26th Annual Research Forum

Wednesday, April 23, 2008
12:00pm - 2:00pm
VCH Theatre
(2221 VCH)

Established and Sponsored by the Vanderbilt University House Staff Advisory Council
Vanderbilt University Medical Center’s Research Forum provides an opportunity for non-faculty VUMC personnel to present research conducted at Vanderbilt. This Forum is open to all Vanderbilt University House Staff and Medical Students.

Research must have been performed at Vanderbilt. Unpublished work is eligible and encouraged. Work already published, or presented at another meeting, is also eligible and encouraged. All submitted abstracts are published in the Vanderbilt University Medical Center Research Forum book.

Abstracts are reviewed and selected for either an oral or a poster presentation by a panel of Vanderbilt School of Medicine faculty members who are actively involved in clinical and basic science research. There are six abstracts selected for oral presentation—three in Basic Science Research and three in Clinical Research. After the oral presentations at the Forum, the best overall project in each category (Basic Science Research and Clinical Research) will be awarded an Elliot V. Newman Award.

The Grant W Liddle Award, which honors a faculty member who demonstrates exemplary leadership in the promotion of scientific research at Vanderbilt University Medical Center, is presented annually at the Forum. Additionally, Vanderbilt School of Medicine students present the Davies, Brittingham, Hillman and Resident Teaching Awards which honor faculty and house staff teaching excellence.
TWENTY-SIXTH ANNUAL RESEARCH FORUM

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Resident, Department of Surgery

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Professor of Medicine and Director
Center for Quality Aging
GRANT W. LIDDLE AWARD

The Grant W. Liddle Award was established in 1983 by the Vanderbilt University Medical Center house staff to recognize faculty members who demonstrate exemplary leadership in the promotion of scientific research at the Vanderbilt University Medical Center.

A native of American Folk, Utah, Dr. Liddle graduated first in his class from the University of Utah in 1943. After obtaining an M.D. degree from the University of California, San Francisco, in 1948, he served as a post-doctoral fellow at the newly formed Metabolic Research Unit at the NIH. In 1956, Dr. Liddle was recruited by Dr. Hugh J. Morgan to become Director of Endocrinology at Vanderbilt University. He was named Chairman of the Department of Medicine in 1968, a position he held until 1983.

Dr. Liddle’s career was marked by commitment to excellence in research, patient care, and the teaching of house staff and medical students. His research accomplishments include developing the dexamethasone suppression test and metyrapone test for assessing pituitary-adrenal gland function; describing a new form of hypertension, pseudohypoaldosteronism (Liddle’s Syndrome); developing spironolactones as useful aldosterone antagonists; and systematically improving methods for treating Cushing’s disease. In 1982, he was elected to the National Academy of Sciences and to the Royal College of Physicians in England.

Past Recipients of the Grant W. Liddle Award are:

2007    Nancy J. Brown, M.D.
2006    Adrian Jarquin-Valdivia, M.D., R.D.M.S.
2005    Marshall L Summar, M.D.
2004    Denis M. O’Day, M.D.
2003    Herbert S. Schwartz, M.D.
2002    John A. Zic, M.D.
2001    Kathryn M. Edwards, M.D.
2000    R. Michael Rodriguez, M.D.
1999    David H. Van Buren, M.D.
1998    Charles Wright Pinson, M.D., M.B.A.
1997    Steven Leach, M.D.
1996    Jason D. Morrow, M.D.
1995    Robert H. Ossoff, M.D., Ph.D.
1994    William O. Richards, M.D.
1993    Barney S. Graham, M.D.
1992    Gordon Bernard, M.D.
1991    Achilles Demetrius, M.D., Ph.D.
1990    David Robertson, M.D.
1989    Robert Collins, M.D.
1988    Stanley Cohen, Ph.D.
1987    John A. Oates, M.D.
1986    David T. Karzon, M.D.
1985    Naji N. Abumrad, M.D.
1984    Fayez K. Ghishan, M.D.
1983    Grant W. Liddle, M.D.
Elliot Voss Newman was a distinguished cardiologist, scientist, medical scholar and teacher. A graduate of Harvard College and Medical School, Dr. Newman came to Vanderbilt from Johns Hopkins University in 1952 to establish a program of clinical physiology and research. The author of the electrocardiography chapter in Harrison’s Textbook of Medicine and of the renal physiology chapter in Cecil and Loeb’s textbook, Dr. Newman was a pioneer in the development of medical engineering and the use of applied mathematics and computer science for clinical problems. Dr. Newman was the first Joe and Morris Werthan Professor of Experimental Medicine at Vanderbilt and was founder of the Clinical Research Center, which bears his name. He was a friend and mentor to medical students and house officers alike and helped to promote the scientific careers of many.

Recent Elliot V. Newman Award recipients:

**2007**

**Eitan Friedman, M.D. - VMS, Class of 2007**
“The Effects of Tadalafil on Cold-induced Vasoconstriction in Patients with Raynaud’s Phenomenon”

**Sachin Patel, M.D. - Resident, Psychiatry**
“Endocannabinoid Signaling Modulates Dendritic Spine Density in Prefrontal Cortical Pyramidal Neurons”

**2006**

**Siam Oottamasathien, M.D. - Clinical Fellow, Urology-Pediatric Urology**
“Formation of Bladder Tissue from Embryonic Stem Cells”

**Jennifer Schuberth, M.D. - Clinical Fellow, Internal Medicine**
“Changes in Residency Work Hours: Impact of the Short Call Team on Length of Stay and Quality of Care for Heart Failure”

**2005**

**Heidi A. Smith, M.D. - Clinical Fellow, Pediatric Critical Care**
“Nitric Oxide Precursors and Congenital Heart Surgery: A Randomized Controlled Trial of Citruline”

**Fred Y. Wu, Ph.D. - VMS, Class of 2007**
“A Paradoxical Role of p27 Tumor Suppressor: Reduction of Cytosolic p27KIP1 Protein Decreases Akt Stability and Inhibits Cancer Cell Motility, Survival, and tumorigenicity”

**2004**

**Jennifer Halpern, M.D. – Resident, Orthopaedics**
“Evolution of an In Vivo Bioreactor: Vascularized Scaffold Generate Novel Host Bone”

**Elisabeth D. Riviello. - VMS, Class of 2006**
“HIV in Botswana:Saliva Test Validation, CD4 Cell Counts, Prevalence, and Incidence”

**2003**

**Hector A. Malave, M.D. - Clinical Fellow, Cardiology**
“Bradykinin and Bradykinin 1-5 Inhibit Thrombin-Induced Platelet Aggregation in Humans”

**John V. Williams, M.D. - Clinical Fellow, Pediatric Infectious Diseases**
“Molecular Analysis of the Infant B Cell Response To RSV”
TWENTY-SIXTH ANNUAL  
VANDERBILT UNIVERSITY RESEARCH FORUM  
Wednesday, April 23, 2008 • 12:00 noon – 2:15 p.m. • 2210 VCH Theatre

12:00 OPENING REMARKS ................................................................. Josh Smith, MD
WELCOME ................................................................................... Jeffrey R. Balser, MD, PhD
FORUM MODERATOR................................................................. Nancy J. Brown, MD

12:15 CLARA CELL PROTEIN (CC16) A MARKER OF LUNG EPITHELIAL INJURY IS DECREASED IN BLOOD AND PULMONARY EDEMA FLUID FROM CRITICALLY ILL PATIENTS WITH ACUTE LUNG INJURY (ALI) AND THE ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) Jon A. Kropski, R.D. Fremont and L.B. Ware

12:30 NON-INVASIVE ASSESSMENT OF CANCER SUSCEPTIBILITY TO THERAPY Roberto Diaz, MD, PhD, Zhaozhong Han, Allie Fu, Hailun Wang, Ling Geng, Halina Onishko and Dennis E. Hallahan, M.D.

12:45 CHARACTERIZATION OF DIFFERENTIAL MICRO-RNA EXPRESSION IN THE TRANSFORMATION OF FOLLICULAR LYMPHOMA TO LARGE B-CELL LYMPHOMA Sara McClintock-Treep, MD, S. Liang, J.D. Hallock, MP Robinson, MA Thompson

1:00 ELECTROPHILIC CYCLOPENTENONE NEUROPROSTANES ARE ANTI-INFLAMMATORY MEDIATORS FORMED DURING PEROXIDATION OF THE OMEGA-3 FATTY ACID DOCOSAHEXAENOIC ACID Erik Musiek, MD, PhD

1:15 POOR GLUCOSE CONTROL IN ADOLESCENTS WITH TYPE 1 DIABETES IS ASSOCIATED WITH INCREASED IL-8 BUT REDUCED IGF-1 Bradley J. Van Sickle, MD, PhD, J.H. Simmons, MD, R. Hall, M. Raines, K.D. Ness, MD and A Spagnoli, MD

1:30 LITHIUM PROTECTS HIPPOCAMPAL CELLS BY ENHANCING DNA-PK-DEPENDENT NONHOMOLOGOUS END JOINING OF RADIATION-INDUCED CHROMOSOMAL BREAKS Shih-Hsin Eddy Yang, MD, PhD, Hong Wang, MD, Dennis E. Hallahan, MD and Fen Xia, MD

1:45 CLOSING REMARKS ............................................................... Donald W. Brady, MD

1:50 POSTER PRESENTATION AWARDS ........................................... Josh Smith, MD

1:55 GRANT W. LIDDLE AWARD .................................................. Christopher D. Willey, MD, PhD

2:00 ELLIOT V. NEWMAN AWARDS ............................................ Nancy J. Brown, MD

2:10 DAVIES, BRITTINGHAM, HILLMAN AND HOUSE STAFF TEACHING AWARDS ........... Brenessa Lindeman and Natalie Jacobowski, VMS III, Class of 2009
2008 ORAL PRESENTERS

JONATHAN A. KROPSKI
Vanderbilt Medical Student IV, Class of 2008

ROBERTO DIAZ, M.D., Ph.D.
Resident, Radiation Oncology

SARA MCCLINTOCK-TREEP, M.D.
Clinical Fellow, Pathology

ERIK MUSIEK, M.D., Ph.D.
Resident, Medicine

BRADLEY J. VAN SICKLE, M.D., Ph.D.
Clinical Fellow, Pediatric Endocrinology

SHIH-HSIN EDDY YANG, M.D., Ph.D.
Resident, Radiation Oncology
CLARA CELL PROTEIN (CC16) A MARKER OF LUNG EPITHELIAL INJURY IS DECREASED IN BLOOD AND PULMONARY EDEMA FLUID FROM CRITICALLY ILL PATIENTS WITH ACUTE LUNG INJURY (ALI) AND THE ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

Kropski JA, Fremont RD, Ware LB

Background
Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) are common clinical syndromes that are underdiagnosed. Histologically, ALI/ARDS is characterized by severe lung epithelial injury. Clara Cell protein (CC16) is an anti-inflammatory protein secreted by the Clara cells of the distal respiratory epithelium. We have previously identified decreased plasma CC16 levels in a cohort of severely ill trauma patients with ALI compared to those at risk for lung injury. In the present study, we sought to determine the diagnostic and prognostic utility of CC16 in patients with non-trauma related ALI compared to a control group of patients with acute cardiogenic pulmonary edema (CPE).

Methods
Plasma and pulmonary edema fluid samples were obtained from medical and surgical patients with ALI/ARDS or CPE requiring intubation for mechanical ventilation. The etiology of pulmonary edema was determined using consensus clinical criteria for ALI/ARDS and CPE and the edema fluid-to-plasma protein ratio. Edema fluid was collected using gentle luminal suction through a #14 French catheter. Plasma was collected within 24 hours (mean = 4.1 hours) of edema fluid samples. CC16 levels were analyzed in duplicate by sandwich ELISA.

Results
Compared to patients with CPE (n=11), those with ALI/ARDS (n=24) had significantly decreased CC16 levels in plasma (27.6 ± 22.4 vs. 81.5 ± 115.1 ng/ml, p = 0.042) and pulmonary edema fluid (2930.0 ± 3699.2 vs. 5374.9 ± 5057.2 ng/ml, p=0.044). Patients in the lowest quartile of edema fluid CC16 were significantly more likely to have ALI than those in the highest quartile (88.9% vs. 37.5%, p=0.05). A similar trend was observed for plasma levels (87.5% vs. 44.4%, p=0.13). Neither blood nor edema fluid CC16 levels were predictive of mortality.

Conclusion
CC16 is a promising diagnostic biomarker for discriminating acute lung injury from cardiogenic pulmonary edema. Larger scale validation is warranted to better characterize the utility of CC16 in diagnosis of this deadly and under recognized syndrome.
NON-INVASIVE ASSESSMENT OF CANCER SUSCEPTIBILITY TO THERAPY

Roberto Diaz, Zhaozhong Han, Allie Fu, Hailun Wang, Ling Geng, Halina Onishko, and Dennis E. Hallahan

Objectives
Cancer patients are treated with therapeutic regimens for 2-3 months prior to assessment of cancer response. To more rapidly evaluated cancer susceptibility to a treatment regimen, we have selected peptides that bind within responding cancers but do not bind in non-responding cancers. These peptides bind to dead and dying cells and can be used to non-invasively image cancer response within 48 hours of initiating a new therapeutic regimen.

Methods
A phage displayed peptide library was administered to mice treated with receptor tyrosine kinase inhibitors (TKIs). Tumors were then removed and the phage binding within the responding tumor were amplified and enriched by serial passages through responding tumors in mice. We identified the HVGGSSV peptide as being the best peptide for sensitivity and specificity for binding to responding tumors. Peptide binding within animal models was determined by near infrared (NIR) imaging over the course of days. We studied a variety of tumor models including B16, LLC, HT22, H460, D54, and MDA-MB-231, PC3. Peptide binding within tumors was correlated with tumor response to therapy.

Results
Tumors treated with Sutent and irradiation showed that peptide binding within tumor microvasculature by immunohistochemistry. NIR imaging with Cy7 conjugated HVGGSSV peptide maintained imaging and provided a more rapid clearance of peptide from the circulation as compared to intact phage peptide. Labeled peptide detected response to all TKIs including PTK787, AEE788 SU5416 and Sutent. Peptide also detected responsiveness in all tumor models including D54 glioma in the brain, H460 in the lung, and PC3 within the prostate of mice. The sensitivity of detecting response to tumors was 30 of 30 responding mouse tumors detected by the HVGGSSV-Cy7 peptide conjugate. In comparison, 0 of the 15 non-responding tumors showed peptide binding (p<0.001).

Conclusions
Rapid non-invasive assessment of the pharmacodynamic response with in cancer promises to speed drug development and minimize the duration of treatment with ineffective regimens in cancer patients. Phase I clinical trials investigating the sensitivity and specificity of the HVGGSSV peptide in cancer patients is planned.
DIFFERENTIAL EXPRESSION OF MICRORNAS IN THE TRANSFORMATION OF FOLLICULAR LYMPHOMA TO LARGE B-CELL LYMPHOMA

SA McClintock-Treep, S Liang, JD Hallock, MP Robinson, MA Thompson

Background
MicroRNAs (miRNAs) regulate mRNA expression via binding to specific regions of mRNAs, causing post-transcriptional repression. MiRNAs are often encoded in chromosomal regions mutated in lymphomas. Follicular lymphoma (FL) is generally low grade; however in a subset of patients transformation to large B cell lymphoma (LBCL) occurs. In this study we analyze miRNA expression differences between FL and LBCL arising in the same patient. Demonstration of a role for miRNA will further our understanding of the process of transformation and will suggest possible new therapies.

Design
VUMC patients diagnosed with both FL and LBCL during their disease course were identified, and the diagnosis was verified by cytogenetics and/or immunophenotype. H&E-stained sections were reviewed for uniform grade and histology. The most uniform paired sample of low grade FL and LBCL was selected for testing. MiRNA was extracted from paraffin sections using the Ambion RecoverAll mRNA isolation kit. MiRNA quality was assessed by TaqMan reverse transcriptase real time PCR (RT-PCR) for ubiquitous miRNAs. Changes in miRNA expression in the paired sample were assessed in duplicate by TaqMan LowDensity Array (TLDA) miRNA panels (ABI) in which simultaneous quantitative RT-PCR of 365 human miRNAs is performed.

Result
Using ABI RQ Manager software, the TLDA raw data was normalized to the internal control (RNU48) and the quality of individual reactions was verified. 201 miRNAs amplified in one or both samples. The relative quantity of each miRNA in the paired samples was calculated from the Ct. 38 miRNAs showed at least a 5-fold decrease in expression in the LBCL sample compared to the FL sample, whereas only 3 showed at least a 5-fold increase. The 6 miRNAs with the largest difference in expression and with the lowest Cts were selected for confirmatory RT-PCR. Of the 5 miRNAs expressed at greater levels in FL, the corroborative reactions showed differences of 2.2-fold to 89-fold (median 15). The miRNA expressed at a higher level in LBCL showed a less than 2-fold difference in the corroborative reaction.

