TWENTY- FIFTH ANNUAL

Stanford Medical Student Research Symposium

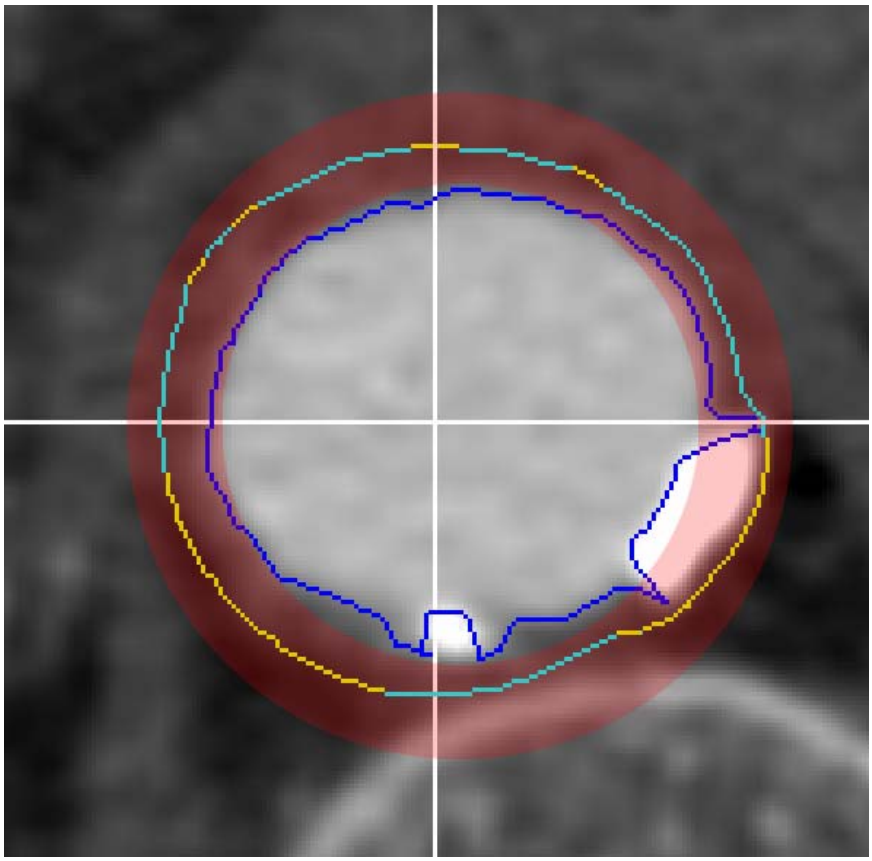
&

FIFTH ANNUAL

POM Population Health Symposium

May 14, 2008
Hospital Atrium

Stanford University Medical Center



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Stanford Medical Student Research Symposium

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Cover Reference:

Bhargav Raman, Raghav Raman, Sandy Napel, Geoffrey D. Rubin
QUANTIFICATION OF SOFT ATHEROSCLEROTIC PLAQUE IN THE SYSTEMIC ARTERIES USING CT ANGIOGRAPHY
A cross-section through the aorta.
Dark Blue: Inner wall excluding calcifications encloses flow channel containing iodine-enhanced blood (bright).
Red: Search Area
Yellow: Well defined outer wall segments used as anchors.
Light Blue: Final calculated outer wall.

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May 14, 2008

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3:00pm-5:45pm

Poster Session

Marissa Aillaud
James Andrews
Jacqueline Baras
Pavan Bendapudi
Catey Bradford
Thea Brennan-Krohn
Bernard Chang
Mark Chao
Yi-Ren Chen
Richard Chiu (2 projects)
B. Elizabeth Delosobera
Harpreet Dhatt
John Downey
Betsy Encarnacion
Alana Frost (2 projects)
Teresa Fu
Michael Galvez
Liana Gefter
Matthew Goldstein
Carly Gomes
Tress Goodwin
Elsie Gyang
Mark Hammer
Stanley Hoang
Paul Hoover
Natalia Isaza
Sha Jones
Gavi Kohlberg
David Krodel
Andrew Lee

Yueyi Irene Liu
Gene Ma
David Meister
Steve Minear
Jayson Morgan
Jason Moss
Sarah Nelson
Phuong Nguyen
Paul Nuyujukian
Erin Palm
Victoria Parikh (2 projects)
Yannis Paulus
Jeremy Pearl
Joe Peraza
Karthikeyan Ponnusamy
James Priest
Bhargav Raman
Jeremiah Ray
Roberto Ricardo-Gonzalez (2 projects)
Chandler Robinson
Keyan Salari
David Shin
Meera Sridhar
Shobha Stack
Emily Tsai
Yana Vaks
Wenshuai Wan
Aaron Wang
Ariel Williams

5:45pm

Closing Remarks

Pat Cross, PhD
Professor of Structural Biology
Associate Dean of Student Affairs

Preetha Basaviah, MD
Associate Clinical Professor of Medicine
Associate Director of Practice of Medicine Course

Awards

Norman Tong, MD
President of the Stanford University Medical Center Alumni Association
Board of Governors

