Research Publication Series

November 2019

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MCW Collaborative Highlights, indicated with the puzzle piece icon, call out articles that are produced by multidisciplinary teams. These articles represent collaborative efforts between researchers from different departments, centers, divisions, or fields of study.

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Namrata Tomar, PhD
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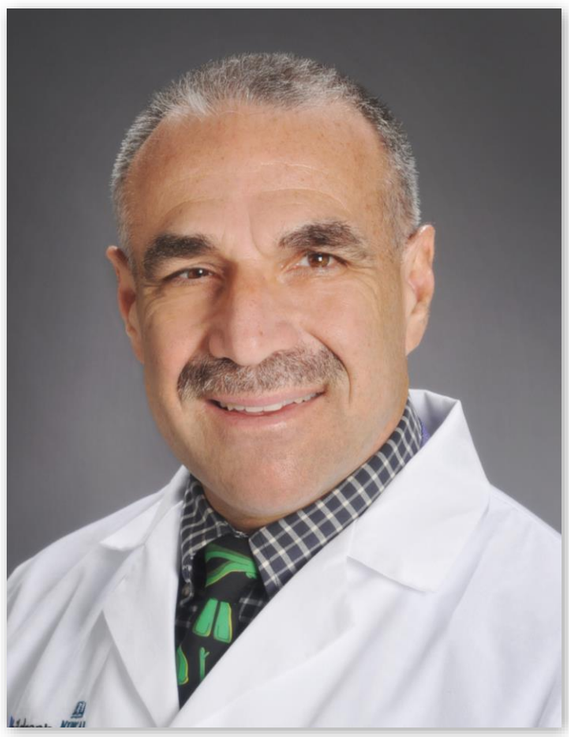
Cover Image from "A Thermodynamically-constrained Mathematical Model for the Kinetics and Regulation of NADPH Oxidase 2 Complex-mediated Electron Transfer and Superoxide Production"



Harry Whelan, MD

Bleser Professor of Neurology and
Pediatrics and Hyperbaric Medicine
Department of Neurology
Medical College of Wisconsin

My Photobiology Research Laboratory is dedicated to supporting my translational bench-to bedside NeuroFibromatosis (NF) research. My successes using PhotoDynamic Therapy (PDT) for pediatric brain cancer are being adapted to NF tumors. I am the Bleser Family Endowed Chair & Professor of Neurology, collaborating with other MDs and research scientists within MCW and at other institutions for this NF research. I am the recipient of the Neuro-Oncology American Cancer Society Clinical Oncology Career Development Award, and I was inducted into the NASA Space Technology Hall of Fame in the year 2000 for my research on near-infrared light PDT and photobiology. I was awarded the Legion of Merit medal from the United States Marine Corps by the President of the United States in 2014.

Photodynamic Therapy for Benign Dermal Neurofibromas

Vincent Riccardi MD,[1] Brendan Quirk PhD,[2] Edit Olasz MD,[2] Suresh Kumar PhD,[2] Donald Basel MD,[2] Harry Whelan MD[2]

[1]The Neurofibromatosis Institute [2]Medical College of Wisconsin

Acknowledgements: This work was supported by the Bleser Endowed Chair in Neurology (to Dr. Whelan), the Baumann Research Endowment (to Dr. Whelan), SUN Pharma (to Dr.Whelan), Children's Tumor Foundation (to Dr.Whelan) -and Ben's Fund (to Dr.Whelan).

Background

Cutaneous neurofibromas involve terminal nerves, are rich in extracellular matrix, and are sparsely populated by the Schwann cells, mesenchymal cells, and mast cells. Patients may, in some cases, develop hundreds of these tumors over their lifetime. Dermal neurofibromas are often overlooked in the "quest for cure" when developing research strategies to aid persons affected with Neurofibromatosis type 1 (NF1). Despite their benign nature, these tumors cause immeasurable stressors to a variety of individuals; the parents of an affected child, the preadolescent affected child who is at risk for developing body dysmorphic disorder, the adult who has to deal with the social stigmatization of the disfiguring lesions as well as their physical discomfort. These small tumors usually begin to develop during adolescence and adulthood, but may develop in early childhood.

Significance

No known medical therapies are beneficial to patients with NF1. Several drug trials have been initiated, looking for medications that slow or halt the growth of neurofibromas. Thus far, none of these medications has demonstrated significant benefit, though various research trials involving chemotherapeutic and other agents are under way, in an attempt to slow the growth of plexiform neurofibromas.

Rationale

Photosensitization of lesions using the Levulan® Kerastick® Topical Solution (Dusa), plus illumination with red light, is the basis for Levulan/ALA-PDT. The topical application of Levulan® Kerastick results in photosensitization through the accumulation of PpIX. When exposed to light of appropriate wavelength and energy, the accumulated PpIX produces a photodynamic reaction which results in a cytotoxic process through the generation of superoxide and hydroxyl radicals.

Part One - Hypotheses

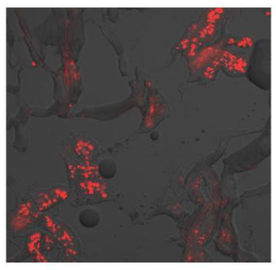
1. Levulan (aminolevulinic acid, ALA) would accumulate, and be converted to Protoporphyrin IX (PpIX), by tumor tissue more than surrounding normal tissue.
2. Tumors incubated with Levulan would show greater fluorescence than untreated tumors and tumors incubated with vehicle only (placebo application).

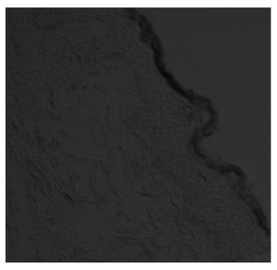
Methods

Levulan applied twice to microneedle prepared tumors, dried, and incubated overnight under occlusion. Placebo lesion treated with vehicle only Kerastick. PpIX signals detected in excised tumors by confocal microscopy.