Conclusion
Global assessment of changes in miRNA expression in low grade FL vs. LBCL by TLDA technology demonstrates significant reduction in expression of 38 miRNAs during transformation. Reduction in miRNAs targeting oncogenes may increase their expression, contributing to the transformation process.
Electrophilic Cyclopentenone Neuroprostanes are Anti-Inflammatory Mediators Formed During Peroxidation of the Omega-3 Fatty Acid Docosahexaenoic Acid

Erik Musiek, M.D., Ph.D.

Objectives
Omega-3 fatty acids have been shown to exert beneficial effects in numerous inflammatory disease states, including atherosclerosis, Alzheimer’s disease, and rheumatoid arthritis. Docosahexaenoic acid (DHA) is the most abundant omega-3 in fish oil and is known to possess potent anti-inflammatory and cytoprotective effects. However, the mechanisms by which DHA inhibits pathogenic inflammation are poorly understood. DHA readily undergoes oxidation to form electrophilic molecules term A4-neuroprostanes, which are similar in structure to other anti-inflammatory lipids. We hypothesized that oxidation to yield bioactive A4-NPs may mediate the bioactivity of DHA.

Results
Following a 20-minute preincubation, oxidized DHA (oxDHA) potently suppressed LPS-induced nitrite accumulation and NF-kB activation, while unoxidized DHA was inactive. The potency of oxDHA correlated with the amount of A4-NP formed during oxidation. Synthetic A4-NP suppressed LPS-induced iNOS and COX-2 expression via inhibition of NF-kB activation, which occurred through inhibition IkappaBalpha phosphorylation by IKappa kinase. Chemical manipulations which abrogated A4-NP bioactivity (reduction, glutathione conjugation) also blocked the effects of oxDHA. Finally, biologically relevant concentrations of A4-NPs were identified in human brain samples, and A4-NP concentrations were elevated in patients with Alzheimer’s disease.

Methods
Novel LC/tandem MS and GC/MS methods were employed to monitor the formation of A4-NPs in oxidized DHA. The effects of synthetic A4-NP and oxidized DHA on NF-kappaB signaling and pro-inflammatory cytokine release were examined in cultured macrophages and RAW 264.7 cells. The metabolism of DHA was also examined using LC-MS techniques in vitro and in human samples.

Conclusions
The oxidation of DHA greatly augments its anti-inflammatory activity in parallel with the formation of A4-NPs, and chemical elimination of A4-NPs abrogate the effects of DHA. Further, synthetic A4-NPs potently suppress NF-kappaB activation and inflammatory cytokine production in macrophages. These results suggest that A4-NPs play a critical role in the anti-inflammatory mechanism of DHA. The elucidation of the anti-inflammatory mechanisms of omega-3 FAs provides new possibilities for the development of novel anti-inflammatory therapeutics.
POOR GLUCOSE CONTROL IN ADOLESCENTS WITH TYPE 1 DIABETES IS ASSOCIATED WITH INCREASED IL-8 BUT REDUCED IGF-1

BJ Van Sickle MD/PhD, JH Simmons MD, R Hall, M Raines, KD Ness MD and A Spagnoli MD

Dysregulation of the growth hormone (GH)-insulin-like growth factor 1 (IGF-1) axis is well-described in adolescents with Type 1 Diabetes (T1DM). Reduced IGF-1 in patients with poor glycemic control is associated with growth retardation and low bone mineral content. Acute and chronic hyperglycemia increase inflammatory cytokines, and IGF-1 is decreased in chronic inflammatory diseases. Therefore inflammatory cytokines may link poor glycemic control with changes in the GH-IGF-1 axis.

Objective
Evaluate the relationship between inflammatory cytokines and IGF-1 in adolescents with poorly-controlled T1DM

Methods
Serum cytokines and IGF-1 were measured in fasting blood samples from adolescents (age 13-18) with T1DM in poor (n=17) and favorable (n=19) glucose control. Poor control (PC) was defined as 3 or more consistent HbA1C > 9% during the previous 2 years. Favorable control (FC) was defined as all HbA1C < 9%. Patients had DM for at least 3 years and were without complications or celiac disease. Data were analyzed by MANOVA and Spearman’s correlation with statistical significance set at p<0.05. Data are mean ± SD.

Results
Groups were similar in gender distribution, BMI, age and duration of DM. HbA1C was 7.5 ± 0.6% in the favorable group, 10.5 ± 0.9% (p<0.001) in the poor control group. IL-8 was significantly elevated in the poor control group (PC: 7.4 ± 4.3 pg/ml, FC: 3.7 ± 4.0 pg/ml; p=0.01) with a trend toward higher MCP-1 (PC: 79.8 ± 82.0 pg/ml; FC: 42.4 ± 38.5 pg/ml; p=0.07). There were no differences for TNF-α or IL-10. IGF-1 was significantly lower in subjects with poor glucose control (PC: 408.9 ± 157.1 ng/ml; FC: 536.5 ± 164.3 ng/ml; p=0.03). Moreover, IL-8 was inversely correlated with IGF-1 (r=-0.40, p=0.03) and positively correlated with HbA1C (r=0.36, p=0.03), MCP-1 (r=0.39, p=0.02) and IL-10 (r=0.46, p=0.005).

Conclusions
Poor glycemic control in adolescents with T1DM is associated with increased IL-8 and reduced IGF-1. The relationship between IL-8 and IGF-1 suggests poor glycemic control may promote systemic inflammation that alters the GH-IGF-1 axis. The mechanism by which inflammation may alter the GH-IGF-1 axis remains to be determined.

Support
Genentech Center for Clinical Research in Endocrinology Grant 305-C02R
VICTR grant 1UL1 RR024975 from NCRR/NIH.
LITHIUM PROTECTS HIPPOCAMPAL CELLS BY ENHANCING DNA-PK-DEPENDENT NONHOMOLOGOUS END JOINING OF RADIATION-INDUCED CHROMOSOMAL BREAKS

Eddy S. Yang, MD, PhD, Hong Wang, MD, Dennis E. Hallahan, MD, and Fen Xia, MD, Ph.D.

Purpose/Objective(s)
Long-term neurological deficiencies resulting from cranial radiation-induced hippocampal cytotoxicity presents a formidable challenge in the treatment of primary and metastatic brain cancers, especially in young children. We have previously shown that lithium protects hippocampal neurons from radiation-induced apoptosis and improves neurocognitive function in treated mice. It is well-established that the most critical lesion induced by radiation involves chromosomal double-strand breaks (DSBs). In this study, we demonstrate a novel role of the non-homologous end joining (NHEJ) DNA DSB repair pathway but not homologous recombination repair (HR) in lithium-mediated protection of mouse hippocampal neurons.

Materials/Methods
Immunohistochemistry was performed on irradiated mouse primary hippocampal neurons, HT-22 hippocampal cells, hippocampal tissue sections from irradiated mice, and GL261 glioma cancer cells with or without lithium prophylaxis. % of cells with γ-H2AX foci (indicative of DNA double strand breaks), DNA-PK T2609 foci (indicative of NHEJ), and Rad51 foci (indicative of HR) were assessed. Activation of apoptosis as revealed by cleaved caspase 3 following irradiation with or without lithium prophylaxis in the presence of the DNA PK inhibitor 1-(2-Hydroxy-4-morpholin-4-yl-phenyl)-ethanone (IC86621) was also examined.

Results
Lithium treatment of mouse primary hippocampal neurons and HT-22 hippocampal cells increases by almost 2 fold radiation-induced DNA-PK Thr2609 foci, a well-characterized surrogate marker for activated NHEJ repair. This coincides with marked reduction (up to 17.5 fold) of radiation-induced γ-H2AX foci, robust in situ markers of DSBs. These findings are confirmed in vivo in irradiated mice. On the contrary, lithium minimally affects radiation-induced Rad51 foci in these model systems. Furthermore, the DNA-PK inhibitor 1-(2-Hydroxy-4-morpholin-4-yl-phenyl)-ethanone (IC86621) abrogates lithium-mediated protection. Importantly, none of these findings are evident in the mouse glioma cancer cell line GL261.

Conclusions
Our results strongly support our hypothesis that lithium protects hippocampal neurons by promoting the NHEJ repair pathway. These findings warrant our current clinical investigations of lithium-mediated neuroprotection during cranial irradiation, especially in the pediatric population. Furthermore, the use of DNA-PK inhibitors as radiosensitizers should be approached with caution, as they may inhibit the ability of normal cells to repair radiation-induced DNA damage.
BASIC SCIENCE RESEARCH

ABSTRACTS

(alphabetically by presenter’s last name)
2008 Basic Science Research Abstracts

19. Madhumita Ananthakrishnan, MD Fellow, Pediatrics
20. Sameer Chopra, AB, AM MSTP
21. Ildiko Csiki, MD, PhD Resident, Medicine
22. Kyle Cuneo Resident, Radiation Oncology
23. Roberto Diaz, MD, PhD Resident, Radiation Oncology
24. Leslie Gewin, MD Clinical Fellow, Medicine
25. Doha Itani, MD Resident, Pathology
26. Jerry Jaboin, MD, PhD Resident, Radiation Oncology
27. Asher Kupperman, BS VMS II, Class of 2010
28. Brandon Litzner VMS II, Class of 2010
29. Daniel Lustig, MD, MA Clinical Fellow, Pediatrics
30. Julia McHugh, BSE MSTP
31. Kenneth Niermann, MD Resident, Radiation Oncology
32. Kaartiga Sivanesan, AB VMS II, Class of 2010
33. Christopher Willey, MD, PhD Resident, Radiation Oncology
34. Shi-Hsin Eddy Yang, MD, PhD Resident, Radiation Oncology
35. David Yu, MD, PhD Resident, Radiation Oncology
L-CITRULLINE INHIBITS CHRONIC HYPOXIA-INDUCED PULMONARY HYPERTENSION IN NEWBORN PIGLETS

Madhumita Ananthakrishnan, M.D., Frederick E Barr, Marshall L Summar, Mark R Kaplowitz, Candice D Fike

Background
Decreased Nitric Oxide (NO) production is one of the proposed mechanisms for chronic hypoxia induced pulmonary hypertension. NO is produced from arginine by nitric oxide synthase. Citrulline is metabolized to arginine and may increase the amount of arginine available for pulmonary vascular NO production.

Hypothesis
Supplementation with L-citrulline will inhibit the development of pulmonary hypertension in piglets exposed to chronic hypoxia.

Methods
Two-day-old piglets were raised in hypoxia (n=15) or normoxia (n=5) for 10 days. Some (n=5) hypoxic piglets received oral L-citrulline twice a day (0.13 mg/kg/dose) for ten days. Piglets were anesthetized for placement of catheters to measure pulmonary artery pressure, left ventricular end diastolic pressure, cardiac output by thermodilution and exhaled NO concentration.

Results
Pulmonary artery pressure (PAP, cm H2O) and calculated pulmonary vascular resistance (PVR, cmH2O ml⁻¹ min⁻¹ kg⁻¹) were higher (p<0.001) in untreated hypoxic animals (PAP 39±3, PVR 0.13±0.01) than normoxic controls (PAP 21±3, PVR 0.04±0.01). In contrast, PAP and calculated PVR in citrulline treated hypoxic animals (PAP 27±1, PVR 0.07±0.004) did not differ from values in normoxic controls. Exhaled NO concentrations (parts per billion) in untreated hypoxic animals (1.5 ±0.2) were lower than normoxic controls (3.4±0.6) p<0.05; while exhaled NO levels in citrulline treated hypoxic piglets (2.5±0.4) were not different from controls.

Conclusions
L-citrulline increases NO production and inhibits the development of pulmonary hypertension in piglets exposed to 10 days of chronic hypoxia. We speculate that enteral supplementation with L-citrulline may limit pulmonary vascular changes in neonates who have prolonged periods of hypoxia.
NON-ELECTROGENIC REQUIREMENTS FOR VOLTAGE-GATED SODIUM CHANNELS IN ZEBRAFISH HEART DEVELOPMENT

SS Chopra, H Watanabe, T Yang, L Batts, DM Stroud, HS Baldwin, CG Burns, S Wells, TP Zhong*, DM Roden* (*co-senior investigators)

Objectives
The voltage-gated sodium channel Na,1.5 (encoded by SCN5A) is required for the initiation and propagation of action potentials in the human heart. Mutations in SCN5A are well-recognized in congenital arrhythmia syndromes. While Na,1.5 is critical to the normal function of the mature heart, the role of sodium channels in the developing heart is unknown.

Methods
The role of Na,1.5 in cardiac development was investigated using pharmacological, genetic, and imaging approaches in zebrafish embryos.

Results
We identified two conserved zebrafish SCN5A genes that are expressed in early embryos and in the developing heart. Knockdown of zebrafish cardiac sodium channels using antisense morpholino oligonucleotides resulted in reduced expression of the myocardial precursor genes nkx2.5, hand2, and gata4, diminished production of cardiac progenitor cells, and marked perturbation of cardiac chamber morphogenesis and looping. Unexpectedly, prolonged exposure of early embryos to drugs that block sodium currents failed to perturb development, suggesting that sodium channel-mediated electrical activity is not required for early cardiogenesis. Cellular transplantation studies revealed that sodium channels act via distinct mechanisms to regulate both cardiac cell fate specification and the viability and proliferative growth of embryonic cardiomyocytes. Both developmental roles preceded any requirement for sodium channels in myocardial impulse propagation.

Conclusions
Voltage-gated sodium channels have evolved multiple roles in the vertebrate heart: in addition to acting as the principal regulators of heart rhythm, sodium channels perform previously-unrecognized, non-electrogenic functions during heart development.
VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR BLOCKADE LEADS TO IMPAIRED HEMATOPOIETIC RECOVERY AFTER MYELOSUPPRESSION

Ildiko Csiki, MD, PhD, Sergey Novitsky, PhD, Mikhail Dikov, Phd, Alan Sandler, MD, David H. Johnson, MD, David P. Carbone, MD, PhD

Objectives
Bevacizumab, a monoclonal antibody against vascular endothelial growth factor (VEGF), has been shown to benefit patients with a variety of cancers. Recently, the addition of bevacizumab to paclitaxel plus carboplatin in the treatment of selected patients with non-small-cell lung cancer has been shown to lead to a significant survival benefit along with increased risk of treatment-related deaths such as infection due to myelosupression. The purpose of this study was to elucidate the mechanism of combination chemotherapy and bevacizumab induced myelosupression and to examine the role of VEGF and its receptors in the proliferation of hematopoietic progenitor cells (HPCs). As VEGF receptors play important role in hematopoietic recovery after myelosupression, we hypothesized that combination of chemotherapy and anti-VEGF receptor treatment may cause delayed hematopoietic recovery and neutropenia in patients.

Methods
Through a retrospective analysis of patients receiving bevacizumab in combination with different chemotherapeutic agents for various cancers compared to matched patients receiving chemotherapy alone, we observed that bevacizumab in combination with chemotherapy significantly delays repopulation of white blood cells (WBC) including the neutrophils. Furthermore, we examined the combination effect of myeloablative agent and VEGF receptor tyrosine kinase inhibitors (VEGFR TKI) on the proliferation of HPCs in-vivo and found that wild-type mice treated with chemotherapy and VEGFR TKI as well as knock-out mice for VEGFR treated with chemotherapy had significantly delayed repopulation of WBC and impaired proliferation of HPCs.

Conclusions
When present, VEGF increases the cell cycling of bone marrow cells and the proportion of HPCs in the bone marrow. These results help explain chemotherapy plus bevacizumab related toxicity and aid in our understanding of the role of VEGF and its receptors in angiogenesis and hematopoiesis.
SUNITINIB SENSITIZES PANCREATIC CANCER TO THE CYTOTOXIC EFFECTS OF IONIZING RADIATION

Kyle C Cuneo, Ling Geng, Allie Fu, Darren Orton, Dennis E Hallahan, Bapsi Chakravarthy

Objectives
Sunitinib (SU11248) is a small molecule tyrosine kinase inhibitor which targets VEGFR and PDGFR isoforms. In the present study the effects of SU11248 and ionizing radiation on pancreatic cancer were studied.

Methods
For in vitro studies human pancreatic adenocarcinoma cells lines were treated with 1 µM SU11248 one hour prior to irradiation. Western blot analysis was used to determine the effect of SU11248 on radiation induced signal transduction. To determine if SU11248 sensitized pancreatic cancer to the cytotoxic effects of ionizing radiation a clonogenic survival assay was performed using 0-6 Gy. For in vivo assays, CAPAN-1 cells were injected into the hind limb of nude mice for tumor volume and proliferation studies.