PRESENTATIONS

Student Name	Mentor Name	Title of Abstract
Aillaud, Marissa	Reaven, Gerald	*Comparison of inflammatory markers in subcutaneous adipose tissue obtained from equally-obese insulin sensitive and insulin resistant individuals*
Andrews, James	Feldman, Heidi	*White matter differences and reading in preterm children*
Baras, Jacqueline	Garber, Alan & Owens, Douglas	*Management of acute kidney injury in the ICU: A cost-effectiveness analysis of daily versus alternate-day dialysis*
Bendapudi, Pavan	Baker, Laurence	*Active case finding for tuberculosis: Feasibility and effectiveness of symptomatic screening in a high-prevalence urban setting*
Bradford, Catey	Grainger-Monsen, Maren	*Burn care at a South African hospital: A photo-essay*
Brennan-Krohn, Thea	Maldonado, Yvonne	*Detection of vaccine and mutant strains of poliovirus serotypes 1 and 3 by PCR*
Chang, Bernard	Shafer, Audrey	*Curriculum change from the trenches: Reflections by a medical student on medical education*
Chao, Mark	Weissman, Irving	*Targeting CD47 eliminates human acute myeloid leukemia stem cells*
Chen, Yi-Ren	Liao, Y. Joyce	*Horizontal eye movement abnormalities in pseudotumor cerebri*
Chiu, Richard	Goodman, Stuart	*Polymethylmethacrylate particles inhibit osteoblastic differentiation of MC3T3-E1 osteoprogenitor cells*
Chiu, Richard	Goodman, Stuart	*Ultrahigh molecular weight polyethylene wear debris inhibits osteoblastic differentiation of bone marrow osteoprogenitors and MC3T3-E1 preosteoblasts in vitro*
Delosobera, B. Elizabeth & Goodwin, Tress	Gilbert, Gregory & Mahadevan, S.V.	*Evaluating efficacy of educational interventions in an international setting*
Dhatt, Harpreet	Biswal, Sandip	*A difference in the pattern of 18F-FDG uptake within the spinal canal in low back pain patients*
Downey, John	Morton, John & Jeffrey, R. Brooke	*Gastric pouch volume correlates with weight loss following Roux-en-Y gastric bypass*
Encarnacion, Betsy	Morton, John	*Primary care physician knowledge and attitudes regarding bariatric surgery*
Frost, Alana	Peebles, Rebecka	*Development of a web-based behavior change program for children and adolescents with obesity*
Frost, Alana	Peebles, Rebecka	*Vitamin D and other micronutrient deficiencies in adolescents after gastric bypass surgery*
Fu, Teresa	Twist, Clare	*Clinical significance of immunohistochemistry-detected MRD in neuroblastoma*
Galvez, Michael	Gurtner, Geoffrey	*Sutureless microvascular anastomosis using thermoreversible poloxamers*
Gefter, Liana	Golianu, Brenda	*Quantitative sensory testing in young women with fibromyalgia: An examination of thermal sensitivity at " tender" and" non-tender" point locations*
Goldstein, Matthew	Levy, Ron	*Cure of large tumors by "immunotransplant" of anti-lymphoma primed lymphocytes into lympodepleted recipients*
Gomes, Carly	Yeomans, David	*An animal model for trigeminal neuralgia*
Goodwin, Tress	(see Delosobera)	
Gyang, Elsie	Jeng, Michael	*Silent infarcts in patients with sickle cell disease at high risk for cerebral vascular accidents on chronic red cell transfusion therapy*
Hammer, Mark	Meyer, Tobias	*Coordinated cell migration in a confluent monolayer is driven by viscous drag*
Hoang, Stanley	Steinberg, Gary	*Netrin-4 enhances angiogenesis and neurological outcome after cerebral ischemia*
Hoover, Paul	Lewis, Richard	*Stoichiometry of STIM1 and ORAI1 in the SOC channel complex*

Student Name	Mentor Name	Title of Abstract
Isaza, Natalia	Dutta, Sanjeev	*Steerable sheath for endoscopic and translumenal surgery*
Jones, Sha	Crawley, LaVera	*Finding HPV vaccine content in black social media*
Kohlberg, Gavi	Butte, Atul	*Discovery of novel AML tumor associated antigens using antibody signatures and gene-expression data*
Krodel, David	Wise, Paul	*Chronically ill children and the hospital safety-net system in California 1998-2004*
Lee, Andrew	Wu, Joseph	*Impact of long term in vitro culture upon mitochondrial function in human embryonic stem cells*
Liu, Yueyi Irene	Butte, Atul	*A human disease "etiome"*
Ma, Gene	Goodman, Stuart	*Polymethylmethacrylate particles alter p38 MAPK activity in MC3T3-E1 preosteoblast cells undergoing differentiation*
Meister, David	Ladd, Amy	*A simple device and method to quickly align, approximate, and attach the deep dermis and superficial fascia system*
Minear, Steve	Nusse, Roel & Helms, Jill	*Wnt-mediated bone regeneration*
Morgan, Jayson	Blau, Helen	*Characterization of the skeletal muscle in microRNA-1-2 knockout mice and screening meaningful microRNAs in satellite cells*
Moss, Jason	Chang, Steven	*Sterotactic radiosurgical treatment of hemangioblastomas in von Hippel-Lindau disease*
Nelson, Sarah	Herschlag, Daniel	*Testing the importance of surrounding residues in positioning hydrogen bond donors in the oxyanion hole of ketosteroid isomerase*
Nguyen, Phuong	Giaccia, Amato	*Using synthetic lethality as a new approach to treat RCC: A novel molecule induces VHL-deficient cell death through inducing metabolic derangement*
Nuyujukian, Paul	Shenoy, Krishna	*HermesC: RF wireless low-power neural recording system for freely behaving primates*
Palm, Erin	Norris, Robert	*Sequelae and patient follow-up after minor head injury*
Parikh, Victoria & Sridhar, Meera	Buckingham, Bruce	*Describing the underlying cause of childhood obesity in India: A case control pilot study*
Parikh, Victoria	Mobley, William	*Effect of ginkgolide A on behavioral and biochemical markers of learning and memory in Ts1Cje, a mouse model of down syndrome*
Paulus, Yannis	Blumenkranz, Mark & Palanker, Daniel	*An analysis of healing following retinal photocoagulation*
Pearl, Jeremy	Goodman, Stuart	*Role of the toll-like receptor pathway in recognition of wear particles*
Peraza, Joe	Morton, John	*The effect of gastric bypass on cognition*
Ponnusamy, Karthikeyan	Helms, Jill	*Engineering a Wnt hodrogel delivery system for tissue regeneration*
Priest, James	Braddock, Clarence	*Comparison of rounding activities and perceptions between hospitals and services*
Raman, Bhargav	Napel, Sandy	*Quantification of soft atherosclerotic plaque in the systemic arteries using CT angiography*
Ray, Jeremiah	Froelicher, Victor	*High intensity resistance training induces left ventricular eccentric hypertrophy without lowering blood pressure or heart rate in young males*
Ricardo-Gonzalez, Roberto	Chawla, Ajay	*PPARδ drives alternative (M2) activation of kupffer cells to ameliorate obesity-induced insulin resistance*
Ricardo-Gonzalez, Roberto	Chawla, Ajay	*TH2 cytokines and STAT6 regulate hepatic fuel selection and enhance insulin action*
Robinson, Chandler	Bundorf, Kate	*Knowledge of medical errors: A significant determinant of risk perception*