Results

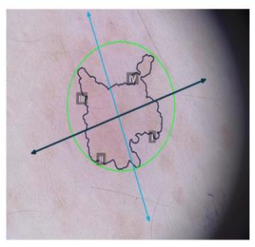
1. ALA was specifically converted to the active agent PpIX in the tumor region as seen by confocal microscopy. Average fluorescence for PpIX positive tumor areas was 318 ± 31 densitometry units/μm^2 (subject n=2). Fluorescence values in the non-tumor areas were zero. Using a one-sample t-test comparing the absolute value to an alternate expected value of zero, the results are significant with p = 0.044.
2. Control and placebo tumors showed no visible fluorescence.

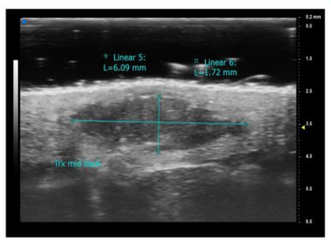




Future Studies

A Phase II clinical effectiveness trial has begun, with the primary endpoint of time to progression, defined as a 50 % increase in tumor size. Tumor area will be measured using FotoFinder digital photography, and tumor volume using VevoMD high frequency ultrasound. Natural history data relating to tumor size and growth rates will be collected.





Part Two - Phase One Trial

1. Dose escalation of light application using Levulan application parameters determined in Part One.
2. Possible tumor size response and clinical evaluation.
3. Histological and light microscopy evaluation of excised tumor tissue.

Methods

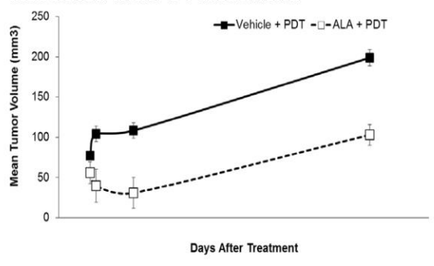
Mean Tolerable Dose (MTD) determined by dose escalation with 3 levels and 3 subjects per level. 630 nm red light at 100 mW/cm^2 applied to both treatment and placebo lesion areas using Omnilux Revive. Doses of 50, 100, or 150 J/cm^2 achieved by irradiation periods of 500, 1000, or 1500 sec. Dose Limiting Toxicity (DLT) defined as pain during irradiation requiring cessation of the light treatment, or any serious cutaneous adverse events.

Results – Dose Escalation

Red light treatment induced marked pain and discomfort in ALA but not vehicle treated tumors, and in one case prompted cessation of treatment due to pain. Due to this marked pain, it was decided that the MTD would be set at 100 J/cm^2, and the highest dose level foregone, even though only one subject experienced DLT.

Results – Tumor Size

Measurement of the tumor size at 2 weeks, 1 and 3 months after treatment did not show significant tumor reduction, likely due to the presence of large amounts of fibrous material not susceptible to PDT treatment.
A non-significant reduction in average tumor growth rates when comparing the area of treated and non-treated tumors on the same subject
(-0.11 ± 0.20 mm^2/day post treatment) was associated with PDT treatment.

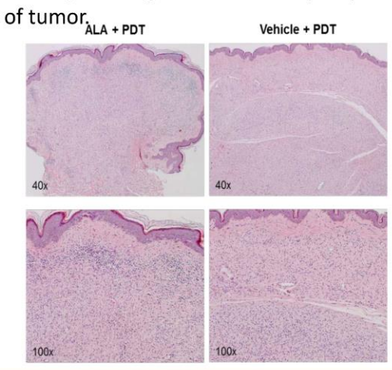


Results – Clinical Evaluation

Red light at 100 mW/cm^2 and a dose of 50 J/cm^2 resulted in marked erythema and edema around ALA (n= 24) but not vehicle (n=20) treated tumors, which lasted for 48 hours. There was no blister formation, erosion or crusting.

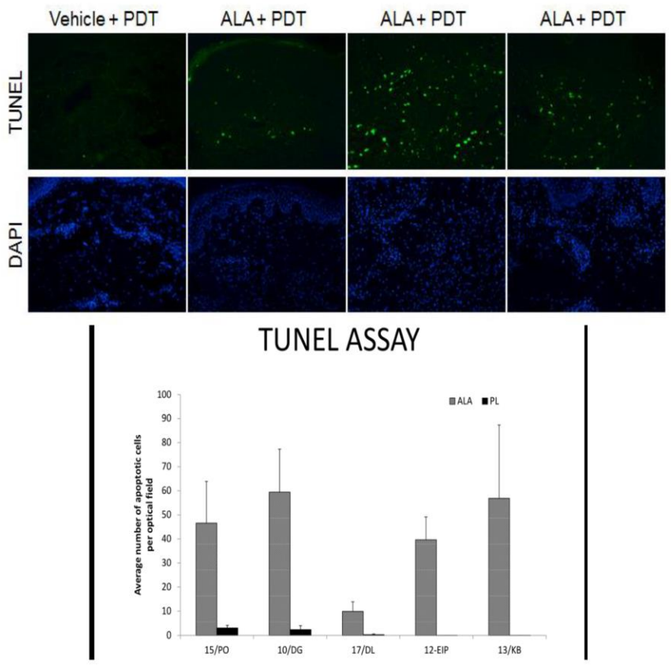
Results – Light Microscopy

Light microscopy showed mixed infiltrate consisting of lymphocytes, neutrophils, and rare eosinophils in ALA but not placebo tumors, showing an inflammatory response and possible necrosis of tumor.



Results – TUNEL Assay

TUNEL assay showed significant increase in number of apoptotic cells as compared to control, further showing cell death in treated tumor tissue.