Results
SU11248 attenuated radiation-induced phosphorylation of Akt and ERK at 0, 5, 15, and 30 minutes. Furthermore, SU11248 significantly reduced clonogenic survival following treatment with radiation (P<0.05). In vivo studies revealed that SU11248 and radiation delayed tumor growth by six and ten days, respectively, whereas combined treatment delayed tumor growth by thirty days (figure below). Combined treatment with SU11248 and radiation further attenuated Brdu incorporation by 75% (P=0.001) compared to control.

Conclusions
Sunitinib sensitized pancreatic cancer to the cytotoxic effects of radiation. This compound is promising for future clinical trials with chemoradiation in pancreatic cancer.
RAPID RECOGNITION OF GLIOMA RESPONSE TO RADIATION THERAPY USING RECOMBINANT PEPTIDES

Roberto Diaz, Ralph J. Passarella, Hongmei Wu, and Dennis E. Hallahan

Objectives
This study assessed the effectiveness of a recombinant peptide at rapidly and non-invasively determining glioma response to radiation therapy within the endothelium of treated tumors.

Methods
Phage-displayed peptide libraries were subjected to biopanning in vivo on GL261 brain tumor xenografts that were treated with 40 mg/kg Sunitinib and 3 Gy of ionizing radiation (IR). After 4 rounds of biopanning in heterotopic tumors and 2 rounds of biopanning in orthotopic tumors, phage peptide recovered from the 6th selection was sequenced to determine identity of phage peptide bound to tumor endothelium specific for Sunitinib+IR. Sensitivity and specificity of peptide detection of tumor susceptibility to IR were studied with immunohistochemical (IHC) staining and near infrared resonance (NIR) imaging. Biological effects of peptide binding in the tumor were assessed by tumor size measurement and histological examination of apoptosis.

Results: Via sequencing analysis of phage recovered from the in vivo biopanning of the T7 library GIRLRG was isolated most frequently (43.75%) along with AARLY (37.5%) and HMWRDSQ (18.75%). NIR imaging was used to monitor the biodistribution of the GIRLRG free peptide in glioma-bearing mice treated with or without IR for 5 consecutive days. At the end of treatment, we found that IR elicited a 24% decrease in tumor volume over the untreated control (p<0.05, Figure 1) and that the GIRLRG peptide showed a > 2.5 fold increase in radiance compared to the untreated controls (p<0.05). IHC staining of the gliomas showed that the GIRLRG peptide binds within treated tumor endothelium but not within untreated tumors.

Conclusions
GIRLRG peptide binds specifically to the vascular endothelium of radiation-treated glioma. Conjugation of the peptide to near infrared imaging agents can be used to show that GIRLRG binds more specifically to IR treated glioma than to untreated glioma, such that delay in tumor growth due to IR treatment correlates to more intense peptide binding. Eventually this could lead to the peptide’s conjugation to a nanoparticle-drug complex and result in radiation targeted drug delivery.
DELETING TRANSFORMING GROWTH FACTOR-β TYPE II RECEPTOR INCREASES RENAL FIBROSIS

L. Gewin and R. Zent

Objectives
Renal fibrosis, the final common pathway of end stage kidney diseases, is primarily characterized by increased collagen deposition in the tubulointerstitium. Transforming Growth Factor-β (TGF-β) plays a critical role in renal fibrosis progression by promoting collagen and other extracellular matrix (ECM) production as well as by inducing epithelial to mesenchymal transformation (EMT). Therefore, disrupting TGF-β signaling in the tubular epithelium should decrease interstitial fibrosis by reducing epithelial collagen production and impairing EMT. As TGF-β requires the TGF-β type II receptor (TβRII) to transduce its effects, we hypothesized that deleting TβRII in the renal collecting system would reduce fibrosis following injury by both abrogating TGF-β signaling and inhibiting EMT.

Methods
Mice lacking the TβRII in the collecting system were generated utilizing the cre/lox technique, and unilateral ureteral obstruction was performed to induce tubulointerstitial fibrosis. Fibrosis was assessed by measuring collagen using immunohistochemistry and immunoblots. IMCD cells were isolated from TβRII mice, and the receptor was deleted in vitro. Active TGF-β in the conditioned media of the WT and null IMCD cells was determined using the PAIL bioassay. EMT was assessed in vitro by determining E. cadherin expression and localization before and after exposure to TGF-β.

Results
There was increased tubulointerstitial fibrosis, characterized by increased collagen expression, in mice lacking TβRII. TβRII null IMCD cells did not undergo EMT and expressed more active TGF-β in the conditioned media compared to that of WT cells.

Conclusions
Contrary to our initial hypothesis, deletion of TβRII in the collecting ducts increased tubulointerstitial fibrosis following injury despite inhibition of EMT in vitro, suggesting that EMT might not play a significant role in mediating the interstitial fibrosis. We hypothesize that the increased fibrosis occurs in the mice lacking TβRII in the collecting system because these cells create an environment of increased active TGF-β that induces fibroblasts with intact TβRII expression to produce more collagen.
PROTEOMIC EVALUATION OF OSTEOSARCOMA: MACROPHAGE MIGRATION INHIBITORY FACTOR AND TUMOR NECROSIS FACTOR-α ARE UPREGULATED IN A HIGHLY METASTATIC MURINE CELL LINE

JMCates1, DMItani1, BWWhited2, WDDupont3, Algonzalez1, NEMuscato1, GEHolt2, HSSchwartz2 and RLCaldwell2

Background
Approximately one-third of patients with osteogenic sarcoma (OGS) die from lung metastasis within 5 years of diagnosis. Molecular signatures that predict pulmonary metastasis from primary OGS and identify those patients at risk would be clinically useful as prognostic markers.

Design
Protein expression profiles of two clonally related murine OGS cell lines with low (K12) and high (K7M2) metastatic potential were compared using two different proteomic technologies [two-dimensional difference electrophoresis (2D-DIGE) and tissue profiling by matrix-assisted laser desorption/ionization mass spectrometry (MALDI) to detect candidate proteins that predict metastatic potential. Two such proteins were subsequently validated by western blotting and immunohistochemistry (IHC) analysis of a tissue microarray (TMA) containing 114 cases of primary human OGS, 56 of which either presented with or developed metastatic disease and 25 of which resulted in death.

Results
Comparison of the proteomic profiles of K12 and K7M2 cells revealed distinct differences in protein expression. Further molecular network analysis suggested several candidate molecules that potentially predict OGS metastasis to lung, including macrophage migration inhibitory factor (MIF) and tumor necrosis factor-α (TNF-α). Western blotting confirmed increased expression of both molecules in lysates of K7M2 cells compared to K12 cells. IHC of human OGS TMA demonstrated that MIF and TNF-α levels are increased in high grade compared to low grade tumors (t-test, p=0.0029 and p=0.257, respectively). However, log rank tests disclosed no significant differences in disease-free survival. IHC scores were not statistically different between patients who developed local recurrence or distant metastasis and those who did not. No significant changes in the levels of MIF or TNF-α occurred after administration of adjuvant chemotherapy or tumor progression.

Conclusion
Proteomic analysis of murine OGS cell lines disclosed several potential markers of pulmonary metastasis. Although upregulation of these candidate biomarkers for OGS metastasis was validated in vitro, IHC of human OGS TMA did not confirm these findings. These results demonstrate the necessity of validation studies in the evaluation of molecular markers derived from animal studies using proteomic techniques.
RADIATION-TARGETED DRUG DELIVERY SYSTEM TO PROSTATE CANCER

Jerry Jaboin, Allie Fu, Ghazal Hariri, Zhaozhong Han, Dennis E. Hallahan

Purpose
We studied a novel radiation-guided drug delivery system in various mouse models of prostate cancer. Peptides that bind to radiation inducible receptors in prostate cancer were used to delivery nanoparticle drug delivery.

Methods
Phage displayed peptide libraries were utilized to identify peptides that bind within irradiated prostate cancer. This in vivo biopanning process is illustrated in Figure 1. We identified the HVGGSSV peptide as the predominant ligand that binds within prostate cancer models in mouse (Fig. 2). This peptide was conjugated to nanoparticles to study the pharmacokinetics and biodistribution in mouse models of prostate cancer. Prostate specific conditional PTEN transgenic mice that develop prostate tumors over the course of five months were studied for tumor drug delivery. We also utilized orthotopic tumor models with PC3 tumors injected into the prostate of nude mice which develop over the course of 2 weeks. In addition, we studied the subcutaneous heterotopic prostate cancer tumors injected into the hind limbs of mice. HVGGSSV peptide was conjugated at the aminoterminus to nanoparticles labeled with near infrared imaging, fluorochrome and with radionuclides. Biodistribution and pharmacokinetics were studied in the space of the Xenogen in vivo imaging system and gamma camera imaging. Prostate tumors were treated with 3 Gy to activate ligand binding to receptors. Peptide-conjugated liposomes were injected by jugular vein.

Results
Microscopic imaging of nanoparticle binding within tumor microvasculature showed binding within four hours of radiation and persistent binding of nanoparticles within prostate cancer for nine days (Fig. 3). In contrast, prostate tumors that were not treated with radiation showed no drug delivery. The nanoparticles labeled with NIR fluourochromes were imaged by Xenogen IVIS which showed specific binding to nanoparticles to irradiated orthotopic (Fig. 4), heterotopic prostate tumors (Fig. 5) and a prostate-specific PTEN transgenic mouse (Fig. 6). A 90-fold increase in nanoparticle binding to prostate tumors over that of background normal organ binding was observed (p=0.001). Radiolabeled nanoparticle cleared from circulation within 24 hours and persistent binding within the irradiated prostate tumors was observed for over nine days. To verify binding within prostate tumors, the prostate was resected and imaged by NIR or gamma camera imaging (Fig. 4). This showed specific binding within prostate tumors with no activity within bladder, intestine or other organs (Fig. 4).

We conjugated Stealth liposomes (Fig. 7) to our peptide (HVGGSSV) and a control scrambled peptide for tumor delivery (Fig. 8). The HVGGSSV-tagged liposomes were able to bind selectively to irradiated tumor vasculature, but not to sham-irradiated tumors. The scrambled peptide-bound liposome was unable to bind irradiated tumors (Fig. 8). We added doxorubicin to the liposomal conjugate, and studied the pharmacokinetics of the HVGGSSV-tagged liposomes. We determined that the HVGGSSV-tagged liposomes were able to accumulate in irradiated tumors at a much higher rate than in nonirradiated tumors. In addition, they were able to deliver more doxorubicin to tumors than a similar dose of free doxorubicin (Fig. 9).

Conclusions
The HVGGSSV peptide targets radiation-induced neoantigens on irradiated prostate tumors (heterotopic, orthotopic or murine transgenic animal models). The Stealth liposome can be successfully conjugated to the HVGGSSV peptide without loss of tumor specificity. In addition, loading of doxorubicin to this nanoparticle allows for increased tumor drug delivery over free drug.
BVES REGULATES AQUEOUS FLOW IN THE TRABECULAR MESHWORK THROUGH ITS ROLE IN TIGHT JUNCTION FORMATION AND CELLULAR CONTRACTION

Asher Kupperman¹, Patricia Russ², Frederick Haselton², Min Chang³

Objectives
Aqueous outflow in the trabecular meshwork (TM) is altered by tight junctions (TJ) and cellular contraction. TJ formation and cell contraction lead to decreased outflow. We have previously shown that Blood Vessel Epicardial Substance (Bves) increases TJ formation and decreases Rho activation in epithelial cells. We hypothesize that Bves in TM plays a role in regulating aqueous outflow by modulating both TJ formation and cellular contraction, a downstream target of activated Rho.

Methods
TM cell lines from normal (NTM) and glaucomatous (GTM) donors, as well as NTM cells stably transfected to overexpress Bves (NT-w), were evaluated by Western blots to determine levels of TJ protein ZO-1 and phosphorylated myosin light chain (pMLC). pMLC is an indicator of cellular contraction that is a downstream target of Rho. Immunofluorescent (IF) localization of Bves and ZO-1 was also performed. In addition, diffusion of sodium fluorescein in a two-chamber assay was measured to assess paracellular flow.

Results
Western blots demonstrated that GTM cells had increased Bves and ZO-1 levels compared to NTM cells. Bves and ZO-1 co-localized to the cell border as shown by IF. Functionally, GTM cells showed decreased permeability compared to NTM cells in the two-chamber assay. NT-w cells (stably overexpressing Bves) exhibited an increase in ZO-1 levels and a decrease in permeability similar to GTM cells. These findings indicate that increased Bves correlates with increased TJs and decreased TM permeability. We measured pMLC to determine Bves’ effect on cellular contraction. NT-w cells showed lower levels of pMLC compared to NTM cells, consistent with decreased Rho activation and cell contraction. GTM cells showed pMLC levels similar to NTM cells, suggesting that diseased GTM cells have an alteration in the Rho pathway.

Conclusions
These studies indicate that Bves is an important modulator of TM TJ formation and cellular contraction. Increased levels of Bves lead to increased TJs and decreased pMLC. Increased TJs lead to decreased flow and decreased pMLC leads to increased flow. Our findings suggest that Bves is an important homeostatic regulator of aqueous flow through its dual, opposing effects on outflow, and that alterations in this pathway may lead to dysregulation of intraocular pressure.
ENDOTHELIN-1 REGULATES MITF EXPRESSION AND FUNCTION THROUGH THE MAP KINASE AND PROTEIN KINASE A PATHWAYS

Litzner, BR,¹ Scheschuk, JP² and Burnett, PE²

Objectives
The endothelins (ETs) are a family of molecules composed of three 21 amino acid peptides: ET-1, ET-2 and ET-3. They produce their physiologic effect by binding to two different G-protein coupled receptors (GPCRs), EdnR and EdnRB. Interest in their role in the skin came when studies demonstrated that Waardenburg’s syndrome (a syndrome involving hypopigmentation and hearing deficits) is caused by mutations in ET-3 or EdnRB, as well as the transcription factor MITF. This observation suggested that signaling through the ET pathway was necessary for melanocyte viability during development. It was later found that the ETs and their receptors played a significant role in multiple human cancers, including melanoma. ET has been shown to induce proliferation, chemotaxis and pigment production in adult melanocytes. In addition, the receptor, EdnRB, is highly upregulated in melanocytic tumors and can be used as a marker of tumor progression. The ET-1/EdnRB pathway has been shown to activate p42/p44 MAP kinase and activation of this pathway is believed to be a critical step in the development of melanoma. Although the importance of the ET pathway has been demonstrated in melanocytes, relatively little is known about the molecular mechanisms underlying these interactions. Our study focuses on these mechanisms that underlie melanocyte proliferation and differentiation.

Methods
We studied these pathways using normal human epidermal melanocytes in cell culture. The downstream effects of activating or inhibiting various segments of the pathway were assayed using reverse transcriptase PCR and western blot analysis.

Results and Conclusions
We were able to demonstrate that ET-1, signaling through EdnRB, in human melanocytes leads to protein kinase C activation and eventually to phosphorylation and activation of MITF, a transcription factor known to be the key regulator of melanocyte differentiation and proliferation. In addition, we show activation of protein kinase A, which phosphorylates CREB, leading to transcriptional upregulation of MITF. In addition, ET-1 activates p38 and a phosphatase that inhibits p42/p44 MAPK activation and MITF phosphorylation.

In summary, ET-1 signaling is characterized by a proliferative stimulus via PKC, p42/p44 MAPK and phosphorylation of Mitf. The proliferative effect is regulated by the activation of the PKA pathway that dampens this response and favors differentiation. Our increased understanding of this pathway in normal melanocytes offers new insight into a potential role for endothelin signaling in melanoma formation and metastasis.
THE NATURAL PROGRESSION OF FATTY LIVER IN THE DIET-INDUCED OBESE MOUSE

Lustig, D; Berglund, E; Correa, H; Damon, B; Wasserman, D

Non-alcoholic fatty liver disease (NAFLD) is fast becoming the most common cause of liver disease in the preadolescent and adolescent age groups. There has been an increase in the prevalence of fatty liver disease that correlates with the dramatic rise in obesity observed in the pediatric population during the past three decades. In some individuals, fatty liver can stimulate hepatic inflammation and progress to steatohepatitis. It is important to study fatty liver prior to the development of an inflammatory response and steatosis.

Objectives
We endeavored to elicit the chronologic progression of fatty liver using a common mouse model.

Methods
We placed C57Bl/6J mice on high fat (35.5% by wt) or chow (5% by wt) diet. At selected time points (2, 4, 8, 12, and 20 weeks after weaning to diet), the liver of these mice were imaged using serial magnetic resonance imaging (MRI). The mice were sacrificed the day of scanning. Liver was obtained and analyzed, then, three different ways: 1) MRI, 2) histologically by a pathologist, and 3) biochemically via high performance thin layer chromatography (HPTLC). Currently we are attempting to elicit the predominant fatty acid in steatosis by measuring inflammatory markers from liver tissue at time points; namely tumor necrosis factor-α (TNF-α).