Student Name	Mentor Name	Title of Abstract
Salari, Keyan	Pollack, Jonathan	*Integrative analysis of genomic and transcriptional profiles in colorectal cancer*
Shin, David	Cooke, John	*Asymmetric dimethylarginine predicts mortality and major adverse cardiovascular events in patients with peripheral arterial disease*
Sridhar, Meera	(see Parikh, Victoria)	
Stack, Shobha	Miller, D. Craig	*Quantitative histology of the mitral valve in an ovine model*
Tsai, Emily	Plevritis, Sylvia	*Overdiagnosis in lung cancer screening and its impact on mortality: Simulation based sensitivity analysis*
Vaks, Yana	Barnes, Patrick	*Magnetic resonance analysis of the hippocampal volumes in children with seizures*
Wan, Wenshuai	Macario, Alex	*Improving safety in the operating room: A systematic literature review of retained surgical sponges*
Wang, Aaron	Liang, David & Taylor, Charles	*Aiding the detection of vascular trauma with image-based models of blood flow*
Williams, Ariel	Brooks, James	*Renal cell carcinoma: molecular markers of histologic subtype assessed by tissue microarray*

COMPARISON OF INFLAMMATORY MARKERS IN SUBCUTANEOUS ADIPOSE TISSUE OBTAINED FROM EQUALLY-OBESE INSULIN SENSITIVE AND INSULIN RESISTANT INDIVIDUALS

Marissa Aillaud, Tracey McLaughlin, Alicia Deng, Shawn Mullen, Oscar Gonzales, Gail Yee, Cindy Lamendola, Fahim Abbasi, Sam Cushman, Gerald Reaven, Phil Tsao. Department of Medicine (Cardiovascular Medicine).

We have described differences in cell-size distribution and expression of genes related to adipocyte differentiation in subcutaneous abdominal fat obtained from equally-obese insulin sensitive (IS) and resistant (IR) persons. To evaluate other possible differences related to insulin sensitivity in equally-obese individuals we quantified markers of inflammatory activity in adipose tissue from overweight, IR and IS individuals.

Subcutaneous abdominal tissue was obtained from moderately obese women, divided into IR (n=14) and IS (n=19) subgroups by determining their steady-state plasma glucose (SSPG) concentration during the insulin suppression test. Inflammatory activity was assessed by comparing expression of 9 relevant genes, and by immunohistochemical quantification of CD45 and CD68-containing cells. SSPG concentrtions were approximately 3-fold higher in IR individuals. Expression of CD68, EMR1, IL-8, IL-6, and MCP/CCL1 were modestly, but signficantly increased ($p<0.05$) in IR subjects. Results of immunohistochemical staining were consistent with gene expression data, demonstrating modest differences between IR and IS individuals. Crown-like structures, in which macrophages surround single adipocytes, were rarely seen in tissue from either subgroup.

A modest increase in inflammatory activity was seen in subcutaneous adipose tissue from IR as compared to equally-obese IS individuals. Since IR and IS women did not differ in terms of adiposity, per se, it appears that modest obesity, by itself, does not lead to inflammatory changes in adipose tissue. These data provide additional evidence that biological properties of adipose tissue may be more closely related to differences in insulin sensitivity than amount of adipose tissue in moderately obese individuals.

Funding provided by the Stanford Medical Scholars Fellowship Program.

WHITE MATTER DIFFERENCES AND READING IN PRETERM CHILDREN

James S. Andrews[1], Michal Ben-Shachar[2], Jason D. Yeatman[3], Beatriz Luna[4], and Heidi M. Feldman[1, 3]. [1]School of Medicine, [2]Department of Psychology, [3]Department of Pediatrics, [4]Department of Psychiatry and Psychology-Carnegie Mellon University.

Children born preterm are at risk of white matter injury. Diffusion tensor imaging (DTI) allows us to characterize microstructural properties of white matter in these children and to compare these properties between preterm and full-term children. Previous studies have found that microstructural white matter measures, including fractional anisotropy (FA), correlate with tests of neuropsychological function, such as reading performance in children. To our knowledge no study has tested for similar associations in preterm children. We tested the hypothesis that FA values correlate with gestational age (GA), birth weight (BW), and reading scores in previously identified white matter regions, including a left temporo-parietal region and anatomical divisions of the corpus callosum (CC).

We studied white matter structure in a cohort of adolescents, ages 11-15 years (9 preterm, 5 full-term controls), with a range of reading scores. GA ranged from 24 to 39 weeks (preterm mean: 30.4wks), BW ranged from 624 to 3000 grams (preterm mean: 1307gm), and reading scores ranged from 90 to 130 (overall mean: 105.3, preterm mean: 101.1, full-term mean: 112.8). Using a Region of Interest (ROI) approach, we failed to identify significant correlations between FA, BW, and/or reading in a temporo-parietal ROI (MNI coordinates: -28, -28, 25), a region where FA correlates with reading scores in adults and children with reading difficulties. However, we discovered correlations between BW and FA and between reading and FA measured in the whole CC ($p<0.05$). Within the CC, BW-FA and reading-FA correlations were found in the genu ($p<0.05$) and body ($p<0.01$).

The results corroborate previous findings of an association between white matter microstructural features in the anterior and middle corpus callosum and prematurity. We report a novel observation that in these regions of the CC, FA correlates with reading scores. In future studies we will apply whole brain, voxel-based analyses to examine additional differences in white matter between adolescents born preterm and full term.

Funding provided by the Stanford Medical Scholars Fellowship Program.

MANAGEMENT OF ACUTE KIDNEY INJURY IN THE ICU: A COST-EFFECTIVENESS ANALYSIS OF DAILY VERSUS ALTERNATE-DAY DIALYSIS

Amar A. Desai, **Jacqueline D. Baras**, Benjamin B. Berk, Aya Nakajima, Alan M Garber, Douglas Owens, Glenn M. Chertow. Division of Nephrology and Center for Health Policy and Primary Care and Outcomes Research, Department of Medicine, Stanford University School of Medicine, Stanford, CA; Veterans Affairs Palo Alto Health Care System, Palo Alto, CA; and Stanford Graduate School of Business, Stanford, CA.

