after sentences on aims of study. Also, it is required that spheroids for synergy study were also made and treated by aforementioned the combination of drugs.
6. In the subsection 3.7 Fluorescence microscopy, it is necessary to provide the information on wavelength used in the excitation filter.
7. In the subsection 3.12, it is necessary to state that data on expression of p21 and acetyl- $\alpha$ -Tubulin in the obtained samples were normalized by the values of their protein amounts.
8. It is necessary to provide the subsection on statistical analyses used in the methods section.
9. In the section Aims before Introduction section, there is Grammar issues with the usage of verbs, use the past tense “was” instead of “is” and “will be”.