Boston University School of Medicine
Division of Graduate Medical Sciences

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ANNOUNCEMENT OF FINAL ORAL EXAMINATION
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

CANDIDATE:	**Dolly Thomas**
DEPARTMENT OR PROGRAM:	**Molecular Medicine, Program in Cell and Molecular Biology**
TITLE OF DISSERTATION:	**"The Role of IGF2 in the Regulation of Hematopoietic Stem Cell Function"**
DATE, TIME, AND PLACE:	**Tuesday, April 1, 2014 at 10:00a.m.** **Boston University School of Medicine** **72 E. Concord Street Room –R 103** **Boston, MA 02118**

EXAMINING COMMITTEE

FIRST READER:	**Dr. Gustavo Mostoslavsky**
SECOND READER:	**Dr. David Sherr**
THIRD READER:	
CHAIRMAN OF THE EXAMINING COMMITTEE:	**Dr. Barbara Nikolajczyk** **Phone: (617) 638-7019** **Email: barb.nikolajczyk@gmail.com**
ADDITIONAL COMMITTEE MEMBERS:	**Dr. Katya Ravid**
	Dr. Kenneth Walsh

Members of the committee are asked to confirm attendance by replying directly to the Chairman of the Examining Committee.

ALL MEMBERS OF THE SCHOOL OF MEDICINE FACULTY ARE INVITED TO ATTEND.

THE ROLE OF IGF2 IN THE REGULATION OF HEMATOPOIETIC STEM CELL FUNCTION

DOLLY THOMAS

Boston University School of Medicine, 2014

Major Professor: Gustavo Mostoslavsky M.D., Ph.D, Assistant Professor, Department of Medicine

ABSTRACT

Maintenance of the hematopoietic system is solely dependent upon the proper regulation and orchestrated functions of the HSC (hematopoietic stem cell) pool. A number of extrinsic signaling pathways and intrinsic regulators have been found to regulate HSC processes. However a full understanding of the ability of HSC to balance the processes of self-renewal, quiescence, and lineage specification is not yet clear. We therefore set out to identify novel HSC regulators, by comparative gene expression analysis of whole genome transcriptomes generated for long-term (LT)-HSC (Hoechst $^{low/-}$ Lin^- $Sca1^+$ $cKit^+$ $CD34^-$), short-term (ST)-HSC (Hoechst $^{low/-}$ Lin^- $Sca1^+$ $cKit^+$ $CD34^+$), and non-HSC (Hoechst$^+$) of the bone marrow. These studies identified IGF2 as one of the most differentially expressed genes within LT-HSC, suggesting a potential role for IGF2 in the regulation of HSC. Using a combination of lentiviral-mediated overexpression and knockdown experiments, we found IGF2 to confer enhanced self-renewal *in vitro* and *in vivo*. Overexpression of IGF2 resulted in a greater percentage of

multi-lineage colonies within colony-forming unit (CFU) assays, without affecting lineage specification. In vivo, serial bone marrow transplantation revealed that IGF2 within HSC enhances short-term and long-term donor contribution. Analysis of the expression of G0/G1/S cell cycle regulators revealed that IGF2 induces upregulation of p57 expression specifically within HSC, potentially due to differences in the methylation status of the p57 promoter in HSC compared to other progenitor populations. p57, a member of the Cip/Kip family of cyclin dependent kinase inhibitors, has recently been shown to be required for regulation of HSC quiescence and long-term self-renewal. Analysis of bone marrow obtained from primary and secondary transplant recipients showed that overexpression of IGF2 resulted in an increased percentage of HSC in a quiescent state. Treatment of IGF2 expressing HSC with LY294002, a PI3K-Akt inhibitor prevented IGF2 mediated induction of p57 expression. These findings demonstrate that within HSC IGF2 induces p57 expression through activation of the PI3K-Akt pathway to regulate HSC quiescence. We have identified a novel role for IGF2 in HSC function, providing new insights into the biology of HSC and opening potential platforms for the development of better therapies involving HSC-mediated hematopoietic reconstitution.