Although evidence suggests that higher hemodialysis dose and/or frequency may be associated with improved outcomes, the cost-effectiveness of a daily hemodialysis strategy for critically ill patients with acute kidney injury (AKI) is unknown. This analysis examines the cost-effectiveness of a daily hemodialysis strategy compared to a conventional (alternate-day) hemodialysis strategy for the treatment of AKI in critically ill patients. We developed a Markov model of the cost, quality of life, survival, and incremental cost-effectiveness of daily dialysis, as compared with alternate-day dialysis, for patients in the intensive care unit (ICU) with AKI. We employed a societal perspective with a lifetime analytic time horizon. We modeled the efficacy of daily dialysis as a reduction in the relative risk of death on the basis of data reported in the 2004 clinical trial published by Schiffl et al. One- and two-way sensitivity analysis was performed across cost, efficacy, and utility input variables. The main outcome measure was cost per quality adjusted life year (QALY).

In the base case for a 60-year-old male, daily dialysis was projected to add 2.14 QALYs and $10 924 in cost compared with alternate-day hemodialysis. The $5084 incremental cost-effectiveness ratio (ICER) reflects the costs of more frequent dialysis during acute hospitalization and prolonged survival associated with a larger proportion of patients alive with chronic kidney disease post-discharge. The small incremental difference in costs between the two strategies is explained by similar rates in the main driver of overall costs, maintenance dialysis following hospital discharge. Cost effectiveness was sensitive to the likelihood of requiring maintenance hemodialysis and to initial ICU survival rates. The ICER became less favorable (>$ 50 000 per QALY gained) when the maintenance dialysis rate of the daily dialysis group was varied to greater than 27%, and when the difference in 14-day post-discharge mortality between the alternatives was varied to less than 0.5%.

A daily dialysis strategy provides a cost effective option for managing AKI in critically ill patients that can be readily implemented in the large majority of hospitals that already provide hemodialysis services.

Dr. Desai was supported by a training grant from the Agency for Healthcare Research and Quality (AHRQ). Dr. Chertow was supported by R33 DK067645. Research was also supported in part by the Department of Veteran Affairs.

ACTIVE CASE FINDING FOR TUBERCULOSIS: FEASIBILITY AND EFFECTIVENESS OF SYMPTOMATIC SCREENING IN A HIGH-PREVALENCE URBAN SETTING

Holger Sawert, M.D., M.P.H. [1], Masami Fujita, M.D. [2], **Pavan K. Bendapudi** [3], Pricha Choontonusuri, M.D., M.P.H. [4], Dhanida Rienthong, M.S. [5] Stanford Advisor: Laurence Baker, Ph.D. 1. Human Development Network, The World Bank, Washington, D.C., USA 2. World Health Organization, Hanoi, Viet Nam 3. Stanford University School of Medicine, Stanford, California, USA 4. Bangkok Metropolitan Administration Public Health Service, Bangkok, Thailand 5. Department of Communicable Disease Control, Ministry of Public Health, Bangkok, Thailand

In contrast with cure rates for tuberculosis, which have risen steadily, case detection has remained relatively low. A large and growing proportion of cases in low-income countries live in urban areas with particularly high disease prevalence. To curb a further rise of the disease burden in these settings, alternatives to the current strategy of passive case finding need to be explored.

We surveyed 1,971 households with a total population of 6,341 in a socio-economically deprived area of Bangkok, Thailand. Socio-economic data and information on symptoms of tuberculosis were collected with a standardized questionnaire. Subjects reporting any symptom underwent diagnostic procedures including sputum smear- and culture examination and x-ray. Confirmed cases received treatment according to WHO standards.

Of 15 persons who were on treatment for tuberculosis at the time of the survey, five were found to be smear- and culture-positive. In addition, 78 subjects reported symptoms of tuberculosis, of which 44 underwent further diagnostic evaluation. In these, 15 cases with smear-positive tuberculosis and five smear-negative cases with positive culture results were found. Adjusting for symptomatic subjects not presenting for diagnostic procedures, the prevalence was 638/100,000 for tuberculosis of all forms, and 528/100,000 for smear-positive disease. Screening for symptoms increased the case detection ratio in smear-positive cases from less than 30% to more than 80%. The cure rate in newly detected smear-positive cases was 87%.

Active case finding based on symptomatic screening is highly effective in urban settings with high disease prevalence. Incremental costs were low at USD 38 per newly detected case. The intervention should be evaluated for wide-spread implementation in resource-poor countries.

This work was funded in part by the Research Fund for International Medical Cooperation, Ministry of Health, Labour and Welfare, Japan and by the Stanford Medical Scholars Research Program.

BURN CARE AT A SOUTH AFRICAN HOSPITAL: A PHOTO-ESSAY

Catey Bradford, Maren Grainger-Monsen, MD, Biomedical Ethics and Medical Humanities Scholarly Concentration, Adelin Muganza, MD, Head of the Chris Hani Baragwanath Hospital Burns Centre

Healthcare in South Africa is fraught with numerous challenges, including high rates of HIV/AIDS, violent crime, and poverty. Despite desegregation, the townships that black South Africans were banned to under Apartheid remain predominantly black, overcrowded, and steeped in poverty. They are also where thousands of burn injuries occur every year. Over the last twenty years, large numbers of rural villagers searching for job opportunities have migrated to South African cities. Lacking any kind of urban planning for such an influx, many South Africans live in informal settlements within the larger townships. These settlements are essentially shantytowns built of cardboard, tin, and wood. They have no electricity and most use paraffin to light stoves for cooking and heating their homes in the winter. In 2001, over 3000 people in South Africa were burned in paraffin-related incidents, both from accidents as well as assaults and suicide attempts.