Results
MRI data revealed insignificant change in hepatic steatosis until weeks 12 and 20 when steatosis increased 174% and 662% respectively when compared to chow controls. Using HPTLC quantification of hepatic lipids as the gold standard, comparison with MRI data was found to closely correlate ($r^2$: 0.8), while visual histologic quantification by a staff pathologist consistently overestimated fat content ($r^2$: 0.6). No fibrosis was appreciated at any time point. HPTLC was used to separate hepatic lipids into four subsets; triglycerides (TG), diglycerides (DG), cholesterol esters (CE), and phospholipids (PL). As expected, the fat fed mice became increasingly steatotic, noting changes as early as 8 wks with increases in lipids (in μg/mg); CE (0.65 vs 0.33), DG (1.87 vs 0.76), and most significantly TG (16.0 vs 5.6) compared with chow mice. Interestingly, PL showed a mild decrease overall in HF mice by 20 wks (21.1 vs 27.3).

Conclusions
MRI combined with HPTLC allows the progression of fatty liver disease to be accurately determined.
THE PRESSOR EFFECT OF WATER: AN ANIMAL MODEL


Water exerts a robust pressor response in patients with autonomic dysfunction and to a lesser degree in older normal subjects. However, little is known about the mechanism by which water exerts this effect. We have developed a mouse model using sinoatrial denervation (SAD) to eliminate the baroreflex to more closely mimic human autonomic dysfunction. Using this model, we observed an increase in systolic blood pressure (SBP) of 15.0 +/- 0.1 mmHg (p<0.0001) appearing 3 minutes after water infusion and peaking at 15-25 minutes. Infusion of an identical volume of physiological saline showed an increase SBP of 3.4 +/- 0.1 mmHg (p<0.0001). In non-SAD mice, infusion of water resulted in an immediate pressor response different in nature than that observed in SAD mice and in patients with dysautonomia. The increase in SBP observed in non-SAD mice occurred over a significantly shorter time course, with SBP returning to baseline 12 minutes post infusion. The effects observed after water infusion in SAD and non-SAD mouse models match well with those observed in dysautonomic patients and normal subjects, respectively, with the pressor effect only in cases where the autonomic nervous system has been impaired. In an effort to uncover the efferent arm of the water response, mice were given 0.5 mg/kg prazosin i.p. 20 minutes prior to infusion of water. Mice given prazosin showed an increase in SBP of 3.6 +/- 0.1 mmHg (p<0.0001), a much reduced response compared to mice given water alone. The lack of a pressor response in mice given saline suggests that osmolality may be the stimulus for the observed response. The greatly attenuated response in mice given prazosin suggests that the increase in SBP is through an α-1 adrenergic mechanism. Our SAD mouse model has proved to be useful in studying the pressor effect of water and we will continue to use this model to gain a better understanding of the mechanisms behind this unexpected response elicited by water.

The effect of intraduodenal (blue) water or saline (pink) on blood pressure.

WATER (n=8)
SALINE (n=6)
ENZASTAURIN: RADIOSENSITIZATION FOR LUNG CANCER

Kenneth J. Niermann, M.D., Christopher Willey, M.D., Ph.D., Dakai Xiao, Ph.D.
Tianxang Tu, M.D., Bo Lu, M.D., Ph.D.

Objectives
Most lung cancer patients present with locally advanced and disseminated disease. Despite advances in concurrent chemoradiation strategies, long term survival is poor. Enzastaurin (ENZ), a selective inhibitor of PKC-β, has demonstrated broad application in preclinical cancer models, but little is known about its interaction with radiation. The purposes herein was to characterize the radiosensitizing effects of ENZ on lung cancer.

Methods
Lung cancer cell lines A549, H460, and H661, and human umbilical vein endothelial cells (HUVEC) were pre-treated with 1-2 μM ENZ prior to radiation treatment. They were analyzed for clonogenic cell survival, western blotting for phosphorylated GSK3 and s6, and MTT mitochondrial cell viability. HUVEC were analyzed for tubule formation and cell gap migration.

Results
ENZ effects on clonogenic cell survival. The clonogenic cell survival assays failed to show radiosensitization in the lung cancer cells, despite inhibition of PKC downstream molecules GSK3 and S6 by ENZ. All lung cancer cell lines demonstrated a decrease in cell proliferation when treated with high doses (>4 μM) of ENZ. The HUVEC cell line was acutely sensitive to even low doses of ENZ, displaying 40-60% relative reduction in cell proliferation.

Radiation-sensitizing ENZ inhibition of tubule formation and angiogenesis. HUVEC treated with ENZ alone demonstrated 12% reduction in tubule formation, with radiation alone resulting in a 38% reduction and combined radiation and ENZ yielding a 63% reduction (See figure). Furthermore, cellular migration was strongly inhibited in the HUVEC population subjected to both radiation and ENZ, with a lesser effect observed with either ENZ or radiation alone.

Conclusions
The effect of ENZ on lung cancer cells and its inhibition of PKC-β has important clinical implications. Interestingly, radiosensitization of lung cancer cells is not critically dependent on PKC activity. Instead, PKC appears to be crucial for vascular maintenance and angiogenesis. These studies provide a basis for studying the in vivo effects of ENZ in preclinical tumor models. A better understanding of the antiangiogenic and functional tumoricidal mechanisms by which ENZ works may lead to significant improvements in treatment strategies in the clinical approach to lung cancer.
ROLE OF MTP ISOFORMS IN LIPID DROPLET MATURATION IN ADIPOCYTES

Kaartiga Sivanesan, Dr. Larry L. Swift

Objectives
Microsomal triglyceride transfer protein (MTP) is a lipid transfer protein essential for the assembly of triglyceride (TG)-rich lipoproteins in the liver and small intestine. Our laboratory discovered MTP in mouse and human adipocytes, as well as in the pre-adipocyte 3T3-L1 cell line. Immunohistochemical studies suggest that MTP is abundant on the surface of small (<5μm) lipid droplets. Our studies have also shown that in mice MTP exists as two isoforms (MTP-A and MTP-B), with MTP-B predominating in adipocytes. The function and localization of MTP imply its importance in adipocyte lipid droplet maturation. We aimed to determine the role of MTP in lipid droplet maturation.

Methods
CHO cells, which do not express MTP, were transfected with MTP A or MTP B cDNA, and incubated with oleic acid to stimulate TG synthesis and lipid droplet formation. Cells were either fixed and stained with Nile Red to visualize lipid droplets or harvested with subsequent analysis of mRNA by RT-PCR, protein by immunoblot, and TG and phospholipid by gas chromatography.

Results
MTP A and B were expressed at similar levels in CHO cells. Expression of either isoform did not affect total cellular TG or phospholipid content. Lipid droplets in cells transfected with MTP B, but not A, were significantly larger (17%, p=0.000309) than control.

Conclusions
The finding of larger droplets in the presence of MTP-B suggests a critical role for this protein in lipid droplet maturation. We hypothesize that MTP enhances droplet maturation by facilitating fusion of small droplets.
PKCε REGULATES RADIATION INDUCED AKT ACTIVATION VIA MYRISTOYLATED ALANINE RICH C KINASE SUBSTRATE (MARCKS) IN VASCULAR ENDOTHELIUM
Christopher D. Willey, Tianxiang Tu, Vijay Rao, Kyle Cuneo, Bo Lu, Dennis E. Hallahan

Objectives
Tumor vascular endothelium likely contributes to radiation response in cancer. As signaling pathways involved in vascular endothelial survival are elucidated, therapeutic interventions can be designed to enhance radiation effectiveness. However, the initial events that occur in response to radiation treatment remain largely unknown. The Akt pathway is one such survival pathway that is triggered by radiation. Therefore, we sought to identify signaling pathways that might influence radiation response.

Methods
Human umbilical vein endothelial cells (HUVEC) were analyzed in response to ionizing radiation. Immunofluorescence (IF) for phosphatidylinositol (3,4,5)-trisphosphate (PIP3) was performed. Western blotting for Akt, phospho-Akt, several PKC isoforms that are known to be expressed in HUVEC as well as the downstream effector, MARCKS were performed. Pharmacological inhibition of PKC (staurosporine, Gö6976, Ro-32-0432, and Enzastaurin) as well as dominant negative PKCε, WT MARCKS and nonphosphorylatable (NP) MARCKS (both with GFP tags) adenoviral infection were studied.

Results
IF analysis of HUVEC after 3 Gy irradiation shows rapid formation of PIP3, first in the nucleus and later at the plasma membrane (Figure A). Western blot analysis shows MARCKS phosphorylation (Figure B), the definitive PKC substrate, in a similar time course which correlates with Akt activation. Moreover, PKC activation occurs immediately following irradiation. The pan-PKC inhibitor, staurosporine blocked both radiation induced MARCKS phosphorylation and Akt activation. Selective PKC pharmacological inhibitor studies suggested that novel PKC isoforms were responsible for MARCKS phosphorylation. When subcellular fractionation was performed on irradiated HUVEC, only PKCε showed a radiation-inducible translocation to the membrane, indicating activation. When, dominant negative PKCε adenovirus was used to infect HUVEC prior to radiation, MARCKS phosphorylation was abolished. GFP-tagged WT MARCKS translocated from the membrane to cytoplasm upon 3 Gy, but the NP MARCKS remained membrane bound upon 3 Gy confirming the importance of MARCKS phosphorylation in this process.

Conclusions
We demonstrate, for the first time, the production of PIP3 in vascular endothelial cells in response to ionizing radiation which was accompanied by PKC activation, MARCKS phosphorylation, and Akt activation which could be blocked by targeting PKCε.
DISSOCIATION OF BRCA1’S DNA REPAIR FUNCTION FROM DNA DAMAGE-INDUCED CYTOTOXICITY: A DEPENDENCE ON BRCA1 SUBCELLULAR LOCALIZATION

Eddy S. Yang, MD, PhD, Hong Wang, MD, Ph.D. Liping Li, MD, PhD, and Fen Xia, MD, PhD.

Purpose/Objectives: The tumor suppressor BRCA1 is a nuclear shuttling protein, and several hereditary mutations are known to cause aberrant localization of BRCA1. It has been hypothesized that BRCA1’s control of vital cellular processes such as DNA repair in the nucleus and cell death in the cytoplasm could determine cellular sensitivity to DNA damage. However, the exact role that BRCA1 subcellular localization plays in these functions remains to be elucidated. We thus investigated whether homologous recombinational repair (HR) of chromosomal breaks is affected by BRCA1 localization. We also tested whether targeted re-distribution of wild type BRCA1 to the cytosol can enhance cancer susceptibility to DNA damage based therapies. Lastly, we explored the therapeutic sensitivity of breast cancer cells expressing various BRCA1 mutants which render cells HR-deficient or alter BRCA1 localization.

Methods: Immunohistochemistry was performed in MCF7 human breast cancer cells to assess radiation-induced Rad51 foci, an in vivo functional marker of HR activity, in relation to BRCA1 localization. Targeted translocation of BRCA1 to the cytoplasm was achieved by ectopic expression of a peptide (tr-BRCA1) containing the BRCA1 NES and BARD1 binding domain. Cytotoxic response to ionizing-radiation (IR) and cisplatin was assessed via colony-formation assays in human breast (MCF7) and colon (HCT116) cancer cells with or without tr-BRCA1. Sensitivity to these agents were also examined in breast cancer cells expressing various BRCA1 mutants deficient in HR (S988A) or altered BRCA1 localization (C64G or P1749R).

Results: We demonstrate that HR requires BRCA1 in nucleus. In addition, sequestering BRCA1 in the cytosol diminishes HR function and sensitizes the cytotoxic response to IR and cisplatin in human breast and colon cancer cells. Dissection of the mechanism of this enhanced cytotoxicity using BRCA1 mutants deficient in HR reveals a dissociation between BRCA1’s function in DNA repair from its effects on cellular sensitivity to DNA damage. Interestingly, DNA damage-induced cell killing is dependent on translocation and accumulation of BRCA1 in cytosol.

Conclusions: Our data suggests that deficiency in HR alone may not be sufficient for initiating the cascade of DNA damage-induced cytotoxicity. Furthermore, cytoplasmic translocation of BRCA1 is vital not only in the regulation of vital cellular processes such as DNA repair, but also in the facilitation of cytotoxic response to DNA damage. We propose that the status of BRCA1 nuclear shuttling may provide a molecular marker to predict tumor response, and targeting BRCA1 localization to the cytoplasm may thus be a novel therapeutic strategy to sensitize cancer cells to DNA damage-based therapy in sporadic breast cancer.
Objectives
The precise replication of the genome and the continuous surveillance of its integrity are essential for cellular survival and the avoidance of various diseases, including cancer. The genome is constantly exposed to environmental and endogenous genotoxic insults. To cope with this challenge, a complex signaling network - the DNA damage response network - has evolved to detect aberrant DNA structures and coordinate diverse DNA repair and cell cycle checkpoint pathways. Two pathways operate during DNA replication to monitor the genome: the ATM (ataxia-telangiectasia mutated)-dependent pathway, which primarily responds to DNA double-strand breaks (DSBs) and the ATR (ATM and Rad3 related)-dependent pathway, which primarily responds to single-stranded DNA (ssDNA) generated by processing of DSBs or at stalled replication forks. ATR function is essential to stabilize stalled replication forks and promote recovery. When ATR-deficient cells are challenged with genotoxic agents that stall replication forks such as ultraviolet radiation or hydroxyurea (HU), replication forks collapse, and DNA synthesis cannot be completed. The objective of this study was to identify the genes and mechanisms involved in mediating recovery after replication fork arrest.

Methods
We completed a loss of function genetic screen using RNA interference in human cultured cells. We optimized an assay for viability following recovery from treatment with HU using non-specific (NS) targeting, ATRIP, or ATR siRNA. We employed a library of 27,976 siRNAs corresponding to four unique siRNA duplexes, targeting each of 6,994 unique human genes arrayed in a one-gene-one well format on 96-well plates. Secondary screens to validate and functionally categorize replication fork recovery (RFR) genes are ongoing.

Results
We identified 146 candidate RFR genes, representing 2.1% of the genes in our primary screen. We optimized a cell cycle recovery assay in which depletion of ATR or ATRIP impairs cell cycle recovery. This assay will be used in our secondary screen.

Conclusions
Assaying for viability following recovery from HU treatment is a robust assay for conducting our siRNA screen. Our screen has yielded several valid replication response genes in the ATR signaling pathway. We expect that novel regulators of the ATR signaling pathway, DNA replication control, and genomic stability will be
CLINICAL SCIENCE RESEARCH
ABSTRACTS

(alphabetically by presenter’s last name)
### 2008 Clinical Science Abstracts

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INFLUENZA VACCINE RESPONSE IN KIDNEY TRANSPLANT RECIPIENTS

Kelly Birdwell MD, T. Alp Ikizler MD, Edith Sannella MT, Mine Ikizler MS, Peter Wright MD

Objective
To compare the immune response to influenza vaccine in kidney transplant recipients and controls.

Methods
Sixty adult kidney transplant subjects and 107 healthy controls were recruited prior to the 2006-7 influenza season to receive the trivalent, inactivated intramuscular influenza vaccine. Transplant subjects were on a tacrolimus-based immunosuppression regimen +/- prednisone. There was no difference in age between control and transplant groups, or in prior influenza vaccination (84% and 95%, respectively). Serum was collected pre-vaccination, one month post-vaccination, and the end of influenza season and tested for antibody responses by hemagglutinin inhibition assay. Primary outcomes were seroresponse (4-fold rise in antibody titer) and seroprotection (post-vaccination antibody titer of ≥ 1:32). Secondary outcomes included associations of immune response with gender, age, immunosuppression, and kidney function. Chi-square testing was used for the primary outcomes. Odds ratios were obtained with binary logistic regression.

Results
For influenza A/H1N1, 27% of controls versus 19% of transplants had a seroresponse (p=0.241); 84.1% of controls and 64.2% of transplants developed seroprotection (OR 2.96, 95% CI 1.38 to 6.35, p=0.005). For influenza B, 11% of controls versus 5.7% of transplants had a seroresponse (p=0.25); 85% of controls and 71% of transplants developed seroprotection (OR 2.43, 95% CI 1.10 to 5.37, p=0.028). The difference in seroprotection for H1N1 and B between groups remained when adjusted for serum creatinine, which was different at baseline (controls 0.9 mg/dL, transplant 1.3 mg/dL, p<0.001). For transplant subjects there was no difference in vaccine response based on prednisone use. Diabetic transplant subjects had statistically significant higher proportion of seroprotection to type B.