Mary Eapen, MBBS, MS
Professor of Medicine
Division of Hematology & Oncology
Senior Scientific Director – Research,
Center for International Blood and
Marrow Transplant Research
Medical College of Wisconsin

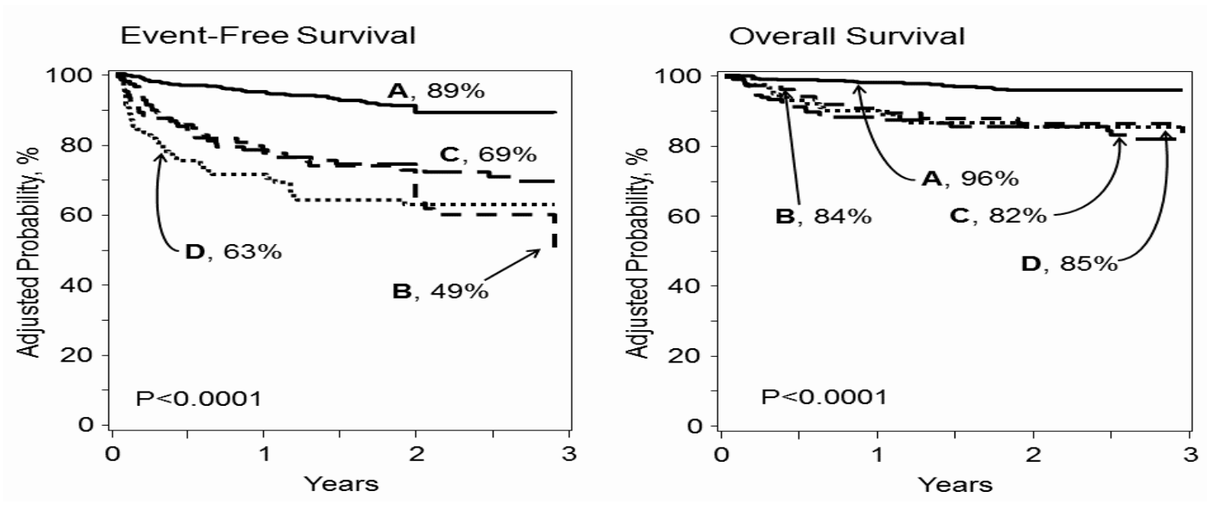
I am a Professor of Medicine in the Division of Hematology-Oncology. My research focuses on extending access to hematopoietic cell transplantation, a life-saving treatment for those with malignant and non-malignant hematologic diseases. Through the CIBMTR, I study the effectiveness of utilizing donors other than HLA-matched siblings for hematopoietic cell transplantation. As HLA-matched siblings are available for less than a third of patients who may benefit from transplantation, most transplants utilize alternative donors. My work has identified strategies to improve outcomes after hematopoietic cell transplantation in regards to HLA-mismatched related and HLA-matched or mismatched unrelated donor transplantation, including umbilical cord blood. The CIBMTR®, a collaboration between the National Marrow Donor Program® (NMDP)/Be The Match® and MCW, facilitates critical observational and interventional research through scientific and statistical expertise, a large network of transplant centers, and a clinical outcomes database.

"Effect of Donor Type and Conditioning Regimen Intensity on Allogeneic Transplantation Outcomes in Patients with Sickle Cell Disease: A Retrospective Multicentre, Cohort Study"

Eapen M, Brazauskas R, Walters MC, et al. *The Lancet Haematology*. 2019;6(11):e585-e596.

In this study on sickle cell disease, we compared the relative risk of outcomes for transplants from HLA-matched sibling donors to alternative donors. We collected data on 996 patients with sickle cell disease who had transplants in 2008–17, reported to the CIBMTR. Our data suggest that event-free survival (alive without graft failure) and overall survival at 3 years were highest with an HLA-matched sibling donor, 89% and 96%, respectively, after adjustment for ag at transplant and conditioning regimen intensity (see figures, line A). For patients without an HLA-matched sibling, 3-year adjusted event-free and overall survival were lower after transplantation from HLA-mismatched relative, (B, 49% and 84%); HLA-matched unrelated donors (C, 69% and 82%); and HLA-mismatched unrelated donors (D, 63% and 85%); $p < 0.0001$. Our data do not favor one alternative donor type over another.

Figure 1. Outcomes after transplant from: A, HLA-matched, sibling donor; B, HLA-mismatched relative; C, HLA-matched unrelated donor; D, HLA- mismatched unrelated donor.



Rat Genome Database Team

Mary Shimoyama, PhD; Mindy Dwinell, PhD; Anne Kwitek, PhD; Jeffrey De Pons, BS; Jennifer Smith, MS; G Thomas Hayman, PhD; Stanley J Lauledеrkind, PhD; Shur-Jen Wang, PhD; Marek Tutaj, MS; Jyothi Thota, MS; Monika Tutaj, PhD; Matthew Hoffman, BS; Harika Nalabolu, MS; Mary Kaldunski, BS; Cagatay Dursun, MS; Santoshi Ellanki, MS; Stacy Zacher, MS; Yiqing Zhao, PhD (members at time of publication).

