Grant Title: Enhancing Bone Marrow Regeneration

Grant Number: MRM 2015 3586

Principal Investigator: Troy C. Lund, MD, PhD

Project Timeline: April 1, 2015 - August 30, 2017

MRM Progress Report

Progress to Date:

Aim 1A. Test chemicals from a naturally occurring compound library for enhancers of engraftment.

We completed pilot studies using luciferase expressing donor marrow cells into pigmentless irradiated recipient zebrafish (so-called *casper*) and showed our system is robust in terms of measuring effects on engraftment within 48 hours of BMT. We have successfully testing over 750 compounds to date.

We have discovered 35 compounds with robust effects on hematopoietic cell engraftment that are now undergoing secondary experiments to verify their activity.

Overall, this aim is 100% complete.

Aim 1B. Verify chemical "hits" in a murine model of HSC homing and transplant.

In this aim, we tested 1-2 compounds (thus far) in murine mouse models of hematopoietic cell transplant. We have performed hematopoietic stem cell expansion assays and colony forming unit assays on our candidate compounds. We have been able to demonstrate that a new compound, ergosterol, promoted the homing of murine hematopoietic cells after BMT and promotes stable and long term engraftment. Furthermore, we discovered that ergosterol has pleiotropic effects where upon it increases cell mobility, expression, cell viability and reduces oxidative stress. The increase in cell mobility seems to be related to CXCR4 expression. CXCR4 is a key cell surface receptor expressed on hematopoietic stem cells and engages the chemokine SDF1 to facilitation the migration of cells to the bone marrow compartment. Ergosterol-mediated upregulation of CXCR4 may, in part, explain why we see accelerated engraftment of cells after ergosterol exposure. Ergosterol is the inactivated form of ergocalciferol, or vitamin D2. This led us to further experiments to test Vitamin D2's effects on hematopoietic stem cells whereupon we also discovered positive effects on cell homing, viability, and cell growth. We are now currently investigating the specific mechanisms by which the Vitamin D class of compounds is able to perform these functions and whether or not they are acting through the Vitamin D receptor (VDR). This will undoubtedly move us into a new and exciting field of research in which novel vitamins have unexplored effects of hematopoietic cell behavior. We even foresee a time where marrow cells from bone marrow donors could be treated with Vitamin D prior to bone marrow transplant in a novel clinical trial to determine if engraftment is improved.

We have verified that a second compound from our screen can modulate hematopoietic cell homing. This is a novel sugar known as Melizitose (504.44 g/mol, $C_{18}H_{32}O_{16}$, PubChem ID 9281). Our pilot data suggest it also regulates CXCR4 expression and assists in the prevention of apoptosis in cultured hematopoietic stem cells of both human and mouse origin.

Overall, this aim is 100% complete.

Publications:

The following two manuscripts were published: Astuti et al. A Functional Bioluminescent Zebrafish Screen for Enhancing Hematopoietic Cell Homing. *Stem Cell Reports*. 2017;8(1):177-90.

Kramer et al. Dermatopontin in Bone Marrow Extracellular Matrix Regulates Adherence but Is Dispensable for Murine Hematopoietic Cell Maintenance. *Stem Cell Reports*. 2017;9(3):770-8.

Grant applications and/or awards:

Data derived from this MRM-funded project has been submitted as an R21 to the National Institute of Allergy and Infectious Diseases (NIAID) as an R21.

Patents

I have meet with the University Office of Technology Commercialization to discuss possible patent applications for ergosterol (the focus of Aim 1B). I am also meeting with the Medicinal Chemistry Group to discuss modifications to ergosterol to improve solubility and function.

Budget Update:

Please report the initial year's funding vs. spending and comment on any variance of >20% of estimated budget. We asked for and received a 6-month no-cost extension to finish generating pilot data on several novel compounds that improve stem cell regeneration.

Overview:

Despite its growing use, there is significant morbidity and mortality associated with bone marrow transplant (BMT) due to infection during the neutropenic state just after transplant, prior to engraftment (which can take up 32 days). One way of ameliorating this morbidity due to neutropenia is to make the transplanted hematopoietic cells engraft in the recipient at a faster rate. Currently, cells migrate on their own and engraft without any pharmacologic modulation. The funded MRM project utilizes the *Danio rerio* model of hematopoiesis in which we have developed the fist functional screen to evaluate small molecule libraries for new and novel enhancers of engraftment using 2 specific aims:

We have **scientifically advanced** the field of hematopoietic cell regeneration by discovering new compounds that accelerate stem cell migration to the marrow. These compounds are both effect and non-toxic providing an excellent basis on which to build entry in to **translational** experiments to reach **clinical** trials. This project was executed in a timely and efficient manner that resulted in the **publication** of two related **manuscripts** in the field of hematopoietic cell homing, both in the respected journal *Stem Cell Reports.* Through this advancement we will be **improving the health** of Minnesotans in the very near future. Not only have we advanced the field and provided **employment** to Minnesotans, but our laboratory efforts have been fertile ground for **training** 2 Master's students and **educating** 3 Undergraduate students during this grant period.