Conclusions
A significantly higher proportion of controls than transplant subjects were seroprotected after influenza vaccination, despite similar percentages of prior history of vaccination. Among transplant subjects, diabetes may modulate vaccine response whereas prednisone use does not.
HIGHLY-SENSITIVE C-REACTIVE PROTEIN (hsCRP) IS ASSOCIATED WITH BODY MASS INDEX (BMI) AND SERUM LIPIDS IN HIV-INFECTED PATIENTS WITH LOW CARDIOVASCULAR DISEASE (CVD) RISK AND VIROLOGIC SUPPRESSION ON ANTIRETROVIRAL THERAPY (ART)

M. Boger¹, A. Shintani², V. Mitchell³, H. Erdem³, L.A. Dageforde¹, D. Haas¹, T. Hulgan¹

Background
Metabolic abnormalities complicating ART may lead to increased CVD risk. hsCRP predicts CVD risk in the general population; less is known about its use in HIV-infection. Our objective was to assess relationships between hsCRP and metabolic parameters in HIV-infected patients on ART.

Methods
Data were from a prospective cohort of HIV-infected adults enrolled June 2005-July 2007. Eligible subjects were receiving ART with ≥ 2 NRTIs, had HIV-1 RNA <10,000 copies/mL plasma, and no known diabetes or CVD. Clinical data were collected at each visit. Non-linear mixed effect regression models were used to assess effects of BMI and lipid changes on hsCRP over time after adjusting for gender, age, race, smoking status, fasting state, PI use, lipid-lowering therapy, CD4 count and HIV-1 RNA.

Results
94 subjects had data available from at least one visit, 65 from two visits, 41 from three visits, and 19 from four visits. Median age was 44 years, 27% were female, 43% were of non-white race, 34% had clinical lipoatrophy, 17% were on lipid-lowering therapy, and 54% were on a PI. Median CD4, HIV-1 RNA, and hsCRP were 515 cells/mm³, <50 copies/mL, and 2.94 mg/L, respectively. Median Framingham risk score was 3 in males and females, reflecting a 10-year CVD risk of 5% and 3%, respectively. In multivariable repeated measures analysis adjusting for the listed covariates, there were significant correlations between increased hsCRP and greater BMI (p=0.005), higher non-HDL (p=0.02) and triglycerides (p=0.04), and lower HDL (p=0.03).

Conclusions
In this group of HIV patients with low CVD risk and virologic suppression on ART, hsCRP was independently associated with BMI and serum lipid changes. These results suggest that hsCRP may be of value for assessing certain HIV-infected patients for metabolic complications and CVD risk on ART. Future studies should assess associations between hsCRP and clinical outcomes.
DEFINING THE SURGICAL TRAINEES' LEARNING CURVE FOR LAPAROSCOPIC COLORECTAL RESECTION

Jaime L. Bohl MD, James M Isbell MD, Paul E. Wise, Roberta L. Muldoon, Alan J. Herline MD

Objectives
Laparoscopic colorectal resections (LCR) have a steep learning curve. Previous studies have measured the learning curve of fully trained surgeons who are integrating LCR into their established surgical practice. We hypothesize that the general surgical trainee's learning curve for LCR can be measured using similar outcomes.

Methods
A retrospective review was conducted for all LCR performed by general surgical trainees under the supervision of two fellowship trained Colon and Rectal Surgeons. Surgical trainees who performed >9 LCR were included in the analysis. The primary outcome was operative time. Secondary outcomes included conversion rate, complication rate and readmission rate. Multivariable regression was used to examine the impact of increasing surgical trainee experience on these outcomes while controlling for patient age, body mass index, attending surgeon, diagnosis, prior abdominal surgery, and type of colon resection.

Results
A total of 408 LCR were performed from September 2002 to June 2007. Twenty surgical trainees performed a mean of 20.4 ± 6.5 LCR. There was a significant decrease in operating time for each consecutive quartile of surgical trainee experience (2.81 minutes, p=0.001), with no significant effect on conversion rate, postoperative complications, or 30 day readmission. Overall, increasing surgical trainee experience resulted in an 8% decrease in average operative time.

Conclusions
Traditional measures of a learning curve for LCR can be used to measure the technical progress of a general surgical trainee. Trainees can demonstrate significant improvement in technical skill without increases in patient morbidity or conversion rates. When establishing a practice, general surgery graduates with LCR exposure may still be on a learning curve even after 20 LCR and may experience continued improvement.
A REVIEW OF THE EFFECTS OF NATURAL AND SYNTHETIC ESTROGENS ON PRECOCIOUS PUBERTY IN AFRICAN AMERICAN GIRLS

Rhea Whitney Boyd, B.A.

Introduction
African American girls are, on average, the earliest sexually developing children in the world. The literature suggests early sexual maturation could be caused by exposure to natural and synthetic estrogens, pediatric obesity, and genetic differences between ethnic groups; each of which may contribute to the unique occurrence of precocious puberty in African American girls.

Objectives
To explore the relative roles estrogen exposure, obesity, and genetic differences may play in the premature onset of sexual development among African American girls and to identify the public health consequences of precocious puberty.

Methodology
The MEDLINE database was searched for articles pertaining to the etiology and nature of precocious puberty and the relationship between precocious puberty and sexual violence. Additional materials were provided by the National Sexual Violence Resource Center and obtained from the American Anthropological Association’s “RACE project” annotated bibliography.

Results
Natural and synthetic estrogens are associated with the occurrence of precocious puberty in girls. In addition, while related, pediatric obesity and genetic differences between “racial” groups do not fully explain the occurrence of precocious puberty in African American girls. Much remains unknown about the causes of early puberty in this population. Evidence does support a potential association with sexual violence and other adverse health conditions.

Conclusions
Scientific explanation of the unique occurrence of early sexual maturation in the United States requires a nuanced understanding of the dynamic interrelationship between human biology and the physical and sociopolitical environment in which that biology functions and gains meaning. More work is needed to achieve the integrated understanding of the ways in which environmental estrogens, pediatric obesity, and the social construction of “race,” may act individually or synergistically to affect adolescent health, necessary to grasp the impact of sexual precocity on African American girls.
AMBULATORY-CARE-SENSITIVE
EMERGENCY DEPARTMENT USE AMONG
LOW-COST MEDICAL HOME PATIENTS

Mariu Carlo, VMSII

Background/Problem
Rates of hospitalization for ambulatory care-sensitive conditions (ACSC, non-emergent or primary-care-treatable) are indicators of access to and effectiveness of primary care. The medical home model has been shown to improve health outcomes, decrease emergency department (ED) visits, and decrease the cost of care. An assessment of whether Bridges to Care (BTC), a program that links Nashville’s uninsured population to a medical home, minimizes ED visits for ACSCs is needed, as well as an explanation for why patients with medical homes use EDs for ACSCs.

Objectives
The objective of this study is to 1) quantify rates of ACSCs among BTC, non-BTC/uninsured, and insured patients, and 2) to determine why patients provided with a medical home utilize EDs for ACSCs.

Methods and Materials
20-item telephone survey concerning barriers to primary care and reasons for ED use was developed and administered to a total of 76 BTC, uninsured/non-BTC, and insured/non-BTC patients with prior exposure to BTC. Rates of ACSCs were obtained for Vanderbilt ED patients between 1/1/07 and 3/31/07 on the basis of ICD-9 and CPT codes. Descriptive statistics were tabulated. This study was approved by VU IRB.

Results
Rates of ACSCs among BTC were not different than those of uninsured/non-BTC or insured. Reasons for using EDs for ACSCs were not different between BTC and non-BTC patients. More BTC than non-BTC patients cited cost of healthcare as a problem that needed to be improved (p=0.04).

Conclusions
BTC patients have similar ACSC rates when compared to both uninsured/non-BTC and insured patients, and it is likely that cost of healthcare is a factor in their accessing healthcare.

Acknowledgements
Dr. James Powers, Internal Medicine
Dr. Mario Davidson, Biostatistics
INCIDENCE AND CLINICOPATHOLOGICAL FEATURES OF ATYPICAL MENINGIOMA: A 7-YEAR EXPERIENCE

Samuel N. Crosby, Michael L. Edgeworth, Kathleen M. Egan, Reid C. Thompson

Objectives
Atypical meningiomas (WHO II) comprise about 5-10% of all meningiomas, have a worse prognosis and require a more aggressive treatment than typical meningiomas (WHO I). In this study, we examined the presentation, demographics, histologic characteristics, and treatment courses of all atypical meningiomas diagnosed over a 7 year period at VUMC.

Methods
We retrospectively examined data from the medical records of all patients diagnosed with meningioma by surgical pathology at VUMC between 2000 and 2006. A total of 196 meningiomas were identified, of which 33 were atypical (17%). Those cases with atypical meningiomas were further investigated.

Results
Of these patients, 22 were female (67%) and 11 were male. 29 were Caucasian (87.9%), 3 African American, and 1 Hispanic. The mean age was 51.4 years (range: 20-80). 10 patients (30%) had a history of cancer other than meningioma with 5 receiving prior cranial irradiation. One patient had NF-2 and another had RB. Common symptoms at presentation were focal deficits in 22 patients (67%), headaches in 14, seizures in 10, and hydrocephalus in 1. Histologically, the average MIB-labeling rate of those reported was 13.9±8.5% (n=11). The average mitotic rate was 3.8±2.0 per 10hpf (n=13). On review of the pathology report, they were characterized by a small cell component in 17 (52%), hypercellularity in 13, sheeting in 13, brain invasion in 9, EMA staining in 8, prominent nucleoli in 6, positive vimentin stain in 4, and choroid features in 3. Treatment involved surgery with gross total resection in 28 (85%) or subtotal resection in. Adjuvant treatments included radiotherapy (n=7), embolization (n=5), and hydroxyurea (n=4).

Discussion
In our experience the prevalence of atypical meningioma at VUMC was slightly higher (17%) than what is typically reported in the literature (5-10%). Of the atypical meningioma cases, 2/3 were women, consistent with the predilection of this disease in females. Approximately 1/3 had a prior history of cancer (30%), 1/2 of which had received previous ionizing radiation to the head, both known risk factors for meningioma. The most common histological characteristics were consistent with the literature. A planned multicenter case-control study will investigate risk factors for these tumors and other histological subtypes of meningioma.
EPIDEMIOLOGY OF SPIDER BITES IN MIDDLE TENNESSEE

NM Curcio, PG Arbogast, KM Egan, LE King, GP Stricklin

Objectives
The aim of the study was to analyze the patient population with documented spider bites with a focus on spider bites due to the Brown Recluse Spider (BRS), in a region in the USA where it is endemic.

Methods
A 12-month long prospective, multi-clinic, case-series study within Vanderbilt University Medical Center (VUMC) was conducted to evaluate the frequency, risk factors, clinical features, symptoms, progression, treatment, outcome and economic impact of clinically documented BRS bites and other spider bites.

Results
Between September 2006 and May 2007, 20 of 30 (67%) patients seen at VUMC had documented BRS bites. Sixteen (53%) patients saw the spider at the time of the bite and were able to identify it from a group of photographs. Five (17%) patients brought the spider for identification. The most common location of the bite was inside of the home (20/30; 67%) and most bite sites were on the extremities (16/30; 53%). All patients had localized erythema, but 14 of 30 (47%) patients had skin necrosis. Nearly half of the BRS bite lesions were moderate (9/20), 7/20 were mild (35%) and 4/20 (20%) were severe. The systemic symptoms occurring more often in patients with BRS bites included nausea, vomiting, chills, generalized rash, and abdominal cramping. The mean cost to spider bite victims including medical therapy and loss of work income was $818 [range $0 – $4350]. The average number of missed days of work due to a BRS bite was 9.2 days (n=17) vs. 0.9 days for patients bitten by other spiders (n=8) (5/30 did not work outside the home).

Conclusions
Of the spider bites examined, the bites due to the BRS were more commonly necrotic, more locally severe and induced more systemic symptoms. BRS bites impact patients’ lives by the financial costs due to medical bills, missed work, and restricted work-related activities. These physical limitations may persist for days to weeks depending upon the severity of the BRS bite and the patient’s occupation.
THE LEGACY OF OLAVIDE IN THE U.S.A.

NM Curcio, F Heras, L Conde-Salazar

Objectives
The aim of the study was to compile a complete list of works published by Dr. J.E. Olavide, father of Spanish dermatology, and to determine which, if any, are in existence in the United States today.

Methods
A historical investigation was conducted using primarily three sources: World Cat (global network of library catalogues), to find books currently in existence worldwide; Index Cat (Index Catalogue of the Library of the Surgeon-General’s Office), to attain a listing of books and journal articles published by Olavide; and Index Medicus, for a complete listing of articles published by the author by year.

Results
World Cat demonstrated four books written by Olavide as primary author, including *Dermatología general y clínica iconográfica de enfermedades de la piel* (1871), his most famous text, and one book in which he was the author of the prologue. There is also one book authored by Joaquin Calap in which Olavide is the subject of the textbook. All of these texts are available at the National Library of Medicine (NLM). Index Cat was helpful in searching for both texts and journal articles. It revealed four of the five books gathered on World Cat, in addition to two others that were no longer in existence worldwide. Index Cat also contained a thorough listing of medical articles published by Olavide from 1860 through 1896. Finally, using Vanderbilt’s complete collection of volumes of Index Medicus from the late 19th century, all years in which Olavide published medical research (1879-82, 1888-92, 1895/96) were determined and the title, journal, and location of those articles was obtained. There were ten articles only found in Index Medicus. Copies of all of Olavide’s articles are available through the NLM.

Conclusions
More than one thousand years after his death, the legacy of Olavide in dermatology continues through his museum of wax moulages, and through his textbooks and medical articles. And although Olavide never crossed the Atlantic Ocean, many of his visionary ideas, case reports, and dermatologic mastery illustrated in his literary works are only preserved today within the NLM. This project led the photographic preservation of several of his textbooks and scanning of many of his articles from the NLM for conservation at the Olavide Museum (Madrid, Spain).
INTENSITY-MODULATED RADIATION THERAPY (IMRT) WITH CONCURRENT TAXANE-BASED CHEMOTHERAPY FOR LOCALLY-ADVANCED HEAD AND NECK CANCER (LAHNC): TOXICITIES AND EFFICACY

Roberto Diaz, Stephen W. Thorpe, Barbara A. Murphy, Wyndee Kirby, Brian B. Burkey, Christine H. Chung, Bashar Shakhtour, Patrick Murphy, Michael Beach, Anthony J. Cmelak

Objectives
The addition of chemotherapy to radiotherapy improves survival in LAHNC at the expense of higher toxicity. IMRT for LAHNC provides a means to minimize dose to normal tissue. Our experience with concurrent IMRT and taxane-based chemotherapy in 128 sequential LAHNC patients is presented.

Methods
From December 2002 to October 2006, 128 patients with LAHNC were treated with IMRT and concurrent taxane-based chemotherapy for curative intent. A single differential IMRT fractionation regimen was developed: 2.1 Gy/day to gross disease with +/-5% homogeneity, and 1.7 Gy to prophylactic nodal sites down to the clavicles. Weekly paclitaxel 30mg/m² and carboplatin AUC 1 were given concurrently with IMRT to all 128 patients. The majority of patients with N2 or N3 disease (78%) also received weekly induction chemotherapy with paclitaxel 60mg/m² and carboplatin AUC 2. Chemotherapy was held for ANC<1000, platelets<100k, or grade 4 mucositis.

Results
Mean follow-up is 20.7 months. One and two year survival was 87% and 79%, respectively. Local control was 95% at 1 year and 91% at 2 years. Regional control is 96% at 1 year and 95% at 2 years. One and two year relapse free survival (RFS) was 77% and 64%, respectively. Distant metastasis developed in 9%. Percent weight loss during IMRT correlated with RFS (p<0.02) and with survival (p<0.1). Mean percent weight loss was 7%. Pegs were placed before beginning treatment in 5% and additional 66% required Peg during treatment; 8% required Peg use > 1 year. Grade 3 mucositis occurred in 70% (1% Grade 4) and grade 3 dermatitis was seen in 64% (4% grade 4). Eleven patients (9%) developed osteoradionecrosis. 32% of patients developed hypothyroidism after their treatment. There were no patients with nadir sepsis nor significant nephropathy or GI toxicity. Late xerostomia is ≤ grade 1 in all patients.

Conclusions
Differential-dose IMRT with paclitaxel and carboplatin is extremely feasible with excellent dose delivery of both radiation and chemotherapy. Minimizing dose (≤25Gy) to sensitive tissues and strict use of IMRT homogeneity criteria appear to prevent significant long-term swallowing difficulties. Early toxicities are low with impressive tumor control. Development of hypothyroidism, however, appears higher with IMRT than historical rates using 3-D conformal radiation.
GENDER AND SEX HORMONE SPECIFIC RISK FOR VENTILATOR ASSOCIATED PNEUMONIA

Lesly A. Dosset, M.D.

