Professor Qingbo Xu, MD, PhD
BHF John Parker Chair of Cardiovascular Sciences
Cardiovascular Division
King's College London
125 Coldharbour Lane
London SE5 9NU
Tel 0207 848 5322
Fax 0207 848 5296
Email: qingbo.xu@kcl.ac.uk

KING'S College LONDON
University of London

EVALUATION

of doctoral thesis of Mr Krzysztof Szade, MSc, the PhD student in the Department of Medical Biotechnology at Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University

Title of the dissertation:
"Protection of hematopoietic stem cells from premature aging – the role of heme oxygenase-1"

I have read the thesis submitted by Mr Krzysztof Szade, and am pleased to provide the comments about the topic, impact and scientific contribution in general. The PhD candidate has involved investigating that whether blood cells in the postnatal organism can be derived from non-hematopoietic Lin-Sca-1+CD45- fraction or only from the defined hematopoietic stem cells (HSC) and elucidate whether heme oxygenase-1 (HO-1) protects the stem cells from premature aging and exhaustion of hematopoietic regenerative potential. The candidate has applied a number of complementary methods in the study. The number of methodologies used is impressive and he and his supervisor are to be commended on this. Through examining the HSCs using a variety of techniques, he did not confirm the hematopoietic potential of Lin-Sca-1+CD45- fraction from adult murine bone marrow, which was called Very Small Embryonic-Like cells

(VSEL). Using appropriate markers he has demonstrated protective role of HO-1 in aging of HSCs, which is consistent with subsequent usage in HO-1 knockout mice. He proposed several potential factors that may be involved in HO-1-depedent protection of LT-HSC from premature aging. Aside from his main project, the candidate has been involved in additional work and publications on that collaborative work are included in the thesis. Overall, he already has a number of publications in good journals from his time in Krakow.

The topic. The bone marrow is the major reservoir of adult stem cells, fulfilling the constant need of new cells in the body. Mariusz Ratajczak group reported the presence of VSELs from the bone marrow which is pluripotent. Despite several papers published in different journals, the precise existence and contribution of VSELs remained a controversy. The present thesis selected the topic of HSCs in bone marrow to clarify if VSELs exist, which is a cutting edge project. In addition, the topic was extended to the role of HO-1 in ageing HSCs that is related to understanding the molecular mechanisms of HSC ageing.

The Introduction. The review of the literature in the Introductory chapter is appropriate and comprehensive. Two potential impacts on the Introduction in its current form are (i) the findings and criteria of VSELs and (ii) the role of HO-1 in stem cell renewal, proliferation and function. It also covered the historic issues on HSCs and some controversial issues. Although the candidate provided a thorough citation, there are some sentences/paragraph lacking appropriate references. This may be because the information is familiar to workers in the field, but even so inclusion of appropriate review/text book references may be desirable as good practice.

The Methods. The Methods are reasonably well described. There are several novel methods used in the present study. Most of them were described in detail. Some techniques, such as single cell gene sequencing are crucial for the studying the objectives in the project, which were well performed by the

candidate. There is a minor issue with the Methods in that the mouse bone marrow transplantation was carried out in other laboratory, which was lacking detailed description about the success criteria for the mouse irradiated. The candidate stated that mice were irradiated in University Children's Hospital of Cracow with help of Dr Jacek Kijowski. All recipient mice were myeloablated by whole body irradiation with 137Cs g source at 110 cGy/min. Mice received total dose of 900 cGy divided in two parts given within 4 hours. It would be better to provide the criteria on the success of bone marrow transplantation, although there is no influence on the understanding the methods by the readers.

The Results/Findings. The Results are concisely presented. Results presentation is clear. There are two main new findings in the thesis. The first one involves the presence/absence of VSELs. Since it is very important issue for the field working in regenerative medicine, the current finding is crucial for clarifying the controversial issue. The author demonstrated that Lin-Sca-1+CD45-FSClow population is heterogenous, enriched in early apoptotic cells, which do not express pluripotent marker Oct-4A. In the study, it was found that Lin-Sca-1+CD45-FSClow did not showed hematopoietic potential in the single cell colony formation assay or after co-culture with OP9 cells, which could be potentially contaminated with the nuclei expelled from the erythroblasts. Thus, this is a landmark confirmation on the absence/presence of VSELs.

The second new finding is to answer the question whether HO-1 influences HSCs during ageing in vitro and in vivo, which is essential for understanding the mechanisms on how HO-1 exerts its role in this process. The candidate demonstrated that HO-1-/- mice possess LT-HSC that show signs of premature aging, resulting in gene expression profile connected with increased oxidative metabolism, in which LT-HSC from HO-1-/- mice lost quiescence and intensively proliferate. The candidate proposed several potential factors that may be involved in HO-1-depedent protection of LT-HSC from premature aging. This finding provided the basic information on the role of HO-1 in HSC functions

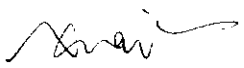
during ageing.

The Discussion. The Discussion and Concluding remarks are reasonable. There are two parts in the Discussion section. The first one is focussing VSELs, which were supported by two publications, i.e. Szade et al. Murine Bone Marrow Lin−Sca-1+CD45− Very Small Embryonic-Like (VSEL) Cells Are Heterogeneous Population Lacking Oct-4A Expression. (2013) **PLoS ONE** 8(5): e63329. Szade et al. Comment on: The proper criteria for identification and sorting of very small embryonic-like stem cells, and some nomenclature issues. (2014) **Stem Cells Dev**. Apr 1;23(7):714-6. It is convincing, and well written. The second part of the Discussion emphasized the Verification if HO-1 protects HSC from premature aging. It is known that studies confirmed the beneficial role of HO-1 in different stress conditions in variety of tissues. Most of these studies concern the HO-1 function in cardiovascular bed, while the action of HO-1 in hematopoietic system was less intensively studied. The candidate pointed out the impact of their findings comparing the presence of evidence in HO-1 research field. It is logical.

In summary, the Introduction section of the thesis provided information covering all aspects of the studied subjects, from hematopoietic stem cells involved, VSELs, cytokines, cell lineage differentiation, to HO-1 molecules. It was very well organised and easy to read. I have cross-checked some of the references, and no errors were found, i.e. the literature was accurately cited. The Materials and Methods sections listed all the reagents and techniques used in the study and this detailed protocol should allow others to reproduce the experiments. This could be a good reference for new students who use the techniques described in the thesis. The results section showed clearly the experimental data and illustrated the important findings. Descriptions of the data are clear and the figures are clearly labelled, although some of the figures are published in the papers. The Discussion section focused on the interpretation and implication of the findings. Thus, the thesis is well organised. The techniques are described in detail. The data are clearly presented and appropriately illustrated. In my opinion,

the Candidate, Mr Krzysztof Szade, MSc, has achieved all aims of the study and his dissertation meets all criteria of doctoral thesis. Therefore I recommend the thesis to the Council of Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University. Owing to the high quality of research, as documented by publication of the results in the very good journal, and due to the comprehensive and sound approach to the topic I propose consideration of the thesis for the prize.

Sincerely yours,



Qingbo Xu, MD, PhD
Professor and BHF John Parker Chair of Cardiovascular Sciences