Grant Title: Enhancing Bone Marrow Regeneration

Grant Number: MRM 2015 3586

Principal Investigator: Troy C. Lund, MD, PhD

Project Timeline: April 1, 2015 - March 31, 2017

MRM Progress Report

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 first functional screen to evaluate small molecule libraries for new and novel enhancers of engraftment using 2 specific aims:

Progress:

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 10 - 20 compounds with robust effects on hematopoietic cell engraftment that are now undergoing secondary experiments to verify their activity. Overall, this aim is 75% 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 (preliminary). 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.

Publications

A manuscript entitled: “A functional bioluminescent zebrafish screen for enhancing hematopoietic cell engraftment” has been submitted to *Stem Cell Reports*

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

Academic Health Center Grant Financial Report

Period covered:	4/1/15 - 3/31/16
Type of Grant:	Minnesota Regenerative Medicine
Title of Grant:	Enhancing Bone Marrow Regeneration (Award # MRM 2015 3586) CON000000052863, Project 00050471
Principal Investigator(s):	Troy Lund, MD, PhD
Grant Award Amount:	250,000

University of Minnesota

	Proposed Grant Budget		Actual Expenses
Personnel Salary and Fringe	89,737		42,075
Consultants, Honoraria			
Operating Supplies and Services	79,105		15,413
Travel	1,500		0
Repairs and Maintenance			
Rents and Leases			
Equipment/ Computer Equipment			
F&A (indirects)	79,658		29,894
Total Expenses (direct + indirect)	250,000		87,382

Prepared by:	Troy Lund, MD, PhD
Date:	3/23/16

We had a variance of > 20 % the first year due to personnel issues that included replacing some personnel, hiring new people in lab and on-boarding them.
Such issues have been resolved, and we anticipate to back on budget Summer 2016.

Overview

Minnesota has been one of premiere institutions in the study of blood forming cells, treatment of leukemia, and bone marrow transplant (BMT) for decades. We have a history of pushing the envelope with examples such as: early descriptions of platelets, neutrophils, and the functions of B- and T-cells; development of the first cytogenetic techniques; developing therapies for the treatment of pediatric leukemia; and performing the world's first successful bone marrow transplant, to name a few. The focus of this work was on the BMT process and how blood forming cells find their way back to the bone marrow immediately after the transplant is performed – a process still not understood. This is important, because despite our advances in blood research, many of our patients fail to have a successful BMT. This may be, in part, due to the slow speed at which blood forming cells reach the marrow cavity. The goal of this research was to find novel compounds that speed up this process using a new drug discovery platform developed in my lab which is built upon tracking transplanted zebrafish cells as they travel to the marrow in real time. In the first year, we have been fortunate enough to have already discovered several new compounds that positively affect this process and improve marrow regeneration. Some of these compounds are very novel and are patentable as new drugs. After testing these compounds, we anticipate some will be developed into clinical trials here in Minnesota utilizing the vast experience and resources we have to develop novel therapies thereby completing our goal of translating the findings of this study into the clinic and advancing regenerative medicine.