Background
Ventilator associated pneumonia (VAP) is a frequent complication of critical illness which significantly contributes to mortality among intensive care unit (ICU) patients. Animal studies suggest that sex and sex hormones modify a patient’s risk for VAP. Our objective was to describe sex and sex hormonal differences in patients with VAP after critical illness or injury.

Methods
A prospective cohort study of adult surgical and trauma patients at high risk for VAP (ICU admission ≥48 hours) was performed. Demographic and clinical data was collected prospectively, and sex hormones were assayed at study entry. VAP was defined by Centers for Disease Control (CDC) criteria with the use of quantitative cultures obtained from bronchoalveolar lavage (BAL). Multivariate logistic regression was used to estimate the adjusted odds of VAP associated with differences in sex.

Results
2,290 patients met entry criteria and 718 patients developed VAP (31%). Males were more likely to develop VAP (34% vs 25%, p<0.001), and this association remained after adjusting for age and illness severity (OR 1.5 95% CI 1.2 – 1.8, p<0.001). Of patients with VAP, there was no difference in mortality related to sex (16% mortality in males vs 18% in females, p=0.40). An anti-inflammatory sex hormone profile (low estrogen, high testosterone) was associated with VAP, but did not fully explain observed sex differences.

Conclusion. Males are 50% more likely to develop VAP than females following critical illness and injury. These differences are not fully explained by sex hormones measured in the early phase of critical illness.
CATEGORIZING THE WORLD OF REGISTRIES

Brian C. Drolet, Kevin B. Johnson

Objectives
The term registry is widely used to refer to any database storing clinical information collected as a byproduct of patient care. Despite the use of this single characterizing term (registry), these databases exist in various forms and support diverse functions. This ambiguous terminology impacts the ability to locate and learn about specific types of registries. The goal of this project was to devise a framework for defining and characterizing registries of the type now prevalent in the literature under that heading.

Methods/Results
This project was conducted in three phases using an immersion/crystallization process used in qualitative research. In the first phase, we constructed a comprehensive list of registry-related papers by examination of the peer-reviewed literature. In the second phase (immersion), we reviewed each paper to determine how well it fit the accepted definition of registry; we also noted the presence and absence of characteristics identified as important by authors. In the final step (crystallization), we distilled and summarized these frequently cited characteristics to construct an inclusive definition for the term medical data registry (MDR) and to generate our categorization framework “MDR-OK.”

Conclusion
This research has resulted in a framework, MDR-OK, that may be used to better distinguish an MDR from a non-registry database, to define the term “registry,” and to score and categorize various data-systems. Our definition is the most thorough to date, and should provide clarity regarding choice of terminology in the literature. Researchers may use our framework to better describe and understand data-systems, and eventually to conduct more accurate literature searches. Likewise, physicians and hospital administrators can use our results to evaluate and utilize registries in medical practice.
INSULIN CLEARANCE: LESSONS FROM BARIATRIC SURGERY

Julia P. Dunn, H. Ayesha Hossain, Robyn Tamboli, James M. Isbell, Pamela Marks, Irene Feurer, Naji Abumrad

Objectives
Metabolic clearance rate (MCR) has been proposed as a measure of insulin efficiency, but its use in type 2 diabetes (T2D) has been controversial. We hypothesized that this could be related to variations in prevailing insulin concentration. Our study examines MCR in a human model of reversal of T2D (obese subjects before and after roux-en-y gastric bypass, RYGB).

Methods
Studies performed in 12 obese (preop and 6 and 12m after RYGB) and 7 lean subjects using a 2-stage sequential (low and high) hyperinsulinemic, euglycemic clamp. MCR calculated as IIR divided by prevailing ISS adjusted for (a) IB or (b) differences in Cpep. Linear regression was used to characterize the relationship between IIR and ISS at each time point. Longitudinal effects were tested using linear mixed models. Insulin sensitivity (IS) was measured using the insulin clamp and HOMA-IR.

Results
HOMA-IR was higher in obese vs. lean and decreased after RYGB (p<0.001). IS was lower in obese vs. lean at all time points (p<0.001). MCR (ml/min) did not differ between obese and lean and did not change after RYGB. MCR decreased from low to high IIR (all p<0.05). All linear relationships of IIR to ISS were significant (all r/slope p<0.05). All intercepts (except of the 12 month low IIR) were positive and significant (p<0.05)

Summaries and Conclusions
MCR (ml/min) is similar in lean and obese (pre- and post-RYGB) despite differences in insulin sensitivity, and this challenges its utility as a measure of insulin efficiency. MCR decreases with higher insulin concentrations. Constructing a mathematical model based on the linear regressions shows that MCR is dependent on and changes with: (a) an insulin-independent component represented by the slope of regression line and (b) an insulin-dependent component represented by the positive intercept. Hence, any use of MCR as a measure of insulin efficiency has to take into consideration the effect of insulin concentration that results from these two variables.
HIGH RATE OF SUB-ACUTE LEAD COMPLICATIONS AND CARDIAC PERFORATION, WITH NEW GENERATION, SMALL CALIBER IMPLANTABLE CARDIOVERTER-DEFIBRILLATOR (ICD) LEADS

Ellis, Christopher R., MD; Jeffrey N. Rottman, MD

Introduction
Increased rates of several types of sub-acute complications related to use of small diameter ICD leads have recently been reported. Aggressive lead design and engineering may be associated with increased risk of cardiac perforation, and sub-acute ICD lead dislodgement or failure. The purpose of this study was to determine the risk of perforation and sub-acute ICD lead failure in a single center from Jan 1, 2007 to August 31, 2007.

Methods
All patients who received percutaneous high voltage right ventricular ICD lead implantation at VUMC, and at Nashville VA Medical Center (TVHS) between Jan 1, 2007 and August 31, 2007 were included in this study (Riata 1580, Riata ST 7000 St.Jude Medical, St.Paul, Minnesota, USA, Sprint Fidelis 6949 and Sprint Quattro 6947 Medtronic, Minneapolis, Minnesota, USA). Information was collected retrospectively.

Results
A total of 305 high voltage ICD leads were implanted during the study period. 136 small diameter leads were implanted, along with 169 standard diameter leads. Eleven lead failures occurred in the small diameter lead group, versus one in the standard group. Failure was defined as development of high pacing threshold, marked sensing change, cardiac perforation, or extracardiac stimulation related to an ICD lead requiring a procedure (pericardiocentesis, lead reposition, lead replacement, NIPS with reprogramming). Implantation of small diameter ICD leads (Riata 1580, Riata ST 7000, or Medtronic Sprint Fidelis 6949) was associated with a significant increase in complication rate 8.1% versus 0.6%, (p=0.0008), when compared to standard size leads (Medtronic Sprint Quattro 6947). Complications were increased with the Riata 1580 and Riata ST 7000, when compared to the Sprint Quattro 6947 lead (1580 versus 6947 p=0.005, 7000 versus 6947 p=0.007) with a trend towards increased risk with the Sprint Fidelis 6949 lead (6949 versus 6947 p=0.07). Symptoms of cardiac perforation or lead migration developed <28 days after implant. One death occurred unrelated to the ICD lead implantation. One case of pericardial tamponade requiring emergent intervention occurred in the Riata ST 7000 group.

Conclusions
During the study period between Jan 1, 2007 and August 31, 2007, lead revision and cardiac perforation was more likely with use of the small diameter ICD lead, (Riata 1580, Riata ST 7000) when compared to the Sprint Quattro 6947 lead. This adds to the available data in recently published reports, and implies that the increased complications may reflect a “class effect” due to different engineering constraints, rather than a function of a single lead.
UNPLANNED PREGNANCIES AND FUTURE NEEDS OF WOMEN INCARCERATED AT AN URBAN JAIL: A STEP TOWARD DEVELOPING APPROPRIATE JAIL-BASED INTERVENTIONS

Kristy Kummerow, BS

Unintended pregnancy is a significant social and public health problem in the United States, affecting mothers, their children, and society. As a population, incarcerated women have higher rates of unintended pregnancy than the general population and their pregnancies are often high-risk due to substance use and inadequate prenatal care. Structured interviews were conducted with 100 incarcerated women at the Davidson County jail in Nashville, TN, regarding the circumstances surrounding their past unplanned pregnancies. 86% of women who had been pregnant had at least one unplanned pregnancy (range 0-9 pregnancies/woman, average 2.9/woman). 147 children were born to these 79 women as a result of unplanned pregnancies. Many pregnancies resulted from circumstances in which women had minimal control. In 40%, women were high (using drugs) at conception, while women reported being drunk at conception 27% of the time. Female inmates reported that they did not use contraception in the past because they had a consistent partner and/or were in love (52%), they were concerned about side effects (39%), and they did not think pregnancy was possible (20%). 55% of female inmates surveyed are at risk for a future unplanned pregnancy. There was a significant association (p<0.001) between women who responded that they were likely to have unprotected sex within three months of release from jail and those who responded that they “felt sex would be less exciting and less natural if birth control was used.” 70% of at-risk women plan to use some form of contraception in the future. Data from previous studies indicate that female inmates are 14 times more likely to initiate contraceptive use when it is offered by the jail upon release than if they are referred to a community provider. Women at Nashville’s county jail may benefit from such a program.
THE FREQUENCY OF A POLYMORPHISM IN THE GLUTAMATE CYSTEINE LIGASE GENE CORRELATES WITH MALARIAL PREVALENCE IN POPULATIONS OF AFRICAN DESCENT

Truc M. Le, George Ayodo, Jeffrey A. Canter, David Reich, Marshall L. Summar

Background
Glutamate cysteine ligase (GCL) is the rate-limiting enzyme in the glutathione biosynthesis pathway, and genetic variation in this gene can affect the ability to produce glutathione and respond to oxidative injury. We previously identified a non-synonymous polymorphism (C1384T) present only in individuals of African descent and demonstrated that this polymorphism encodes an enzyme with no activity in vitro.

Objective
To determine whether the 1384T polymorphism is correlated with development of severe malaria in populations of African descent.

Methods
Using high-throughput genotyping, we have genotyped Kenyans from malaria-endemic and non-endemic regions as well as several African-descent populations.

Results
In a malaria-endemic region of Kenya, the 1384T polymorphism is found in 7.8% of individuals. In contrast, within a non-endemic region of Kenya, the 1384T polymorphism is present in only 3.8% of the population (p<0.05). Similarly, individuals from a non-endemic region of Ghana have a 5% prevalence of this allele, whereas African-Americans from North Carolina exhibit 7.4% prevalence. Within the endemic regions we studied, we note that the 1384T allele is present in 6.3% of patients with severe malaria, whereas it is present in 9.0% of controls. Though not statistically significant, these results suggest the 1384T polymorphism is associated with less severe malarial disease.

Conclusions
We have previously described a glutathione production pathway polymorphism common in individuals of African descent with little or no in vitro activity. Here, we demonstrate an increased frequency of the 1384T GCLC allele among malaria endemic populations as compared to populations from non-endemic areas. In addition, our results suggest that this polymorphism is associated with less severe disease.
A LOST OPPORTUNITY FOR EDUCATION OF PEDIATRIC PATIENTS ABOUT TESTICULAR TORSION

John H. Makari, John C. Pope IV, Mark C. Adams, John W. Brock III, and John C. Thomas

Background
At our institution, a recent review of patients undergoing exploration for testicular torsion revealed that two-thirds of patients lose a non-viable testicle, most frequently due to initial delay seeking medical treatment. In an effort to better target public health initiatives, we investigated pediatric patients are routinely educated about this potentially devastating diagnosis by primary care physicians (PCPs) during routine well child care (WCC).

Methods
We sent an anonymous survey addressing routine genitourinary (GU) health education during well child care to 533 PCPs who refer to our institution for Pediatric Urological care. Surveys which had insufficient responses for evaluation or were returned as undeliverable were excluded.

Results
154 returned surveys (28.9%) were sufficient for evaluation. Mean practitioner age was 44.6 y, with a mean length in practice of 14.3 y. 94.8% of respondents evaluate between 0-1 patients on average per week for the chief complaint of scrotal pain and refer means of 31.2% to Pediatric Urology and 13.8% to the ER. 81.8% indicated that they routinely provide GU health education to boys during WCC, beginning at a median age of 11 y. 42.8% specifically discuss testicular pain with their patients. 5.8% indicated that they specifically mention the diagnosis of testicular torsion. During WCC, PCPs alternatively provide GU health education to boys about testicular cancer/self-examination (59.7%), sexual education (39.6%), hygiene (17.5%), normal growth and development (11.7%), sports injury/trauma prevention (4.5%) and other topics (9.1%).

Conclusion
Individual PCPs rarely see more than one patient per week with scrotal pain. While the vast majority of PCPs routinely provide GU health education during WCC, less than half specifically discuss testicular pain with their pediatric patients; very few discuss the specific diagnosis of testicular torsion. Boys must be educated that testicular pain is not normal and that they must tell their parents if it occurs. WCC visits provide an underutilized forum for this important education.
INTRA-OPERATIVE COMPLETION ANGIOGRAPHY AFTER CABG.

Mohan K. Mallipeddi

Coronary artery bypass graft (CABG) surgery is the standard of care for severe, multi-vessel coronary artery disease. Nevertheless, CABG suffers from a 20-25% early graft failure rate. Since many factors contributing to failure arise during surgery, intra-operative detection and correction can potentially improve outcomes. Thus, we set out to assess the effectiveness of intra-operative completion coronary angiography (IOCA) in identifying graft defects and guiding appropriate revision. We conducted a retrospective chart review of 239 consecutive patients who underwent CABG with IOCA in the Vanderbilt Hybrid OR between April 2005 and July 2006. Operative outcomes were examined. Completion angiography revealed that 82 out of the 517 grafts performed (16%) were defective and needed major (11%) or minor revision (5%). Approximately 42% of major revisions were to the conduit itself. Overall, 54% were corrected by percutaneous coronary intervention. CABG with IOCA produced statistically similar operative outcomes when compared to national averages. Among those who received IOCA, cardiopulmonary bypass time and length of stay were extended for those who underwent revision; most other major outcomes were statistically similar to those who did not undergo revision. IOCA enabled detection and revision of graft defects without adversely impacting perioperative outcomes. However, follow-up on our patients is necessary to determine whether this process improves long-term graft patency.

Acknowledgements
Mentor: John Byrne, MD (Chair, Cardiac Surgery).
David Zhao, MD (Chair, Interventional Cardiology), Marzia Leacche, MD (Fellow, Cardiac Surgery), Ram Umakanthan, MD (Fellow, Cardiac Surgery), Jim Balvich, Jodi Weinstein, Frank Zhao (VMS 2010).
CONJUNCTIVAL CONTAMINATION IS SIGNIFICANTLY REDUCED WITH APPROPRIATE EYE PROTECTION

Alfred A. Mansour, III, MD; Jesse L. Even, MD; Sharon Phillips, MSPH; Jennifer L. Halpern, MD

Background
Conjunctival contamination from splashed solid or liquid debris during orthopaedic procedures places surgeons at risk for communicable diseases such as human immunodeficiency virus (HIV) and Hepatitis B/C. Various studies have demonstrated the importance of protective eyewear, and in fact most institutions mandate protective eyewear. Yet compliance with recommended precautionary measures remains low, and 30% of surgeons do not wear any protective eyewear. Many surgeons use prescription glasses alone as “protective eyewear.”

Objectives
The purpose of this study is to compare the effectiveness of various types of protective eyewear in preventing conjunctival contamination.

Methods
Average intra-operative surgeon head position was determined by analysis of operating room photographs, and a simulation model was constructed. In this model, a mannequin head was placed at an appropriate distance from the surgical field and a femoral osteotomy was performed on cadaveric bone. Colored saline was used to irrigate the osteotomy site. Six experimental groups were tested for their ability to prevent splash contamination of the conjunctiva: 1) low-profile prescription glasses, 2) standard loupes, 3) contoured hard plastic glasses, 4) disposable plastic glasses, 5) combination facemask/eye shield, and 6) no protection (control). Thirty laterally-based femoral osteotomies were performed and contamination on both the protective devices and simulated conjunctival surfaces was recorded.

Results
No protective device that was tested was 100% effective. The low-profile prescription glasses and the control both had conjunctival contamination rates of 83.3%. The other eye protective devices showed significantly lower rates of overall contamination – loupes, 50% (p=0.04); facemask/eye shield, 30% (p=0.0001); hard plastic glasses, 16.7% (p=0.0001); and disposable plastic glasses, 3.3% (p<0.0001).