Chris Hani Baragwanath Hospital is a 3000-bed public community hospital in Soweto, South Africa. The Burns Unit admits approximately 200 patients with burn injuries each year. Burn injuries are devastating to an individual and their family. Not only is there a high rate of mortality (50% in patients with 70% total body surface area affected), but patients also require long hospitalizations and many operations before they are able to return to their home and work. Their injuries are extremely painful in the acute stage, and they are disfiguring and disabling in the long term. Patients are often malnourished and greatly weakened by their injuries, requiring significant physical and occupational therapy to be able to return to their homes and work. With these photos depicting my experiences at the Chris Hani Baragwanath Hospital Burns Unit, I hope to provide a better understanding of the issues, how people are affected, and their challenges and struggles as they work to be able to return to their lives and families.

DETECTION OF VACCINE AND MUTANT STRAINS OF POLIOVIRUS SEROTYPES 1 AND 3 BY PCR

Thea Brennan-Krohn, Devasena Gnanashanmugam, Stacy Huang, Yvonne Maldonado. Department of Pediatrics – Infectious Diseases.

Attenuation of poliovirus to create the Sabin trivalent live attenuated oral poliomyelitis vaccine (OPV) is associated with point mutations in non-coding regions of the three virus serotypes (P1, P2 and P3). Back-mutations at these positions create revertant mutants which are capable of causing Vaccine Associated Paralytic Poliomyelitis (VAPP) and which spread to unvaccinated individuals through fecal-oral transmission of shed virus. A rapid, simple and inexpensive method of testing for shedding of revertant OPV will allow monitoring of OPV mutation and aid in management of VAPP in developing countries where more advanced diagnostics are not readily available.

We used reverse transcription to create cDNA from RNA samples of vaccine and revertant strains of all poliovirus serotypes. A single reverse primer was used for both strains of each serotype. We then identified forward primers that could distinguish between the vaccine strain of each serotype and the corresponding revertant strain on gel electrophoresis. The primers matched the sequence of interest except at the ultimate position (which corresponds to the mutation site) and the penultimate position. This method of sequence design has been shown to be effective in distinguishing sequences with single point mutations. We then applied a range of primer concentrations and of annealing temperatures during the PCR step to determine the optimal conditions for the assay. We have identified primers that detect only the revertant form of P1 or P3 at one annealing temperature but detect both vaccine and revertant forms of the same serotype at a different annealing temperature. In preliminary tests on RNA extracted from stool samples of children recently vaccinated with OPV, this assay has identified the presence or absence of shed vaccine and mutant strains of P1.

The next step in the project will be to identify a primer capable of distinguishing between the vaccine and revertant strains of P2, so that all three serotypes can be identified. The availability of this simple assay, which requires only a thermal cycler and basic laboratory supplies, will make the detection of poliovirus shedding much more feasible in developing countries. The WHO goal of polio eradication may likely be achieved in the coming years, and this achievement will probably be followed by cessation of OPV administration, creating a large unvaccinated population. This population will be vulnerable to VAPP, and thus an understanding of the dynamics of OPV circulation is critical for the development of post-eradication vaccination policies.

Funding provided by the Stanford Medical Scholars Fellowship Program.

CURRICULUM CHANGE FROM THE TRENCHES: REFLECTIONS BY A MEDICAL STUDENT ON MEDICAL EDUCATION

Bernard P Chang, PhD. & Audrey Shafer, M.D. Stanford Center for Biomedical Ethics

The past decade has seen a flurry of innovative curriculum changes to predoctoral medical education. This report follows the evolution of one medical student through medical school and his efforts at initiating content/learning style changes in the curriculum.

A brief discussion of the specific education contributions of his work are presented, from the initial design of problem based problem sets in preclinical courses, to the creation of new course material in clinical clerkships, and ultimately the design of a freestanding course on medicine and behavior, offered at the university.

A focus of this poster will be informational for students/faculty interested in the actual process and pitfalls often encountered by medical students invested in medical curriculum development.

Funding provided by the Stanford Medical Scholars Fellowship Program.

TARGETING CD47 ELIMINATES HUMAN ACUTE MYELOID LEUKEMIA STEM CELLS

Mark P. Chao[1], Ravindra Majeti[1,2], Ash A. Alizadeh[1,2], Irving L. Weissman[1,3]
[1]Institute for Stem Cell Biology and Regenerative Medicine
[2]Department of Medicine, Division of Hematology
[3]Departments of Pathology and of Developmental Biology
Stanford University School of Medicine, Stanford, California.

The long-term prognosis of patients with acute myeloid leukemia (AML) is extremely poor. A permanent cure requires elimination of the leukemic stem cell (LSC), the only cell population capable of initiating and maintaining leukemic disease. Therefore, the identification and targeting of unique LSC markers is essential for permanent cure. We have identified CD47 as a cell surface protein with high expression on human AML LSCs. Interestingly, the main function of CD47 is inhibition of immune phagocytosis by macrophages through binding its macrophage ligand Sirpα. In mouse leukemia models upregulation of CD47 is necessary for leukemogenesis. Thus, leukemic pathogenesis appears to be partly regulated by evading immune phagocytosis through CD47 upregulation. Using a monoclonal antibody, we investigated whether disrupting the CD47-Sirpα interaction could eliminate LSCs by macrophage phagocytosis.

A monoclonal antibody against human CD47 that blocks the CD47-Sirpα interaction was administered *in vitro* to LSCs isolated from human AML patients in a macrophage-containing cell culture. LSCs from all four AML patients underwent significant phagocytosis with treatment of anti-CD47 antibody compared to isotype controls. *In vivo*, coating LSCs with anti-CD47 antibody prior to xenotransplantation into NOG immune-deficient mice blocked disease engraftment while controls did not. Moreover, preliminary results demonstrate that *in vivo* administration of anti-CD47 antibody to AML-engrafted NOG mice significantly decreases their leukemic disease. Finally, gene-expression profiling from 122 AML patients showed that high CD47 expression predicts worse overall survival ($p=0.03$), independent from Flt3/ITD status ($p=0.02$), an established marker of poor prognosis in AML. This data thereby supports a possible role for an anti-CD47 antibody therapy in the clinics.