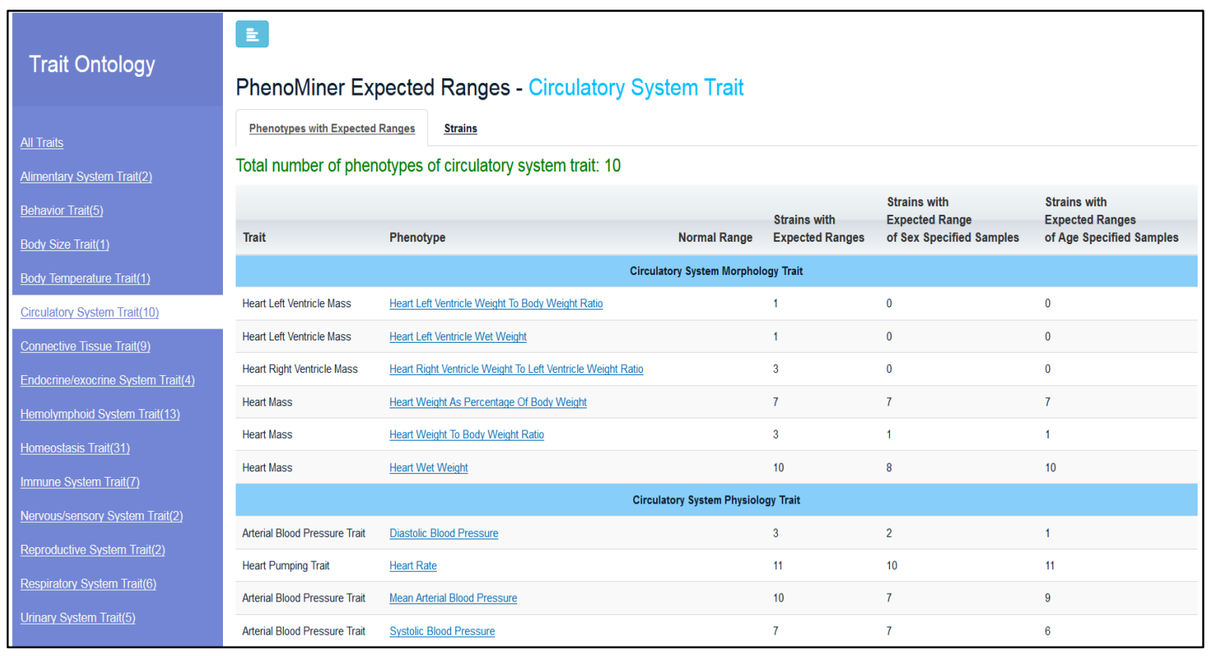
Our Rat Genome Database (RGD) is nearly 20 years old, and these years have been fruitful for rat research. A large amount of rat data has been taken into the database and RGD now grows to host biological data from chinchilla, bonobo, dog, ground squirrel and pig as well. RGD provides a catalogue of genomic elements for these species and their associated phenotype data. The data from multiple species can be analyzed by tools developed at RGD. For example, the Gene Annotator (GA) tool, Gene and Ortholog Location Finder (GOLF) and Multi-Ontology Enrichment Tool (MOET) are tools that allow gene analysis for multiple species.

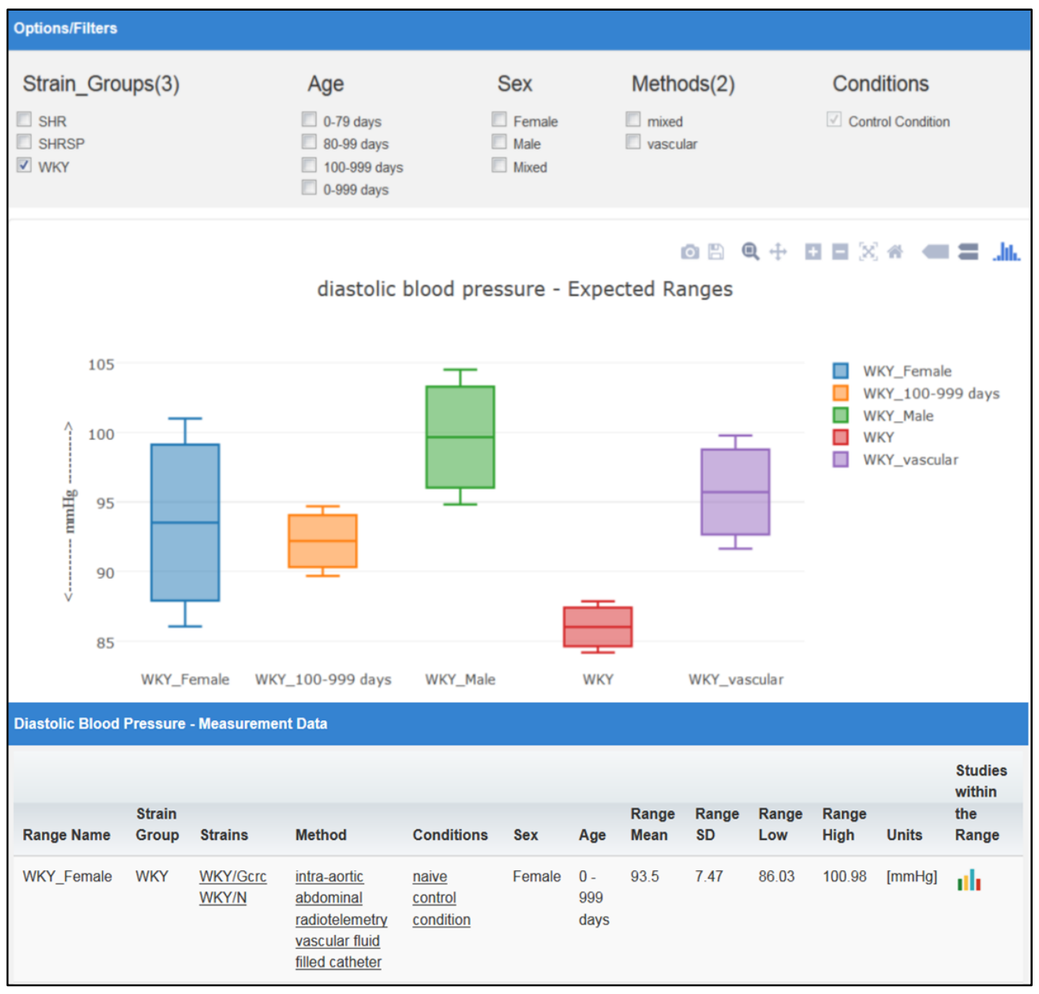
"Integrated Curation and Data Mining for Disease and Phenotype Models at the Rat Genome Database"

