Dear members of the Society of Pediatric Urology Executive Committee:

I am writing to update you on the progress our lab has made on the project “The role of bone marrow derived cells in bladder obstruction and fibrosis,” which was generously supported by the 2008-2009 Society for Pediatric Urology Research Grant.

The main goal of our proposal was to study the role of bone marrow derived cells in bladder fibrosis. Chemokines recruit bone marrow derived cells to sites of tissue injury. Blockade of chemokines in other organ systems has resulted in reduced fibrosis. In the bladder, identification of bone marrow derived cells and the chemokines involved in their recruitment may provide potential targets for antifibrotic therapies. In order to study the role of the bone marrow derived cells in bladder fibrosis, we used chimeric mice whose bone marrow cells were labeled with green fluorescent protein (GFP). Briefly, wild type C57BL/6 female mice underwent lethal irradiation. Their bone marrow was reconstituted using fetal liver cells from transgenic mice ubiquitously expressing GFP. Their bone marrow was reconstituted using fetal liver cells from transgenic mice ubiquitously expressing GFP. The chimeric mice then underwent partial bladder outlet obstruction with periurethral collagen injection.

We describe below our progress on each of our specific aims:

**SPECIFIC AIM 1:** To characterize the bone marrow derived cells associated with partial bladder outlet obstruction. We will establish the identity of bone marrow derived cells in the bladder after urethral obstruction and their temporal appearance in relation to histologic and physiologic changes in the bladder

- We found that periurethral bladder outlet obstruction caused histologic changes in the bladder. At 4 weeks of obstruction, bladder smooth muscle hypertrophy was present. At 12 weeks of obstruction, increased collagen within the detrusor layer was consistently present.
- We found that periurethral bladder outlet obstruction caused urodynamic changes. At 4 weeks of obstruction, mice had decreased bladder capacity but no significant difference in compliance. At 12 weeks of obstruction, bladder capacity was less than half the capacity of unobstructed mice, and mean bladder compliance was less than one-third the compliance of unobstructed mice.
- Bone marrow cells are recruited to the bladder and persist long term after bladder outlet obstruction. GFP-positive bone marrow derived cells were consistently present in the urothelial and stromal layers of obstructed mice from 1 to 12 weeks after obstruction.
- Although we were unable to identify any bone marrow derived myofibroblasts which we hypothesized may be responsible for fibrosis in the obstructed bladder, we have been able to use fluorescence activated cell sorting to establish the
identity of some of the bone marrow derived cells. We found that less than 5% of the GFP-positive cells were also positive for the murine macrophage marker F4/80. Other possible cell identities are currently being investigated.

- We also discovered that bone marrow derived cells may affect the injured bladder not only by differentiation but also by release of factors associated with hypertrophy and fibrosis. We found clusters of cells with activated EGF receptors around GFP-positive bone marrow derived cells after 12 weeks of obstruction but not in unobstructed controls. These studies are ongoing.

**SPECIFIC AIM 2:** Identify mechanisms involved in recruitment of bone marrow derived cells after bladder outlet obstruction. Chemokines involved in the recruitment of bone marrow derived cells to the bladder will be identified. We will determine if blockade of chemokine / chemokine receptor interactions with neutralizing antibodies will decrease the number of recruited bone marrow derived cells, decrease fibrosis and/or preserve normal bladder function.

- The chemokines CCL2 and CXCL12 which are associated with fibrosis in other organ systems were observed at 1 week and persisted up to 12 weeks following bladder outlet obstruction.
- Treatment of mice with neutralizing antibodies to the chemokines CCL2 and CXCL12 remain ongoing. We will study the effect of these neutralizing antibodies on the reduction of pathologic histologic and pathologic urodynamic changes.

We were able to present our initial findings as a moderated poster at the 2008 American Academy of Pediatrics meeting in Boston. The associated manuscript has been accepted for publication in the Journal of Urology Pediatric Supplement. We hope to complete our pending experiments as indicated and submit our additional findings for publication in the near future.

Thank you again for your generous support of our research.

Sincerely,

Stacy T. Tanaka, MD
Clinical Fellow, Division of Pediatric Urology
Monroe Carell Jr. Children’s Hospital at Vanderbilt