Conclusions
Low-profile prescription glasses provided no added benefit compared to the control in our experimental model and therefore should not be used as sole eye protection during surgical procedures. Eye protective devices that provide protection above and below the eye as well as contoured side protection – similar to the disposable plastic glasses – minimize risk of contamination.
PERINATAL ISSUES FOR PREGNANT IMMIGRANT WOMEN FROM MULTIPLE GLOBAL REGIONS

Laura Meints, Nancy Chescheir, Katherine Hartmann, Melissa McPheeters

Objective
To describe and compare pregnancy outcomes and feeding preferences for immigrant women from multiple global regions.

Methods
We retrospectively reviewed the charts of all women born outside the United States who delivered at Vanderbilt University Medical Center (VUMC) during 2003-2006. These persons were identified via the birth-certificate applications for their children. The primary outcomes were delivery type, estimated gestational ages (EGA) at delivery, birth weights, APGAR scores at 1 and 5 minutes, rates of admission to the NICU, and feeding preferences.

Results
During the years 2003-2006, 1767 immigrant women gave birth at VUMC. The women drew from Africa (19.2%), Asia (23.4%), Europe (9.1%), Latin America (27.6%), Middle East (18.4%), and North America (1.7%). Entry to prenatal care trended increasingly later among immigrant women during 2003-2006: 0.45 months (95% CI = 0.37, 0.53) for every year later the infant was born (p < 0.001). No statistical difference was observed in delivery type among Census regions (p = 0.75). Median APGARS at 1 and 5 minutes were 8.0 and 9.0 for all groups. Median EGA at delivery was greater than 38 weeks for all groups. Median birth weights ranged 7.1-7.2 lb. Percent admissions to the NICU were greatest for Europeans 13% (22/170) and Latin Americans 15% (80/522); infants of Latin American women were significantly more likely to be admitted to the NICU (p = 0.004). Feeding preferences varied across the groups. Compared to other immigrant groups, Latin American women were significantly more likely to have a teen pregnancy (p < 0.001) and significantly less likely to prefer breast feeding their infant (p < 0.001).

Conclusions
Overall, immigrant women who delivered at VUMC gave birth to term gestations with high APGAR scores at 1 and 5 minutes. Entry to prenatal care trended increasingly later among immigrant women during 2003-2006. NICU admissions for infants, teen pregnancies, and preference for bottle feeding were highest among Latin American women.
SCRENNING FOR INFECTIOUS DISEASES IN PREGNANT IMMIGRANT
WOMEN FROM MULTIPLE GLOBAL REGIONS

Laura Meints, Nancy Chescheir, Katherine Hartmann, Melissa McPheeters

Objectives
To describe screening practices for infectious diseases among pregnant women born outside of the United States who delivered at VUMC between 2003 and 2006.

Methods
We retrospectively reviewed the charts of all women born outside the United States who delivered at Vanderbilt University Medical Center (VUMC) during 2003-2006. Foreign born status was determined via the birth-certificate applications for their children. The primary outcomes were results Pap, PPD (TB), rubella immunity, RPR (syphilis), Hepatitis B (HepB), HIV, gonorrhea and Chlamydia (G/C), and Group-B streptococcus (GBS) screening tests in the chart.

Results
A total of 1767 immigrant women gave birth at VUMC during the analysis period. The women drew from Africa (19.2%), Asia (23.4%), Europe (9.1%), Latin America (27.6%), Middle East (18.4%), and North America (1.7%). Abnormal Pap smears were highest among Latin Americans (20%, 27/137). Reactive PPD’s occurred in 29% (26/89) of Africans, 23% (5/22) of Europeans, and 20% (16/82) of Asians tested. Reactive RPRs occurred in 0-1% in all groups. HepB seropositivity was highest among Africans (5%, 17/329) and Asians (6%, 24/405). Positive screens for HIV were highest among Latin Americans (2%, 11/459) and Africans (2%, 8/330) and lowest among Middle Easterners (0%, 0/209). Screening rates for G/C were 0-2% in all groups. GBS was cultured in 19-30%. Likelihood of testing differed as follows: Compared to other immigrant groups, European women were significantly less likely to be tested for HIV (p = 0.0021) and G/C (p < 0.001), Latin American women were significantly less likely to have been tested for HIV (p = 0.0039), and Asian women were significantly less likely to have been tested for G/C (p < 0.001).

Conclusions
There are substantial differences in both testing of infectious diseases and results of tests that do occur among the immigrant population delivering at VUMC. In particular, the high rate of abnormal Pap smears among Latin American women in the analysis may suggest a need for concern and certainly a focus on ensuring appropriate follow up in this population. Small numbers per origin of interest made statistical comparisons difficult; however, statistical differences in HIV testing, given recommendations for increasing screening among pregnant women are of interest. Further studies would be helpful to determine if such differences are due to clinical practice or patient choice for testing. Clinicians should have a high index of suspicion for tuberculosis among pregnant immigrants, particularly persons from Africa and Asia. This finding may be due to BCG vaccination among African and Asian women and/ or clinical TB.
TIMING OF HAART INITIATION IN RELATION TO PREGNANCY AND CHANGE IN HIV-1 RNA AND CD4

Vlada Melekhin, M.D.

Objectives
We previously found that pregnancy was associated with a lower risk of HIV disease progression among women in the HAART era, suggesting a beneficial interaction between pregnancy and HAART. We therefore hypothesized that women who initiated HAART during pregnancy would have greater improvement in CD4 and HIV-1 RNA compared to women starting HAART before or after pregnancy.

Methods
We conducted a retrospective cohort study among pregnant HIV+ women with > 1 clinic visit between 01/01/97 and 12/31/05 at the Comprehensive Care Center, and in whom baseline CD4 and HIV-1 RNA were available ≤180 days prior to the start of first HAART. There were 3 patient groups according to time of HAART initiation: before pregnancy (A), during pregnancy (B) and after pregnancy (C). Change in CD4 and HIV-1 RNA were measured within 180 days of HAART start, while women were still on 1st HAART regimen and in the same patient group. Fisher’s exact and Kruskal-Wallis tests were used for comparisons. Linear mixed-effect models analyzed repeated measurements.

Results
There were 8 women in group A, 59 in B and 29 in C who met the inclusion criteria. Median baseline CD4 were 462, 408 and 447 cells/mm³, respectively (P=0.98). Median baseline HIV-1 RNA were 5.2, 3.9 and 3.6 log₁₀ copies/mL, respectively (P=0.003). Age, race, and duration of 1st HAART were similar between the groups. After adjusting for baseline CD4 and HIV-1 RNA, CD4 following initiation of HAART in group A had similar increase compared to group B (0.19 log₁₀ cells/6 months in group A and 0.18 log₁₀ cells/6 months in group B; P=0.88). Group C had slower CD4 increase (0.017 log₁₀ cells/6 months; P=0.24) than group B. After adjusting for baseline CD4 and HIV-1 RNA, HIV-1 RNA following initiation of HAART changed more slowly in group C compared to group B (0.066 log₁₀ copies/6 months in group C vs. -0.36 log₁₀ copies/6 months in group B; P=0.04), but group A had similar rate of HIV-1 RNA decline compared to B (-0.27 log₁₀ copies/month; P=0.95). These findings persisted when adjusted for age, race, prior use of non-HAART ART and use of PI-based HAART.

Conclusions
In our study, women initiating HAART during pregnancy had a greater decrease in HIV-1 RNA than those initiating HAART post-partum, but the increase in CD4 was not significantly greater. Studies in a larger population are warranted.
BIOLOGICAL MARKERS IN BLOOD OF PATIENTS WITH CEREBRAL
VASOSPASM FOLLOWING SUBARACHNOID HEMORRHAGE

Okwueze M, Cunningham G, Magarik J, Weisner A, Banerjee A, Summar M, and
Thompson R

Cerebral vasospasm is one of the most significant complications of aneurysmal subarachnoid hemorrhage (aSAH).

Objective
The goal of this study was to determine whether Nitric Oxide (NO) substrates or intermediates in blood are predictive markers for onset of cerebral vasospasm.

Methods
This prospective clinical study compared 17 patients who had cerebral vasospasm following aSAH and 16 patients who did not develop cerebral vasospasm following aSAH. Early plasma samples were obtained within 1-4 post bleed days (PBD) and late plasma samples were collected 7-14 post bleed days (PBD). Citrulline, Ornithine, Arginine levels were analyzed using an amino acid analyzer which uses an ion exchange column for separating the amino acids monitored with chromatography.

Results
Comparison of plasma samples collected 1-4 PBD in patients without and with vasospasm

<table>
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<th>Parameter</th>
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<th>With vasospasm</th>
<th>p-value</th>
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</thead>
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<tr>
<td></td>
<td>n=16 Mean±SE</td>
<td>n=17 Mean±SE</td>
<td></td>
</tr>
<tr>
<td>Citrulline</td>
<td>21.3 ± 2.6</td>
<td>16.6 ± 1.9</td>
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<tr>
<td>Ornithine</td>
<td>41.6 ± 3.3</td>
<td>29.3 ± 3.4</td>
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<td>Arginine</td>
<td>39.4 ± 4.2</td>
<td>26.8 ± 1.6</td>
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*p-value < 0.05 for statistical significance using student t-test.

Comparison of plasma samples collected 7-14 PBD in patients without and with vasospasm

<table>
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<th>Parameter</th>
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<tbody>
<tr>
<td></td>
<td>n=16 Mean±SE</td>
<td>n=17 Mean±SE</td>
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<tr>
<td>Citrulline</td>
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<td>Ornithine</td>
<td>43.7 ± 3.5</td>
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<tr>
<td>Arginine</td>
<td>44.9 ± 5.8</td>
<td>31.9 ± 3.1</td>
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</table>

*p-value < 0.05 for statistical significance using student t-test.

Ornithine and Arginine levels are significantly lower on 1-4 PBD in patients with vasospasm compared to those without vasospasm but 7-14 PBD the difference is less probably due to NO stores being replenished.

Conclusion
Immediately following aneurysmal subarachnoid hemorrhage, Nitric Oxide substrates levels are lower in patients with cerebral vasospasm. Thus, Nitric Oxide substrates may be important early markers for cerebral vasospasm.
DELIRIUM AND SEDATION IN THE INTENSIVE CARE UNIT (ICU): 
SURVEY OF BEHAVIORS AND ATTITUDES OF 1,384 
HEALTHCARE PROFESSIONALS


Objective
A 2001 survey found that most healthcare professionals considered ICU delirium as a seri-
ous problem, but only 16% used a validated delirium screening tool. Our objective was to
assess beliefs and practices regarding ICU delirium and sedation management.

Design and Setting
Between October 2006 and May 2007, a survey was distributed to ICU practitioners in 41
North American hospitals, 7 international critical care meetings and courses, and the Ameri-
can Thoracic Society email database.

Study Participants
A convenience sample of 1,384 health care professionals including 970 physicians, 322
nurses, 23 respiratory care practitioners, 26 pharmacists, 18 nurse practitioners and physi-
cians’ assistants, and 25 others.

Results
Of respondents, 59% (766/1300) estimate that over 1 in 4 adult mechanically ventilated pa-
tients experience delirium. Over half [59% (774/1302)] screen for delirium, with 33% of
respondents (258/774) now using a specific screening tool. A majority of respondents use a
sedation protocol, but 29% (396/1355) still do not. A majority (76%, 990/1309) has a writ-
en policy on spontaneous awakening trials (SATs), but the minority of respondents (44%,
446/1019) practice SATs on more than half of ICU days.

Conclusions
Delirium is considered a serious problem by a majority of healthcare professionals, and the
number of practitioners using a specific screening tool has doubled since the last published
survey data. While most respondents have adopted specific sedation protocols and scales
and have an approved approach to stopping sedation daily, few actually report even modest
compliance with daily cessation of sedation as a formal spontaneous awakening trial or
daily wake-up.
HEALTH CARE UTILIZATION BY ADULT HISPANIC LONG TERM SURVIVORS OF HEMATOPOIETIC CELL TRANSPLANTATION (HCT): REPORT FROM THE BONE MARROW TRANSPLANT SURVIVOR STUDY

Pinki K. Prasad, M.D., Canlan Sun, M.D., Ph.D., K. Scott Baker, MD, MS, Liton Francisco, Stephen Forman, Smita Bhatia, M.D., M.P.H, Sadhna Shankar, M.D., M.P.H

Objectives
A third of long-term HCT survivors report severe or life-threatening chronic health conditions, placing significant demands on the healthcare system for a prolonged period of time. However, utilization patterns of healthcare services among minorities differ from those of non-Hispanic whites in the general population. The aim of this study was to evaluate healthcare utilization by adult long-term Hispanic HCT survivors.

Methods
A mailed questionnaire was used to assess self-reported health care utilization in three domains: general contact with healthcare system, general physical examination (GPE), and cancer/HCT-related visit. Eligible individuals had undergone HCT between 1974 and 1998, at 21 years of age or older and survived 2 or more years after HCT.

Results
The entire cohort consisted of 818 individuals, including 137 Hispanic survivors. The median age at HCT was 38.3 years (range: 21-68 years) and the median length of follow-up was 6.6 years (range: 2-24 years). A larger proportion of Hispanic survivors had family income < $20,000 (45.6% vs. 8.8% in non-Hispanics, p<0.001), high school or lower education (37.5% vs. 6%, p<0.001), and lacked health insurance (22.4% vs. 4.6%, p<0.001). Overall, 97% of non-Hispanic whites and 92% of Hispanics reported medical contact 11+ years after HCT (p=0.15). Compared to 2-5 years after HCT, the prevalence of GPE increased among non-Hispanic whites (67% to 76%, p for trend =0.05) but remained unchanged among Hispanics (66% to 61%, p for trend=0.68) 11+ years after HCT. On the other hand, while the proportion of non-Hispanic white survivors reporting a Cancer/HCT-related visit decreased from 94% at 2-5 years after HCT to 54% 11+ years after HCT (p for trend<0.001), the comparable figures in Hispanics were 96% and 81% (p for trend=0.03).

Conclusion
As compared to non-Hispanic whites, Hispanic survivors are less likely to establish contact with a primary care provider years after the HCT and continue to receive care primarily at Cancer/HCT center for many years following HCT. Future studies of this population are needed to establish the factors responsible for this pattern of healthcare utilization.
EQ-5D Preference Weights in Liver Transplant Patients: Effects of Transplantation, Hepatitis C Virus, and Recurrence

Russell R, Feurer I, Wisawatapnimit P, Pinson CW

Background
Health utility instruments measure quality of life and assess the value placed on specific health states, which can be converted to quality-adjusted life years and applied in cost-utility analyses. US preference weights (PW) for the EQ-5D survey were reported by Shaw, and vary from worse than death (-0.109) to perfect health (1.0). This study evaluates the effects of liver transplantation, HCV infection, and HCV recurrence on EQ-5D PW.

Methods
EQ-5D dimension scores were converted to PW using Shaw’s model. Data were stratified by measurement period: pre-transplant, early- (≤12 m), intermediate- (>12&≤36 m), and late-post (>36 m). Pre-transplant HCV infection and its recurrence prior to assessment were recorded. Data were analyzed using nonparametric methods and summarized as mean ± SD.

Results
285 patients completed surveys and follow-up averaged 33±36 months (range:0.8-133). For those with recurrent HCV, mean time to recurrence was 18±18 months. The percentage of patients with HCV recurrence differed by follow-up periods (11%, 57%, 81%;p<0.001). EQ-5D PW for the cohort were significantly improved from pre- to late-post transplant. Time post-transplant was associated with improved PW in HCV- (r=0.31;p<0.001) but not in HCV+ recipients (r= -0.09;p=0.23). However, time post-transplant had a negative effect in HCV+ patients with recurrence (r= -0.27;p=0.07), but no effect on PW in HCV+ recipients without recurrence (r=0.01;p=0.49).
ACUTE RENAL FAILURE DURING EXTRACORPOREAL SUPPORT IN THE PEDIATRIC CARDIAC PATIENT

Andrew H. Smith, Daphne C. Hardison, Christy R. Worden, Geoffrey M. Fleming, Mary B. Taylor

Objectives
End-organ dysfunction has been associated with increased mortality in pediatric cardiac patients requiring extracorporeal support. We sought to further characterize mortality risks specifically associated with acute renal failure (ARF) in this population.

Methods
Records of all cardiac patients in our pediatric intensive care unit receiving ECMO support from 1 July 2005 through 30 July 2007 were reviewed for data with respect to their ECMO course. ARF while on ECMO was defined as a fluid retention or electrolyte disturbance resulting in institution of CRRT, or a GFR of <35ml/min/1.73m².