These data indicate that disrupting CD47 function in human AML can eliminate leukemic disease both *in vitro* and *in vivo*. Thus, an antibody-based therapy blocking CD47 function could have a profound effect as a potential cure for leukemia.

Funding provided by the Howard Hughes Medical Institute Medical Fellows Program and the Stanford Medical Scholars Fellowship Program.

HORIZONTAL EYE MOVEMENT ABNORMALITIES IN PSEUDOTUMOR CEREBRI

Yi-Ren Chen, Y. Joyce Liao. Department of Ophthalmology, Stanford University School of Medicine.

Pseudotumor cerebri (PTC), also known as idiopathic intracranial hypertension, is an important cause of vision loss, headache, and diplopia. It is a disorder of unknown etiology that is encountered most frequently in overweight women between the ages of 20 and 45.

To determine the prevalence of eye movement abnormalities in PTC, regardless of symptoms, we evaluated consecutive PTC patients and consented 7 (4 female, 3 male; age 18-75 years; body mass index 34-41) for 500 Hz binocular infrared oculography (non-ingvasive video eye tracker). Each eye was calibrated to 13 positions, and post-hoc adjusted for improper parameters related to cranial neuropathy. Eye movement data was outputted in a comma-separated format and analyzed using a MATLAB program. While only one patient reported binocular horizontal diplopia, 4 had measurable horizontal misalignment (3 esodeviation, 1 exodeviation) in primary or eccentric gaze. On eye movement recording, all 7 patients demonstrated horizontal eye movement abnormalities: bilateral abduction (4), unilateral abduction (1), abduction and adduction (1), and bilateral adduction (1).

Findings from saccade, smooth pursuit, and optokinetic responses were consistent with a peripheral nerve involvement. In newly diagnosed PTC patients, there was an excellent correlation between the severity of eye movement abnormality and the degree of papilledema, suggesting raised intracranial pressure as the cause of eye movement abnormality. In 3 patients who were monitored during treatment, there was rapid normalization of eye movement changes, well before noticeable changes in disc swelling, strongly supporting the clinical utility of quantitative eye movement studies.

POLYMETHYLMETHACRYLATE PARTICLES INHIBIT OSTEOBLASTIC DIFFERENTIATION OF MC3T3-E1 OSTEOPROGENITOR CELLS

Richard Chiu*, Ting Ma, R. Lane Smith, Stuart B. Goodman. Department of Orthopaedic Surgery

Wear debris particles generated from orthopedic implants have been implicated as a significant inhibitory factor of osteprogenitor differentiation. Polymethylmethacrylate (PMMA) bone cement particles have been previously shown to inhibit the differentiation of osteoprogenitor cells in heterogeneous bone marrow cell cultures. However, these heterogeneous bone marrow cell cultures contain hematopoietic cells such as monocyte-macrophages that may potentially influence the response of osteoprogenitor cells to particles. The purpose of this study was to determine whether the inhibitory effects of PMMA particles could be reproduced in pure osteoprogenitor populations. We examined the dose- and time-dependent effects of PMMA particles on the osteogenic differentiation of MC3T3-E1 cells, a murine osteoprogenitor cell line, and determined whether these cells release soluble factors that can inhibit MC3T3-E1 osteogenesis after exposure to PMMA particles.

MC3T3-E1 subclone 14 osteoprogenitor cells (American Type Culture Collection) were induced to differentiate in osteogenic medium containing ascorbic acid (50 μg/ml) and β-glycerophosphate (10 mM). These cells were challenged with PMMA particles (1-10 μm, Polysciences) at concentrations of 0.038, 0.075, 0.150, 0.300, and 0.600% v/v on the first day of differentiation in osteogenic medium. Cultures were assessed for the quantity of bone mineralization, cell proliferation (DNA), alkaline phosphatase activity, and osteocalcin production after 20 days of culture, and once every 4 days for cultures challenged with particles at concentrations of 0.075 and 0.300% v/v. Additional cultures of MC3T3-E1 cells were grown in conditioned medium taken from confluent MC3T3-E1 cultures that had been challenged with PMMA particles (0.300% v/v) for 24 hrs; control cells were grown in conditioned medium taken from MC3T3-E1 cultures that were not exposed to particles. Each well of cells received equal volumes of conditioned medium and fresh osteogenic medium to ensure the adequate supply of nutrients. Outcome parameters were measured after 20 days of treatment with conditioned medium. Statistical analysis of all data was performed using ANOVA and Fisher's PLSD with p-values < 0.05 considered significant.

MC3T3-E1 osteoprogenitors challenged with PMMA particles showed a dose-dependent decrease in bone mineralization, cell proliferation, and alkaline phosphatase activity. MC3T3-E1 cells challenged with a high dose of PMMA particles (0.300% v/v) showed no rise in rise in these outcome parameters over time, while those challenged with a low dose of particles (0.075% v/v) showed a delayed or reduced rate of increase relative to the control. Osteocalcin production, however, was not significantly affected by PMMA particles at all doses tested. MC3T3-E1 cells grown in conditioned medium from particle-treated MC3T3-E1 cultures showed a significant 54% decrease in bone mineralization compared to the control, but no significant changes in the other outcome parameters were observed. This study has shown that PMMA particles inhibit the osteogenic differentiation of MC3T3-E1 osteoprogenitors, and that these cells produce soluble factors that can partially inhibit mineralization but not differentiation or proliferation.

Funded by Zimmer Inc, the Stanford Medical Scholars Fellowship Program, the Stanford Orthopaedic Research Fund, and the Ellenburg Chair in Surgery.

ULTRAHIGH MOLECULAR WEIGHT POLYETHYLENE WEAR DEBRIS INHIBITS OSTEOBLASTIC DIFFERENTIATION OF BONE MARROW OSTEOPROGENITORS AND MC3T3-E1 PREOSTEOBLASTS IN VITRO

Richard Chiu*, Ting Ma, R. Lane Smith, Stuart B. Goodman. Department of Orthopaedic Surgery

Osteolysis and implant loosening of total joint replacements may result from the biological effects of wear debris particles generated from polyethylene implants. Polyethylene particles not only trigger inflammation and bone degradation, but also inhibit bone formation through the suppression of osteoblast function. However, whether polyethylene particles also inhibit the osteogenic differentiation of osteoprogenitor cells is unknown. In this study, we examined the effects of ultrahigh molecular weight polyethylene (UHMWPE) particles on the ability of primary murine bone marrow osteoprogenitors and MC3T3-E1 cells, a murine preosteoblast cell line, to differentiate into osteoblasts in vitro.