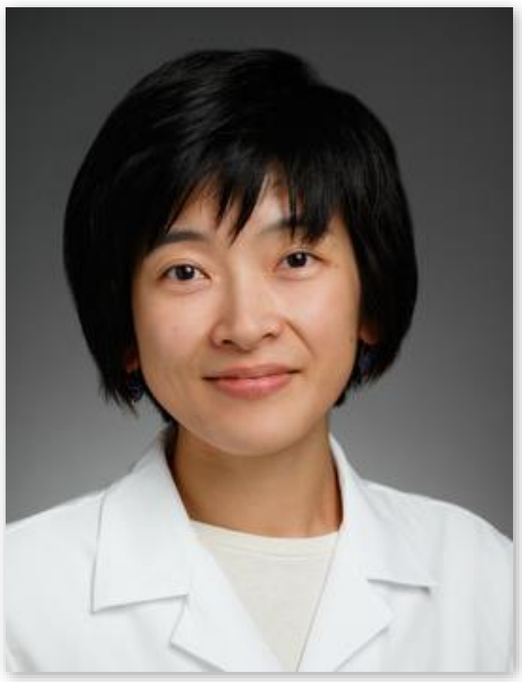
Wang S, Laulederkind SJF, Zhao Y, et al. *Database*. 2019;baz014. https://doi.org/10.1093/database/baz014

To identify appropriate disease models, RGD provides the research community with the Disease Portals and the Phenotypes & Models Portal, where qualitative and quantitative disease and phenotype data are systematically organized and integrated. Related tools are embedded in the portals to allow direct data transfer between tools. Among RGD tools, OLGA (Object List Generator & Analyzer), and PhenoMiner have been used to identify cardiovascular disease models in the publication. A selected disease model can be further characterized by comparing its phenotypic values with Expected Ranges established through a meta-analysis pipeline using data in PhenoMiner.

Figure 4. (C) A summary of the traits for expected ranges analysis is listed on the left and the list of the available expected ranges under the selected circulatory system trait is shown in blue print. Each phenotype is hyperlinked to the associated expected ranges data. (D) The expected ranges of diastolic blood pressure of the selected WKY strain group. Each color-coded box is an interquartile range graph in which the data median is shown as the line in the middle, the upper quartile is shown on the top and lower quartile is shown on the bottom.







Melissa Wong, MD, FACS
Assistant Professor
Division of Transplant Surgery
Department of Psychiatry
Medical College of Wisconsin

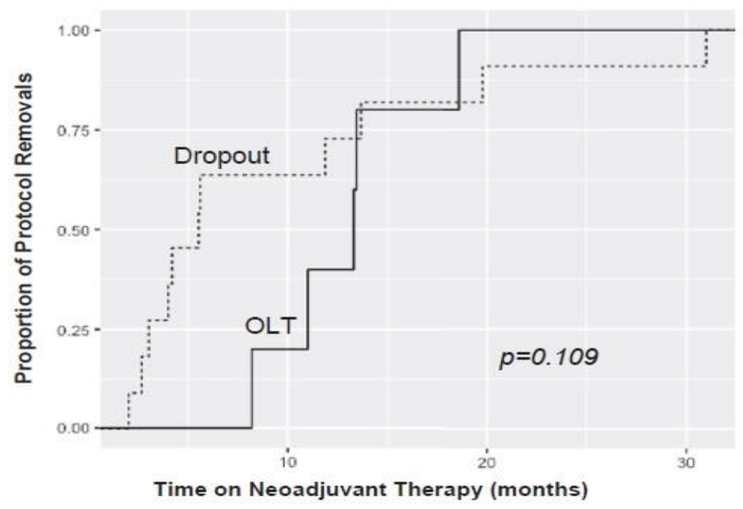
I am an Assistant Professor of Surgery in the Division of Transplant Surgery at MCW, under the leadership of Johnny Hong, MD, FACS, Division Chief and Director of Solid Organ Transplantation. My clinical practice includes liver and kidney transplantation, organ procurement, and the critical care of patients with liver failure. My research interests focus on cholangiocarcinoma, cirrhotic pathophysiology including portal hypertension, and transplant outcomes.

"Downstaging Locally Advanced Cholangiocarcinoma Pre-Liver Transplantation: A Prospective Pilot Study"

Wong M, Kim J, George B, et al. *The Journal of Surgical Research*. 2019;242:23-30.

For patients with cholangiocarcinoma, complete tumor removal offers the best chance for long term survival. Unfortunately, many tumors are unresectable at diagnosis, leading to death within months. Treatment protocols using neoadjuvant radiation and systemic chemotherapy followed by liver transplantation for unresectable cholangiocarcinoma have achieved excellent long term recurrence-free survival, but focus on patients with early stage tumors and exclude those with locally advanced disease. Our prospective study reports the early results of our treatment protocol for unresectable cholangiocarcinoma. Select patients manifested favorable tumor biology during neoadjuvant therapy, while others progressed to protocol dropout within months. This suggested an optimal timeframe for transplant in patients who respond favorably to neoadjuvant therapy. We demonstrated successful downstaging of unresectable, locally advanced cholangiocarcinoma before liver transplantation, and achieved acceptable short term recurrence-free survival.

Fig. 2. NT protocol removals by reason for removal: dropout versus OLT.



Patients who ultimately dropped out received a median of 5.5 mo of NT treatment before removal, whereas those who were removed due to OLT received a median of 13.5 mo of NT (P = 0.109).

Table 3. Preneoadjuvant chemoradiation clinical staging compared with explant pathology for the five patients who underwent liver transplantation.

Patient	Pre-NT clinical staging				Explant pathology					
	cTNM, stage	Intrahepatic	Hilar	Comments	ypTNM, stage	R0	Tumor grade	Liver histology	LN met	LVI
A	cT1N0M0, I	X			ypT1N0Mx, I	Yes	WD	Cirrhosis, PSC	No	No
B	cT2N2M0, IVB		X	PET-positive LNs	ypT1N0Mx, I	Yes	WD	Periportal fibrosis, PSC	No	No
C	cT1N0M0, I	X			ypT2bNxMx, IIB	Yes	MD	Cirrhosis	No	No
D	cT2N1M0, IIIB		X	PET-positive LNs, vascular encasement	ypT2bN0Mx, IIB	Yes	WD	Periportal fibrosis	No	No
E	cT3N0M0, IIIA		X	Vascular encasement	ypT2aN0Mx, IIA	Yes	WD	Cirrhosis	No	No

LN = lymph nodes; LVI = lymphovascular invasion; Met = metastases; MD = moderately differentiated; R0 = microscopically tumor-negative margin; PSC = primary sclerosing cholangitis; WD = well-differentiated.

