

## Cystinosis Research Foundation Progress Report

**Title:** *Elucidating the role of aberrant macrophage activation in nephropathic cystinosis*

**Grant number:** 414010090101

**Investigators:** Daryl Okamura M.D.

**Funding Period:** September 1, 2013 to August 31, 2015

**Progress Report:** September 1, 2014 to February 28, 2015

### OVERVIEW

The project is progressing and we have started to characterize the *Ctns*<sup>-/-</sup> macrophage (MΦ) phenotype in response to kidney injury. However, we have had a major setback in that my primary technician has moved on to another position in the beginning of February and we are still in the process of hiring another technician. We are still characterizing our in vivo studies (Aim#2) to help focus our in vitro investigations (Aim#1). Manuscript is currently in preparation.

Aim #1: To determine the *Ctns*<sup>-/-</sup> macrophage phenotype in response to cytokine activation and the mechanisms that lead to its altered behavior

Our initial hypothesis was that aberrant response to cytokine activation in *Ctns*<sup>-/-</sup> MΦ resulted in more severe injury, however, our preliminary data below suggests that they attenuate injury. It is not clear if this is due to other factors such as proliferation/apoptosis or chemotaxis. Therefore, we will further characterize the in vivo data to help focus our in vitro investigations on mechanism.

AIM #2. To investigate the functional impact of the *Ctns*<sup>-/-</sup> macrophage phenotype on regeneration and fibrosis after renal injury.

We generated *Ctns* ko/wt chimeric mice through bone marrow transplantation. After 8-10 weeks to allow complete engraftment of the monocyte/macrophage population, *Ctns* ko/wt mice and *Ctns* wt/wt chimeric controls underwent unilateral ischemia reperfusion (IR) injury. After 14 days, both groups underwent contralateral nephrectomy to investigate kidney function of the IR kidney. Each mouse underwent blood draws at days 17, 21, and 28 after IR injury. Mice were sacrificed at day 28 and kidneys analyzed for fibrosis severity (n=5-8/group). As mentioned in our previous report, there was actually a preservation of kidney function in the *Ctns* ko/wt mice. We are currently analyzing macrophage infiltration, myofibroblast accumulation and ECM expression, tubular injury, as well as proinflammatory and profibrotic gene expression.

In order to track the *Ctns* ko population of macrophages during kidney injury, we performed bone marrow transplantation using DsRed *Ctns* ko marrow into wild-type mice and examined 5 time points (3 mice/group). We are currently correlating *Ctns* macrophage infiltration with tubular injury.

Also, we have generated another group of Ctns ko/wt chimeric mice and performed UUO on them and sacrificed them at 14 days. Total collagen assays support our IR findings that fibrosis is reduced with Ctns ko macrophages. Similar analyses to our IR study are being performed.



Grantor Agency: Cystinosis Research Foundation Total Award: \$256,208.00  
 Title of Study: Elucidating the Role of Aberrant Macrophase Activation in Nephropathic Cystinosis  
 Principal Investigator: Daryl Okamura, MD  
 Co. Principal Investigator: \_\_\_\_\_ Effective Date of Grant: 9/1/2013  
 Research Fellow: \_\_\_\_\_ Period of this Report: 8/01/14 - 01/31/15

### Report of Receipts and Expenditures

**Receipts:**

Payments Received to Date: 191,156.00  
**Total Available for Expenditure:** 191,156.00

	Current Expenditures [Dates]	Cumulative Expenditures	
Salaries and Wages	\$29,045.61	\$79,077.84	
Supplies and Expenses	\$15,225.15	\$39,878.89	
Equipment	\$0.00	\$0.00	
Travel	\$0.00	\$0.00	
Staff Benefits	\$9,679.94	\$23,611.59	
Other - Subcontract			
Subtotal - Direct Costs	<u>\$53,950.70</u>	<u>\$142,568.32</u>	
Indirect Costs - 10%	\$5,394.32	\$14,255.89	
<b>Total Expenditures</b>	<u><b>\$59,345.02</b></u>	<u><b>\$156,824.21</b></u>	<u><b>\$156,824.21</b></u>

Unexpended Balance as of : 1/31/2015 \$34,331.79

2/20/15 *Valerie Baldwin*  
 Date Authorized by: Valerie Baldwin  
Title: Manager, Research Finance