UHMWPE particles (0.5 μm $\pm$ 0.2 μm) generated from wear simulator tests performed at the Hospital for Special Surgery were isolated from serum by density gradient centrifugation. UHMWPE particles were coated onto the culture well surfaces of 12-well plates at concentrations of 0.038, 0.075, 0.150, 0.300, and 0.600% v/v with a layer of type I collagen matrix. Control wells (0.00% v/v particles) were covered with a layer of type I collagen without UHMWPE particles. MC3T3-E1 subclone 14 preosteoblasts (American Type Culture Collection) and primary bone marrow cells isolated from the femur and tibia of C57BL/Ka mice were cultured on these particle-coated plates and induced to differentiate in osteogenic medium containing ascorbic acid (50 μg/ml), β-glycerophosphate (10 mM), and dexamethasone (0.1 μM). After 20 days of osteogenic growth, these cultures were measured for the quantity of bone mineralization, cell proliferation (DNA), alkaline phosphatase activity, and osteocalcin production. Statistical analysis was conducted using ANOVA and Fisher's PLSD, with p-values < 0.05 considered significant.

Bone marrow osteoprogenitors and MC3T3-E1 preosteoblasts exposed to UHMWPE particles showed a dose-dependent decrease in bone mineralization, cell proliferation (DNA), alkaline phosphatase activity, and osteocalcin production, with complete suppression of these outcome parameters observed at particle concentrations $\geq$ 0.150% v/v. The mechanism of polyethylene particle-induced implant loosening may therefore involve not only inflammation and osteolysis, but also decreased bone formation due to the inhibition of osteoprogenitor differentiation.

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EVALUATING EFFICACY OF "HANDS-ON" VS "HANDS-OFF" EDUCATIONAL INTERVENTIONS IN AN INTERNATIONAL SETTING

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Medical education utilizes a variety of teaching modalities, including traditional didactic lectures, case-based learning, independent study, videos, computer programs, and human simulators. The efficacy of these modalities, assessed by scores on written exams and practical skill tests, has not been conducted in an international setting where both language barriers and availability of professional instructors effect curriculum design. The goal of this study is to determine the effectiveness of an educational intervention in an international setting utilizing three different teaching modalities: a "hands-on" with a live lecture and mannequin case simulations, and two "hands off" interventions, one assigned to independent reading and the other to technology using both a video and a cased-based, interactive computer game.

The study population was comprised of paramedic students at the Emergency Medicine and Research Institute (EMRI), a post-graduate paramedic training facility located in Hyderabad, India. The educational topic intended to refresh material covered in the Advanced Cardiac Life Support (ACLS) training course, taken by all students one month prior to the intervention. Students were randomized to one of three groups: "simulation" (lectures and mannequin simulated cases), "technology" (video and computer game), and "reading" (students studied independently with textbooks). The "simulation" group received a combination of lecture and cases with a mannequin simulator. The technology group was shown the American Heart Association ACLS videos and played simulated cases on a video game. The reading group was assigned to read from their textbooks. All groups learned through their assigned method for a total of 3 hours. Prior to the interventions, all subjects took a pre-test consisting of both a written, multiple choice exam and a skills assessment with two simulated cases. Immediately following their intervention, each participant completed a post-test with a different version of the written exam and skills assessment. The difference between the pre-test and post-test was compared among the three groups.

120 students participated in the study, and 40 were assigned to each group (simulation, technology, and reading). All groups improved on the written test following the intervention, and there was no statistically significant difference among the averages (2%, 4%, and 3%) ($p>.05$). For the skills assessment, the simulation or "hands-on" group improved by 9%, while the "hands-off" technology and reading groups improved by only 4%. The difference between the performance on the skills assessment between the "hands-on" and "hands-off" interventions was statistically significant ($p<.05$).

Performance on written exams improved following review of the material in any format. Skills as measured by performance on simulated cases significantly improved with "hands-on" education as compared to "hands off" education by personal reading, watching a video, or playing a computer game. While the use of "hands off" teaching is especially appealing in an international settings, where both people and resources can be limited, "hands on" teaching more closely resembles real life situations and is more effective for paramedic training.

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A DIFFERENCE IN THE PATTERN OF 18F-FDG UPTAKE WITHIN THE SPINAL CANAL IN LOW BACK PAIN PATIENTS

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Understanding spinal cord and cauda equina glucose metabolism may provide insight into causes or manifestations of low back pain. We have retrospectively characterized the distribution of 2-deoxy-2-[18F]-fluoro-D-glucose (18F-FDG) within the entire length of the spinal canal in subjects with and without low back pain using whole body PET/CT.

Approval of the study was attained by our institutional review board. A retrospective review of 26 negative whole body 18F-FDG PET/CT studies with non-CNS cancers was performed. Thirteen of the subjects had described 'low back pain' (LBP) and the remaining 13 subjects (control) described 'no pain' on their entrance questionnaire. Studies with vertebral marrow hyperplasia, severe spinal arthritis, cervical/thoracic kyphosis or motion artifact had been previously excluded. Using the transaxial CT to define the spinal canal, oval region of interests (ROIs) were placed within the canal and excluded the bony elements of the spine. Using this ROI, corresponding PET measurements were obtained at every slice of the study C1 to S1. ROI measurements included maximum standard uptake values (SUVmax). Transaxial measurements at L5, where minimal neural tissue is present, served as an internal control. For each vertebral level, mean SUVmax was calculated. For comparative analysis, we defined the canal-to-background (CTBmax) ratio as canal to mean SUVmax - L5 SUVmax. Taking the max SUV values for each slice, and normalizing each subject's vertebral segments by (1) calculating the maximum max SUV of all the slices in each segment for that subject, then (2) subtracting the value for L5 from the value of each segment, thus normalizing it. Data was analyzed using RT Image analysis software and a Mann-Whitney test stratified across vertebral segments. Significance is $p<0.01$.