Stephanie Olivier-Van Stichelen, PhD

Assistant Professor

Department of Biochemistry

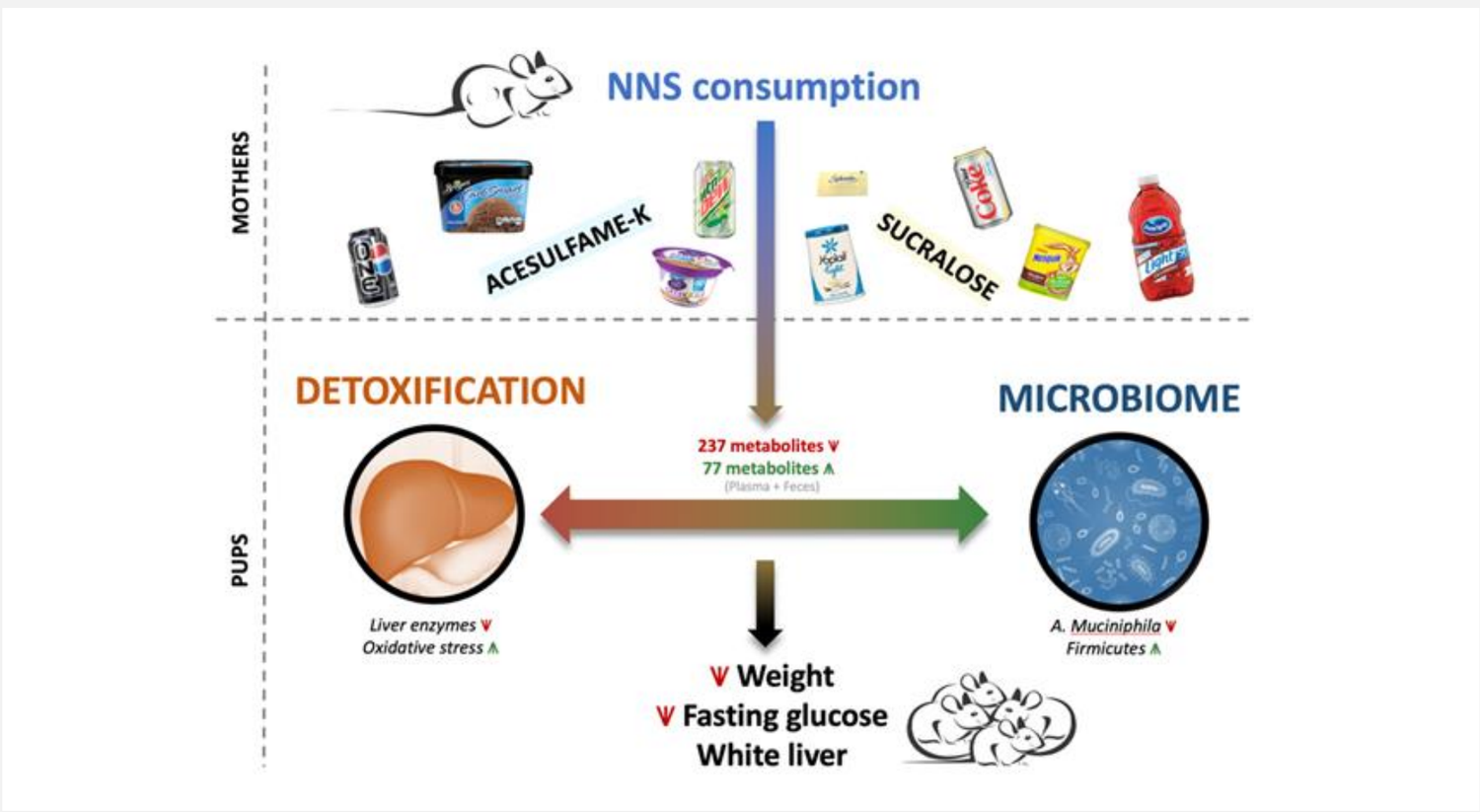
Medical College of Wisconsin

I received my PhD in Biochemistry from the University of Lille (France) in 2012. My work was focused on the understanding of the nutrient-sensing post-translational modification (O-GlcNAcylation) in colorectal cancer development with a special interest in diet-dependent modification of the oncogene beta-catenin. I completed a post-doctoral Fellowship at the National Institute of Health where I worked on different aspects of O-GlcNAcylation during development including X-inactivation of the O-GlcNAc Transferase gene. I also developed a brain O-GlcNAcase knockout model and studied the impact of sugar consumption during pregnancy on O-GlcNAc-dependent development of metabolic homeostasis. More recently, I've developed interests in understanding the importance of artificial sweeteners for offspring's metabolism and microbiome. At MCW, the OVS lab is at the crossroad of sweeteners, pregnancy, development and metabolism.

"Maternal Exposure to Non-nutritive Sweeteners Impacts Progeny's Metabolism and Microbiome"

Olivier-Van Stichelen S, Rother KI, Hanover JA. *Frontiers in Microbiology*. 2019;10:1360.

NNS are marketed as sugar alternatives providing sweet taste with no calories. Yet their consumption has been linked to metabolic dysfunction and microbiome dysbiosis. NNS exposure mostly originates from diet beverages and sweetener packages in adults or breastmilk in infants. Consequences of early life exposure remain largely unknown. We exposed pregnant and lactating mice to NNS at doses relevant for human consumption. While the pups' exposure was low, metabolic changes were drastic, indicating extensive downregulation of hepatic detoxification mechanisms and changes in bacterial metabolites. Microbiome profiling confirmed increase in firmicutes and a striking decrease of Akkermansia muciniphila. Similar microbiome alterations in humans have been linked to metabolic disease and obesity. Our findings need to be reproduced in humans, they suggest that NNS consumption during pregnancy and lactation may have adverse effects on infant metabolism.



