**REPORT DUE DATE: October 13, 2014**

McGILL UNIVERSITY
Graduate and Postdoctoral Studies

**MASTER'S EXTERNAL REPORT**

NAME OF STUDENT: Samuel Meyer OHAYON
DEGREE / UNIT: Master of Science / Department of Pathology
THESIS TITLE: BORIS/CTCFL is an epigenetic modifier of melanoma tumorigenicity

Use the following scale:

**EXCELLENT, VERY GOOD, GOOD, SATISFACTORY, or UNSATISFACTORY**
**(Choose one grade for each category)**

| Criteria | Excellent Top 10% | Very Good | Good | Satisfactory | Unsatisfactory |
|---|---|---|---|---|---|
| 1. Evidence of originality and creativity | | ✓ | | | |
| 2. Resourcefulness, alertness to significance of findings | | ✓ | | | |
| 3. Diligence, care, technical skill in the research | | ✓ | | | |
| 4. Usefulness of the results to other workers in the field; value as a contribution to knowledge | | ✓ | | | |
| 5. Grasp of subject, powers of criticism and general adequacy in review of previous work | ✓ | | | | |
| 6. Quality of presentation (coherence, lucidity, grammar, style, freedom from typographical errors) | | ✓ | | | |

7. **OVERALL JUDGEMENT (circle one)** **PASSED or NOT PASSED**

8. If the overall judgement is '**PASSED**' please provide:
Comments explaining your evaluation of the thesis including recommendations for minor revisions to be included in the final thesis.

9. If the overall judgement is '**NOT PASSED**', at least one of the criteria above must be graded unsatisfactory.

Please provide:
   a. Comments explaining your evaluation of the thesis, including a detailed description of the shortcomings that have informed your decision that it does not meet the requirements of a passing thesis.
   b. An itemized list of the substantive issues that you expect the student to address for the written thesis to receive a passing grade upon re-examination. Please refer to appended instructions for examiners.

In order not to jeopardize the student's anticipated graduation timeline (and potential associated costs), we must receive your report no later than **October 13, 2014**. Please return your report to: Thesis Office by fax 514-398-6283, email sandra.gibson@mcgill.ca or mail, Thesis Office, McGill University, James Administration Bldg, Room 400, 845 Sherbrooke St. W. Montreal, QC Canada H3A 2T5 Tel No. 514-398-3783 Ext. 00711

DATE: Oct 7, 2014 SIGNED: _____
(Dr S. Richard)

**Samuel Ohayon, Master of Science / Department of Pathology**
**BORIS/CTCFL is an epigenetic modifier of melanoma tumorigenicity**
**Reviewer: Dr. Stéphane Richard, External**

The thesis is well presented with an introduction, results, discussion and a bibliography. Samuel Ohayon's work is not published, however, the work within the thesis is a testimony of the excellent experience acquired during his M.Sc. degree. Overall the work is of high quality. My suggestions below are to enhance the presentation of the thesis.

**Synopsis of thesis:**
The thesis examines the role of Boris, a CTCF homolog, in melanoma cell lines. The expression of Boris is mainly restricted to testis in adults, but its expression is 're-activated' in certain cancer types such as melanoma. The approach that Mr. Ohayon chose to study the role of Boris in melanoma is by the loss-of-function in melanoma cell lines. He generated 1 stable cell line for three separate melanoma cell lines with reduced Boris mRNA (and presumably protein) by using an shRNA approach. The inability to obtain several clones is likely due to the fact that reduced Boris levels inhibits cell proliferation. One clone mm111shB3 reverted after 3 weeks likely due to methylation of the promoter driving the Boris shRNA, a well-known phenomenon. Phenotypic expression was performed on the remaining two cell lines (mm102shC5, mm117shB6) where reduced cell proliferation was observed by the MTT assay and cell morphology was assessed by phase contrast microscopy. Mr. Ohayon then performed colony formation assays and cells were also injected *in vivo* into nude mice. Reduced (60%) tumor formation was observed with mm102shC5, while less convincing evidence was obtained with mm117shB6. To identify the genes regulated by Boris, a candidate approach (X chromosome-codeset of 702 genes) was used using Nanostring and the data were normalized to MAGEA11, a gene whose expression did not change between cell lines. SLC10A3 was identified as the gene most highly expressed which is located on the X-chromosome subtelomeric region Xq28. Modified CTCF ChIP assays were performed to examine CTCF looping near the *SLC10A3* and this was lost in the clone mm102shC5. It is suggested that the observed gene changes appeared in clusters and are likely correlated with CTCF-mediated chromatin loops. The loops would lead to gene activation in the case of *SLC10A3*.

**Comments:**

1. Some figures are of low resolution and appear fuzzy and are not legible in some cases. The text should be replaced to improve the quality. In some case higher resolutions of images should be shown or the size of the image should be increased.

This applies for Fig. 9, Fig. 16, Fig.19B, Fig. 19C, Fig. 20, Fig. 21, text of Fig. 22, text of Fig. 26, (e.g. Fig. 29 and Fig. 30 the text is excellent – all figures should be like this), Fig. 31 the whole Figure is fuzzy, Figure 32 should be rotated or reduced to be portrait-style, Fig. 33 - font is too small. Fig. 34 text is fuzzy and the circled part of the figure is not legible and the legend should be changed to left justified. Fig. 40 and table 2 should be deleted, as they add no value to the thesis.

2. Fig. 17, 18: Why not express Boris, CTCF and MAGEA2 levels as in Fig. 14 i.e. mRNA copy no/mg total RNA.

3. Fig. 18. Clarify why are 3 different points for mm7? And two points for mm5?

4. Fig. 24 legend 'reestablishes' should read re-establishes

5. p31 its m117 or mm117?

6. p46 technic, technique

7. Table 1. Boris knockdown is expressed as % so 85% means there remains 15 % Boris mRNA levels. Why do MAGEA2 and VEGFA have negative %?

8. p56 a period is needed after '…on the FN1 gene.'

9. Fix 'Whether FN1 level(s) were...'

**Overall assessment:**

1. The other targets obtained by Nanostring should be described in some detail and their correlation with cancer and/or melanoma. Can any information be obtained with Boris expression and the 702 genes?
2. What is the role of the solute carrier *SLC10A3* or its absence in cancer? What do you think it is transporting?
3. Which genes are responsible for the reduced growth of Boris-deficient cells? Are there candidate genes within the 702 list?
4. Is there a migration, attachment defect of the shBoris cells that contribute to their slow growth?
5. What is their cell cycle profile?
6. Mr. Ohayon should describe approaches were Boris can be studied. For example, epitope-tagged Boris could be stably integrated into melanoma cell lines using a retroviral approach and puromycin selection to obtain bulk populations. Then the chromatin interactions and CTCF interactions of Boris can then be studied, until an antibody becomes available.
7. The thesis mentions dox-inducible knockdown approach with is currently being used. This still does not solve the fact that depletion of Boris will arrest the growth the cells. Knockdown/rescue with mutants of Boris may be required and should be discussed or proposed.
8. The xenograft experiments are difficult to interpret because of the proliferation defects of the shRNA Boris cells. This should be stated.