

**Ph.D. COURSE:** “The biological aspects of controlling animal growth and health maintenance.”

## **DISSERTATION REVIEW REPORT**

**Thesis Title:** “Characterization of canine mammary cancer stem-like cells and their interaction with tumor associated macrophages.”

**Ph.D. Candidate:** Agata Marta Rybicka

**Supervisor:** Prof. Magdalena Król

### **GENERAL EVALUATION**

- *Originality, relevance to the field and possible applications:*

*The manuscript is fully completed with original work that represent an interesting advancement in the field of cancer microenvironment. The idea beyond experimental work is simple: evaluation of miRNAs profile of subpopulations of stem-like cells from canine mammary tumors in order to single out possible specific contributions to macrophages chemoattraction. The work carried out in the thesis represents a solid demonstration of CCL2 (monocyte chemoattractant protein 1) overexpression in aggressive tumor cell lines as a result of miRNAs deregulation and lay the basis for a deeper understanding of epigenetic regulation in cancer development. The study may open novel therapeutic windows that include possible interventions on upregulated miRNAs in cancer stem-like cells.*

- *Technical quality, consistency of theory and experimental work, completeness:*

*The experimental design is convincing and adequately carried out by using FACS analysis with appropriate antibodies for cell stemness assessment, microarray based detection of miRNAs and RT-PCR for microarray data validation. Much work has also been dedicated to stem-like cells isolation and co-culture with macrophagic cells. Bioinformatic search has been performed in collaboration with experts of the field. The author has been thus faced to diverse methodologies, and, according to the statements by co-authors she took a leading part in the project development.*

- *Outputs (publications, patent applications..):*

*Three publications on international peer reviewed journals (all of them first author) is a good package for a PhD.*

- *Writing quality and clarity:*

*Throughout the whole manuscript writing is clear and excellent, measurements are described in depth, introduction and discussion are consequent and essential.*

## Specific comments

*The intercellular crosstalk between cancer stem-like cells and cells of the monocytic lineage is a key topic for the understanding of cancer development and the results shown can be considered as a pioneering step forward to the comprehension of the molecular processes at the basis of such complex interactions. The experimental work lays its ground on previous observations related to miRNAs deregulation in canine mammary tumor cell lines, as correctly referenced in the introduction section (ref. 31-35). The novelty of the present investigation resides in the focus given to cancer stem-like cells, hypothesized to be the initial contributors to macrophages recruiting effect. Contemporary presence of SCA-1, EpCAM and CD44 have been considered as the hallmark of stemness, although there are contrasting evidences in the literature on the expression of Sca-1 as an “early” or “late” pre-differentiation marker. The results obtained confirm the hypothesis as demonstrated by the observed correlation between upregulated miRNAs and overexpression of 18 messengers related to tumor linked genes. Overexpression of CCL2 mRNA appears to be particularly significant and statistically validated. Effective traduction of the CCL2 mRNA has not been assessed, although there are no evidences that translation process is further regulated at the ribosome compex and the assumption that all mRNA is entirely processed may hold. The further evidence of CCL2 receptor downregulation in the cancer cell lines offer an unexpected interesting finding that may account for selective recruitment of macrophages expressing CCR2.*

## Concluding remarks

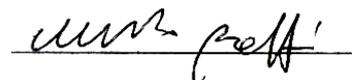
*The present thesis highlight very interesting results obtained in the framework of a consistent experimental work and well designed objectives. The reported publications indicate that the candidate has fulfilled his aims and current results pave the way to far reaching research objectives for the next future. The present thesis fulfill all criteria for a PhD Dissertation.*

*Date\_February, 06 2017*

Name of the referee

ALBERTO BOFFI

(signature)

A handwritten signature in black ink, appearing to read 'Alberto Boffi', is written over a horizontal line.