Jonathan S. Ellison, MD
Assistant Professor
Division of Pediatric Urology
Department of Urology
Children's Hospital of Wisconsin
Medical College of Wisconsin

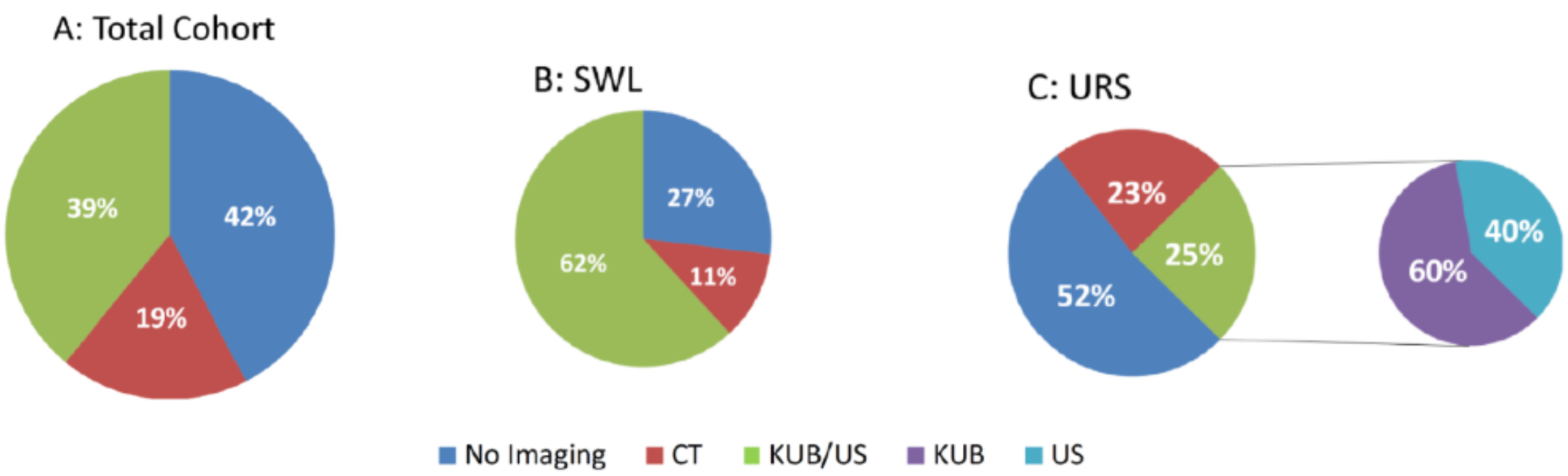
I am an Assistant Professor of Urology within the Division of Pediatric Urology. My clinical and research interest include pediatric kidney stones, including the etiology, surgical management, and health care utilization for these children. I currently am working on projects to investigate the microbiome of pediatric kidney stone formers as well as working within multi-institutional outcomes to define the recurrence patterns and surgical outcomes in pediatric stone disease.

"Postoperative Imaging Patterns of Pediatric Nephrolithiasis: Opportunities for Improvement"

Ellison JS, Merguerian PA, Fu BC, Holt SK, Lendvay TS, Shnorhavorian M. *The Journal of Urology.* 2019;201(4):794-801.

This study evaluated children undergoing the two most common types of surgery (shock wave lithotripsy and ureteroscopy) for kidney and ureteral calculi. We looked at a national administrative claims database of insured patients (Marketscan™) to evaluate post-operative imaging studies in these children. Imaging practices are important after kidney stone surgery in order to ensure no post-operative renal obstruction and evaluate stone clearance. Ideal imaging strategies would minimize radiation exposure. We found only 58% of children received any post-operative imaging within 90 days of surgery. Computed tomography, with higher associated radiation exposure, was more likely for older children and females. Overall, non-ionizing post-operative imaging is underused, while ionizing radiation is likely overused. These data serve as a starting point to inform surgeons on appropriate imaging practices following pediatric kidney stone surgery.

Figure 1: 90-day post-operative imaging strategies following shock wave lithotripsy (SWL) and ureteroscopy (URS), by modality (CT – computed tomography; KUB – plain film x-ray of the kidney, ureter, and bladder; US – renal ultrasound)

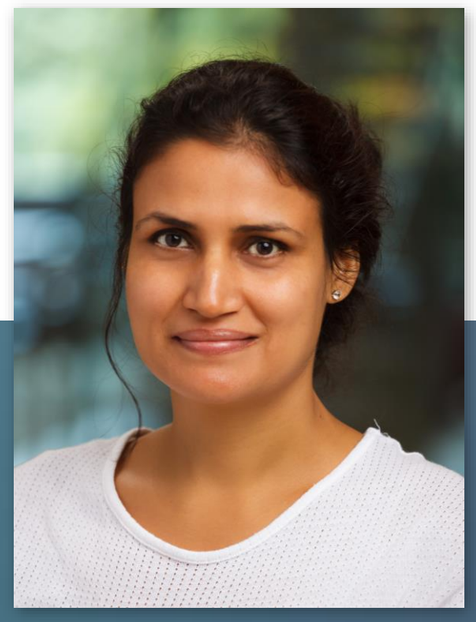


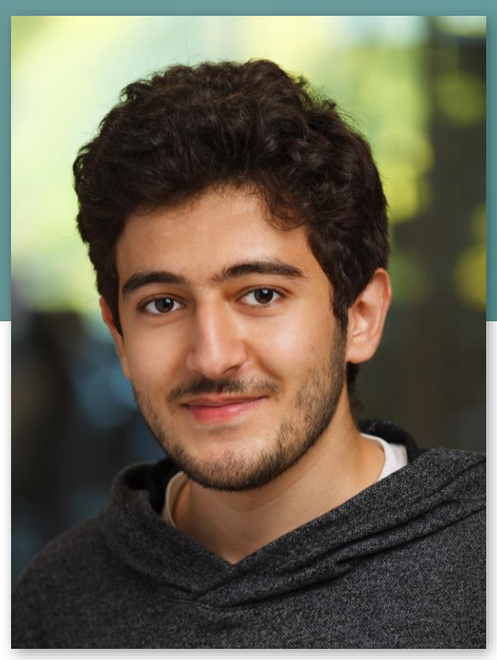
“A Thermodynamically-constrained Mathematical Model for the Kinetics and Regulation of NADPH Oxidase 2 Complex-mediated Electron Transfer and Superoxide Production”