Results
There were 49 ECMO cases (98% VA) in 48 patients. Median age was 16 days (0-945 days) and median weight was 3.4 kg (1.7-57.0 kg). ARF was present in 71.7%, and CRRT was initiated in 58.7%. Odds for developing ARF increased by 68.3% per day of ECMO support (β 1.68, 95% CI 1.13-2.49, p=0.01), independent of both patient age and weight. Overall survival to hospital discharge was 37.5%. ARF during ECMO, despite adjusting for weight and patients with single-ventricle physiology, remained associated with a significant decrease in odds of survival to discharge (OR 4.5, 95% CI 1.02-19.5, p=0.047).

Conclusions
Renal failure is a common manifestation of end-organ dysfunction in pediatric cardiac patients requiring extracorporeal support. Increasing duration of ECMO support is associated with development of acute renal failure. Independent of single-ventricle physiology and patient weight, ARF while on ECMO is associated with a significant decrease in the odds of survival in the pediatric cardiac patient.
PREDICTING SLEEP APNEA IN CHILDREN WITH DOWN SYNDROME

Karen L. Summar, MD

Objectives
Obstructive sleep apnea (OSA) is common in the general population and has been associated with morbidity including hypertension, obesity, coronary artery disease and stroke. In addition, OSA has been implicated in behavior problems in typically developing children. Adults and children with Down syndrome are at increased risk of OSA as compared to the typical population, but specific risk factors for children with Down syndrome have not been elucidated. This pilot study was performed to identify risk factors for OSA in children with Down syndrome.

Methods
Twenty children, ages 6 through 16 years, with Down syndrome participated in this study. A structured interview with the child’s parent included a medical history, a sleep history, as well as a history of maladaptive daytime behaviors. Parents also completed two well validated questionnaires, the Child Sleep Habits Questionnaire and the Child Behavior Check List. The children then underwent 2 consecutive nights of polysomnography (PSG). Statistical analysis was performed with SPSS, version 16.

Results
Twelve of the 20 participants (0.60) had evidence of OSA on PSG, including 5 girls and 7 boys. One half of the children who had OSA had a history of tonsil and adenoid removal in the past.

<table>
<thead>
<tr>
<th>Risk Factor</th>
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</tr>
<tr>
<td>Hospitalization croup/RSV</td>
<td>0.026</td>
</tr>
<tr>
<td>Hospitalization all others</td>
<td>0.288</td>
</tr>
<tr>
<td>Snores loudly</td>
<td>0.107</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>0.298</td>
</tr>
<tr>
<td>Impulsive</td>
<td>0.827</td>
</tr>
<tr>
<td>Seems tired during day</td>
<td>0.028</td>
</tr>
</tbody>
</table>

*Correlations were calculated using Spearman’s rho for ordinal variables. P values are reported as 2 sided.

Conclusions
We found that OSA in children with Down syndrome is frequent co-morbidity and that it is difficult to predict who will have OSA based upon common symptoms. The first line of treatment for OSA in children is tonsillectomy and adenoidectomy (T & A) and this study found that half of the children with OSA had previously undergone T & A. Further study should be done to evaluate whether all children with Down syndrome should be screened for primary OSA and then screened after T & A for recurrence of OSA.
MULTI-INSTITUTIONAL COMPARISON OF OPEN VS. LAPAROSCOPIC PEDIATRIC PYELOPLASTY: DO BENEFITS OF LAPAROSCOPIC APPROACH RECEDE AT YOUNGER AGES?

Stacy T. Tanaka, John A. Grantham, John C. Thomas, Mark C. Adams, John W. Brock III and John C. Pope IV

Objectives
Ureteropelvic junction obstruction is the most common cause of congenital hydronephrosis. Although open dismembered pyeloplasty has been the standard surgical treatment, the laparoscopic approach has been increasingly used. The potential benefits of laparoscopic pyeloplasty may recede in younger age groups. We used a multi-institutional database to address the effect of laparoscopic approach on length of stay, postoperative parenteral narcotic use and postoperative antiemetic use in specific pediatric age groups.

Methods
We performed a retrospective study of 5,261 children with an ICD-9 procedure code for correction of ureteropelvic junction obstruction from Pediatric Health Information System (PHIS), a database of freestanding children’s hospitals. Discharge dates from January 1, 2002 to June 30, 2007 were included. Laparoscopic cases were identified by ICD-9 procedure codes and hospital equipment charges. To investigate the effect of laparoscopic approach on length of stay, parenteral narcotic use and antiemetic use, we used multivariate linear regression while controlling for other variables in the following age categories: infant (³ 1 month and < 2 years), preschool (³ 2 and < 6 years), grade school (³ 6 and < 10 years), pre adolescent (³ 10 and < 13 years), and adolescent (³ 13 and < 19 years).

Results
Laparoscopic approach decreased length of stay and the number of parenteral narcotic pharmacy charges in the pre adolescent (p = 0.03, p = 0.005) and adolescent (p = 0.03, p = 0.006) groups but not in the any of the younger groups. Laparoscopic approach did not affect the number of antiemetic pharmacy charges.

Conclusions
The laparoscopic approach was associated with a shorter hospital stay and decreased parenteral narcotic use in pre-adolescents and adolescents but not in younger children.
PEDIATRIC URETEROSCOPIC MANAGEMENT OF KIDNEY STONES

Stacy T. Tanaka, John H. Makari, John C. Pope IV, Mark C. Adams, John W. Brock III and John C. Thomas

Objectives
Although kidney stones are uncommon in children, its incidence does seem to be increasing. There are many minimally invasive methods to remove kidney stones including endoscopic treatment. Data addressing ureteroscopic management of intrarenal calculi in prepubertal children are limited. We reviewed our experience from January 2002 through December 2007.

Methods
We retrospectively reviewed ureteroscopic procedures for intrarenal calculi in children less than 14 years of age. Stone free status was determined with postoperative imaging. Multiple logistic regression was used to assess the influence of preoperative factors on the need for additional procedures.

Results
Intrarenal calculi were managed ureteroscopically in 52 kidneys in 50 children with a mean age of 7.9 years (range 1.2 to 13.6). Mean stone size was 8 mm (range 1 to 16). Stone free rate after a single ureteroscopic procedure was 25 of 50 (50%) on initial postoperative imaging and 29 of 50 (58%) with extended follow up. Additional stone procedures were required in 18 upper tracts. Both younger age (p = 0.04) and larger preoperative stone size (p = 0.002), but not lower pole stone location (p = 0.49) were significant predictors of the need for additional procedures. Additional procedures were required in over half of stones greater than or equal to 6 mm but in no stones less than 6 mm.

Conclusions
Ureteroscopy is a safe method for the treatment of intrarenal calculi in the prepubertal population. Our ureteroscopic stone free rate for intrarenal stones is lower than that reported for ureteral stones. Parents should be informed that additional procedures are likely especially in younger patients or patients with stones larger than 6 mm.
USE OF SCENE VITAL SIGNS IMPROVES TRISS PREDICTED SURVIVAL IN INTUBATED TRAUMA PATIENTS

Igor V. Voskresensky, B.S., Tanya Rivera-Tyler, M.D., Robert O. Carpenter, M.D., M.P.H., William P. Riordan, Jr., M.D., Bryan A. Cotton, M.D.

Objectives
The Trauma Related Injury Severity Score (TRISS) has been previously validated to predict outcomes in non-intubated, non-paralyzed trauma patients. The purpose of this study was to assess the impact of scene vital signs on predicting survival in intubated trauma patients.

Methods
Our Trauma Registry of the American College of Surgeons (TRACS) was reviewed for all trauma patients admitted between 10/01/04-09/30/06 and arriving by aeromedical transport. TRISS was evaluated for each patient based on their (1) scene vital signs and (2) arrival vital signs. Additionally, the “TRISS-like” score was calculated for each patient. Expected mortality for each score was measured against observed mortality.

Results
4499 TRACS patients were admitted during the study period. 695 (15%) were transported by air. 163 patients (23%) arrived intubated. 480 arrived non-intubated. Observed survival in the intubated group was 76%. Observed survival in the non-intubated group was 100%. TRISS using scene vital signs more closely predicted mortality among intubated patients than the other scoring systems (69% versus 39% using TRISS-arrival versus 80% using TRISS-like). Scene vital signs with TRISS also resulted in less “unexpected” outcomes (survivors and deaths).

Table 1. Results for different TRISS methods in intubated patients.

<table>
<thead>
<tr>
<th>Survival score methodology</th>
<th>Predicted survival</th>
<th>Observed survival</th>
<th>“Unexpected ” survivors</th>
<th>“Unexpected ” deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRISS by scene vital signs</td>
<td>112 (69%)</td>
<td>124 (76%)</td>
<td>20 (16%)</td>
<td>13 (33%)</td>
</tr>
<tr>
<td>TRISS by arrival vital signs</td>
<td>64 (39%)</td>
<td>124 (76%)</td>
<td>74 (60%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>“TRISS-like” by scene vital signs</td>
<td>130 (80%)</td>
<td>124 (76%)</td>
<td>6 (5%)</td>
<td>26 (67%)</td>
</tr>
<tr>
<td>“TRISS-like” by arrival vital signs</td>
<td>130 (80%)</td>
<td>124 (76%)</td>
<td>5 (4%)</td>
<td>21 (54%)</td>
</tr>
</tbody>
</table>

Conclusions
Traditionally, patients arriving to trauma centers intubated are either excluded from the trauma registry or have their physiological score “modified” to account for pharmacologically altered respiratory rate and GCS. In intubated patients, TRISS using scene vital signs more reliably predicts survival and does so with far fewer “unexpected” outcomes than with other available scoring systems.
THE IMPACT OF SPECIFIC TREATMENT PATTERNS ON SURVIVAL FOR CHILDREN WITH LOW AND HIGH GRADE GLIOMAS: ANALYSIS OF THE 1973-2002 SEER DATA

W. Woods-Swafford, J. F. Kuttesch, J. Barnholtz-Sloan

Objectives
Gliomas account for greater than 50% of all pediatric CNS tumors. Investigations into treatment effects and long-term prognosis are limited to single institutional reviews with small sample sizes. We examined trends in survival of pediatric gliomas in the United States using population-based data from the Surveillance, Epidemiology and End Results (SEER) program.

Methods
Study subjects from the SEER Public-use database included 2,799 pediatric and adolescent patients diagnosed with microscopically confirmed primary glial tumors from 1973-2002 with follow-up through 2004. Frequencies of prognostic variables of interest were assessed for differences by time period (1973-79, 1980-89, 1990-02) using chi-square tests. Survival analyses were performed using Kaplan Meier and Cox regression models.

Results
Evaluation of histologic types over time indicated a shift in distribution from astrocytoma, NOS to WHO Grade I/II and oligodendroglial tumors. WHO grade III/IV and Glioma histology types had worse survival when compared to those with astrocytoma, NOS for all periods. In the most recent period, Blacks had an increased risk of death compared to Whites (HR=1.51, CI 1.06,2.14). Surgical management alone was consistently and significantly associated with improved survival (35% decreased risk of death in 1973-79 improving to a 77% decreased risk of death in 1990-02) whereas radiation alone following biopsy (~2 fold increased risk of death in all time periods) and biopsy alone were associated with worse survival when compared to a combination of radiation and surgery. Subset analyses evaluating extent of resection on survival showed any surgical intervention significantly decreased the risk of death (~80% decreased risk of death) but the addition of radiation counteracted this decrease.

Conclusions
Gliomas treated with radiation alone following biopsy had increased risk of death. Surgical resection, regardless of extent, decreased the risk of death significantly. However, combining radiation with surgery eliminated this survival advantage. In a therapy era focused on reducing neuropsychological deficits, these findings mandate further investigation into the role of radiation therapy in this population.
INJURIES IN ULTIMATE FRISBEE PLAYERS AT THE 2007 UPA COLLEGE CHAMPIONSHIPS

Lesianne Yen, M.D.

Abstract
Ultimate Frisbee is a seven-on-seven field sport that was introduced in the 1970s. Jumping, sharp direction changes and sudden deceleration are known risk factors for severe knee injury and are common movements in ultimate frisbee. Despite these high risk features, little has been documented on ultimate frisbee-related injuries.

Objectives
To quantify and describe the injuries incurred by both male and female ultimate players participating in the 2007 Ultimate Players’ Association (UPA) College Championships.

Methods
Each of the 90 games of the 2007 UPA College Championships was observed by a member of the research staff. Every athlete who left the field of play after calling an injury time-out (a stoppage of play for any injury) was interviewed regarding the type and circumstances of their injury. Missed playing time was quantified.

Results
There was only one injury that prevented return to play in each of the two divisions: men’s and women’s. Rate of injuries preventing return to play in both divisions was 1.6 per 1000 athlete-exposures. Among men, there were 1.5 injury time-outs per game. In the women’s division, there were 0.91 injury time-outs called per game. Greater than 50% of injury time-outs for both men and women were related to contact. Most were not associated with a rules violation. The majority of injuries was to the lower extremities and occurred in association with jumping and laying out (diving).

Conclusions
There was a low rate of play-ending injuries at the 2007 UPA College Championships compared to what is observed in less elite tournaments. Despite the fact that Ultimate Frisbee rules prohibit contact, there was frequent contact that resulted in an injury time-out.
HYBRID CORONARY REVASCULARIZATION: COMBINED CORONARY ARTERY BYPASS GRAFTS AND PERCUTANEOUS CORONARY INTERVENTIONS

Frank Zheng Zhao

Background
Current treatment for cardiac ischemia due coronary atherosclerosis include: coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI). The Vanderbilt hybrid operating room allows us to treat select patients with a combined approach. One-stop hybrid coronary revascularization applies the advantages of both types of procedures for complex patient populations in a single operative setting.

Objectives
To evaluate hybrid coronary revascularization safety and efficacy at Vanderbilt Medical Center.

Materials and Methods
From April 2005 to July 2006, 239 patients with multi-vessel coronary artery disease (CAD) underwent coronary bypass surgery. 78 (32%) patients received hybrid revascularization. These patients received the LIMA to LAD bypass graft with a combination of PCI and/or vein grafts. Indications for hybrid revascularization were divided as follows: 23 (29%) patients had no graftable target vessels but favorable lesions for PCI; 4 (5%) patients had poor or no conduits; 14 (18%) patients required decreased operative risk; 27 (35%) patients required PCI to revise defects identified on completion angiogram. All patients had completion angiograms and received 300 mg of Plavix. 150 grafts and 146 stents were placed during hybrid therapy.

Results
Post-operative results were compared using the patient group receiving standard CABG (n=161) versus hybrid revascularization (n=78). There were no significant differences between the two groups in hospital mortality, perioperative MIs, chest tube drainage, post-operative bleeding, wound infection, stroke, renal function, and length of stay. We also reduced the average aortic cross-clamping time by 7 minutes in the hybrid group, resulting in improved intra-operative cardiac perfusion.

Conclusions
We believe that hybrid CABG-PCI is feasible and safe with comparable clinical outcomes to standard CABG surgery. This approach may prove to be the revascularization method of choice in complex patient populations. Long-term benefits of the procedure are currently being investigated.
Background
Epilepsy has been reported in almost 1% of the population. The best diagnostic tool for this disease is electroencephalography (EEG) and is done in patients who are suspected to have epilepsy. EEG helps to get a syndromic diagnosis of epilepsy to select the most appropriate treatment. The yield of the first routine EEG is only 27-55% which increases with subsequent EEGs to the maximum yield of 90%. Continuous inpatient video EEG monitoring provides the ability to get ictal and interictal recordings with the withdrawal of antiepileptic drugs. However the limiting factors including the cost, long wait periods, availability only at big centers etc. makes this unavailable for most patients. As an alternative, prolonged outpatient video-EEG monitoring seems promising. However this diagnostic tool has not gain much acceptance. At Vanderbilt University Comprehensive Epilepsy Program, we perform outpatient long term video EEGs for two and four hours. This study compared the diagnostic yield of the regular EEGs (REEG) with two hours outpatient video EEG monitoring (O vem).

Methods
Patients above age 16 were included with at least one outpatient regular EEG and two hour OVEM done at Vanderbilt from January 2005 till October 2007.

Results
Total 402 patients underwent OVEM while 70 patients had both REEG and OVEM. Out of these 70 patients, REEGs were able to capture typical events in two patients (2.8%) while OVEMs captured typical spells in nine patients (12.8%) (Chi square OR=5.0 (0.95-26.8)). OVEM confirmed the findings of abnormal REEG except in 2 patients. OVEM found epileptiform abnormalities in 18 patients and non-epileptiform abnormalities in 10 patients, which were missed by REEG (Chi square, \( p<0.0001 \)).

Conclusions
OVEM is more likely to capture a typical spell, than REEG. OVEM is significantly likely to be conclusive in evaluation of epilepsy, compared to REEG (\( p<0.0001 \)).