Tomar N, Sadri S, Cowley AW Jr, et al. *Free Radical Biology & Medicine*. 2019;134:581-597.

We have developed here a thermodynamically-constrained mathematical model for the kinetics and regulation of NOX2 complex based on published experimental data on the NOX2 complex function. The model incorporates (i) thermodynamics of electron transfer from NADPH to O2 through different redox centers of the NOX2 complex, (ii) dependence of the NOX2 complex activity upon pH and temperature variations, and (iii) inhibitory effects of drugs on the NOX2 activity. The model provides the first quantitative and integrated understanding of the kinetics and regulation of NOX2 complex, enabling simulation of diverse experimental data. Specifically, the model enables examining the effects of specific targeting of enzymatic sources of pathological ROS which could overcome the limitations of pharmacological efforts aimed at scavenging ROS which has resulted in poor outcomes of antioxidant therapies in clinical studies.

Namrata Tomar, PhD
Postdoctoral Fellow
Department of Biomedical Engineering





Ammar J. Alsheikh, MBBS
PhD Candidate
Department of Physiology

“Renal Nerves and Leukocyte Infiltration in the Kidney During Salt-Sensitive Hypertension”

Alsheikh AJ, Lund H, Dasinger JH, Abais-Battad JM, Fehrenbach DJ, Mattson DL. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*. 2019;317(1):R182-R189.

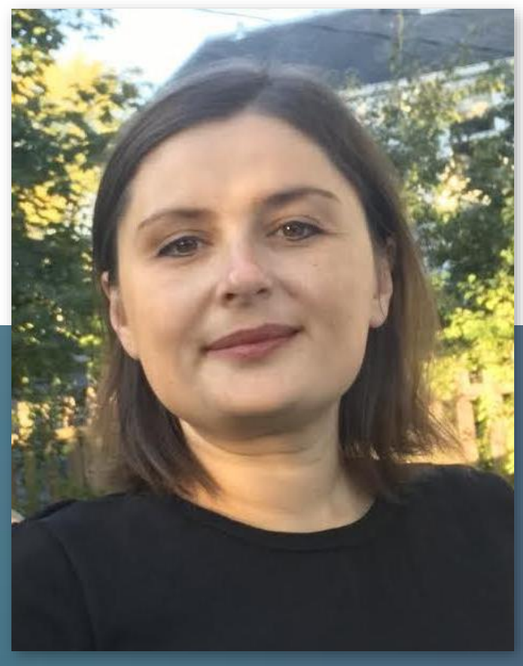
Hypertension remains the most important individual contributor to mortality worldwide. There has been great renewed interest in renal nerves’ role in hypertension clinically with potential therapeutic applications. We performed studies to test the hypothesis that renal nerves contribute to hypertension and renal disease by increasing immune cell infiltration into the kidneys. We used two complementary in-vivo studies to test this hypothesis in a widely accepted rat model (Dahl salt-sensitive rat) to study hypertension. Contrary to our hypothesis, the results of this work suggested that immune cell infiltration in the kidney is not mediated by the renal nerves.

“Characterization of the Distribution of Spin-Lattice Relaxation Rates of Lipid Spin Labels in Fiber Cell Plasma Membranes of Eye Lenses with a Stretched Exponential Function”

Stein N, Mainali L, Hyde JS, Subczynski WK. *Applied Magnetic Resonance*. 2019;50(7):903-918.

Many spectroscopies in a biomedical field generate multi-exponential outputs. Multi-exponential decays are notoriously difficult to separate given their non-orthogonal nature. We used a stretched-exponential function to characterize the saturation recovery electron paramagnetic resonance signals that arise from the spin labeled intact membranes. The method to create a probability density distribution of rates in a sample is described. The distribution can be used to determine a fraction of signal that arises from a particular range of relaxation rates. Also, the use of fitting parameters in a k-means clustering multivariate analysis is demonstrated.

Natalia Stein, PhD
Postdoctoral Fellow
Department of Biophysics



Daniel R. Hanna
MD Candidate, Class of 2021
Center for Advancing Population Science

“Prevalence and Correlates of Diagnosed and Undiagnosed Hypertension in the Indigenous Kuna Population of Panama”

Hanna DR, Walker RJ, Smalls BL, Campbell JA, Dawson AZ, Egede LE. BMC Public Health. 2019;19(1):843.

The purpose of this study was to determine the prevalence of hypertension and investigate sociodemographic correlates of a unique indigenous population in Panama. We investigated the Kuna Indians living on the San Blas islands off the Eastern coast. We conducted surveys and collected anthropomorphic measures. Two hundred and eleven adult indigenous Kuna participated. Overall, prevalence of hypertension was 6.2% as defined by 140/90 mmHg and 16.6% as defined by 130/80 mmHg. Hypertension was found to be significantly higher in men and those with higher income. Investigating these factors remains vitally important in helping improve the health of the Kuna through targeted interventions to address chronic disease.

Medical College of Wisconsin
Office of Research
8701 W Watertown Plank Road
Milwaukee, WI 53226-0509
mcw.edu/office-of-research.htm