The resulting normalized max SUV values are higher for pain patients than normals. Significantly higher mean CTBmax values were observed in the lower thoracic segments, in particular, T7 and T10 (although tests for those segments independently are not significant; jointly tested is significant, however). Of note, the mean L5 SUVmax was 0.75 and 0.82 for LBP and control patients which was not statistically different ($p<0.38$).

Increased 18F-FDG uptake is observed in the distal thoracic spinal canal in subjects describing low back pain. Increased neurosensory and neuromotor activity in the spinal cord at these levels related to LBP may partly explain this observation.

GASTRIC POUCH VOLUME CORRELATES WITH WEIGHT LOSS FOLLOWING ROUX-EN-Y GASTRIC BYPASS

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Bariatric surgery is the only effective and long-lasting treatment for morbidly obese patients. Gastric pouch size is an important factor for weight loss following laparoscopic roux-en-Y gastric bypass (LRYGB). Our study demonstrates the utility of CT scans to estimate gastric pouch volume and to correlate initial pouch size with 12 month weight- loss. A retrospective chart review was conducted on 628 patients at a major bariatric surgery center, and identified 50 patients with CT scans less than one year following surgery. All 50 scans were reviewed by separate readers to measure maximal AP (anterior-posterior), lateral, and vertical dimensions of the gastric pouch in a non-distended native state. Ellipsoid volumes were estimated for all pouches. A multivariate linear regression analysis of weight and percent-excess-weight-loss at 12 months, weight and percent-excess-weight-loss at 6 months as a function of gastric pouch volume, controlling for pre-operative weight/BMI, age, sex, race, and diabetes status were conducted.

A significant positive correlation between pouch volume and weight at 12 months (β_{VOL} = 19.6, 95% CI: 3.25 – 36.0; $p<0.021$) and 6 months (β_{VOL} = 14.56, 95% CI: 1.0 – 28.1; $p<0.037$) was found. A significant negative correlation between pouch volume and percent-excess-weight-loss at 12 months (β_{VOL} = -23.4, 95% CI: -38.1 – -8.7; $p<0.003$) and 6 months (β_{VOL} = -14.78, 95% CI: -27.8 – -1.72; $p<0.028$) was also found.

CT scan is an effective means of estimating gastric pouch volume following LRYGB surgery and that greater 12 month weight is seen in patients with larger pouches and that greater 12 month weight-loss is seen in patients with smaller pouches. Future studies will be conducted to prospectively measure gastric pouch size and follow-up at 12 months to minimize selection bias inherent in a retrospective chart review.

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PRIMARY CARE PHYSICIAN KNOWLEDGE AND ATTITUDES REGARDING BARIATRIC SURGERY

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Bariatric surgery has emerged as a powerful tool in the management of the growing obesity problem in the United States. However, despite the growing number of patients electing to undergo this procedure, few studies to date have sought to understand the knowledge and attitudes of primary care physicians (PCPS) who refer patients for it. As PCPs often provide patients with dietary and exercise counseling and identify potential candidates for bariatric surgery, it is important to understand their current knowledge and attitudes regarding obesity and bariatric surgery. Further, it is important to identify areas for future PCP training and outreach. To do this, we mailed a previously validated 40-item questionnaire to 3000 PCPs in Northern California and then calculated the frequency of each response.

From the responses we obtained, we learned that there is a discrepancy between the training PCPs believe is necessary to care for obese and post-surgical patients, and the training they actually receive. We also found that PCPs were not well informed regarding the indications for bariatric surgery. Additionally, between approximately 12% and 26% of PCPs were not likely to refer family members or themselves for surgery mainly out of concern for complications, death, and concern they would not be able to follow post-op lifestyle changes.

Our study suggests that there is a need to train PCPs in managing the health care needs of their obese patients, as well as to educate PCPs on caring for the post-bariatric surgery patient. Moreover, PCPs themselves strongly feel that such training should be required and that they should follow post-op patients. Finally, our study suggests that education regarding the safety of gastric bypass as a tool in obesity management and the efficacy of patient support groups in helping patients achieve maximal weight loss are important factors in battling against the obesity problem.

DEVELOPMENT OF A WEB-BASED BEHAVIOR CHANGE PROGRAM FOR CHILDREN AND ADOLESCENTS WITH OBESITY

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The United States is experiencing an epidemic of pediatric obesity and an explosion in serious comorbid medical conditions. Behavioral modification of lifestyle is an important part of weight management and successful maintenance of weight loss. Multiple studies have shown internet-based nutrition counseling and education to be useful in adults with obesity; however, no studies to date have studied the efficacy of interactive web-based behavior change programs as an adjunct to a standard clinic based treatment of pediatric obesity.

To examine the feasibility, acceptability, and short-term efficacy of an interactive web-based behavior change nutrition and activity program as an adjunct to the treatment of pediatric obesity. It is hypothesized that this adjunct will improve patient satisfaction, knowledge, and self-efficacy while also increasing exercise time and decreasing total caloric intake and sedentary behaviors.

Develop and pilot an interactive behavior change nutrition and activity website in a randomized, controlled clinical trial with patients from the Pediatric Weight Management Clinic at Stanford. Primary outcome variables include: patient satisfaction, nutrition and activity knowledge, self-efficacy scores. Secondary Outcome Variables include: hours exercise per week, total caloric intake, percent total calories from fat, hours spent on sedentary behaviors per week.

Developed a website for use in an internet-based behavior change nutrition and activity program. Behavior change theory was used to design the website modules. Modules include: Proteins, Fats, Carbohydrates, Beverages, Meal Times, Activities, and Sick, dude! Interactive components include a profile page, weekly activity sheets, and weekly focus messages. Focus group testing will begin this month with launch of a randomized, controlled trial in early summer.

If this website proves to be feasible, acceptable, and efficacious, it will be linked to the LPCH website, potentially assisting many in their efforts to provide pediatric patients streamlined, integrated, and state-of-the-art care in their battle against obesity.

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VITAMIN D AND OTHER MICRONUTRIENT DEFICIENCIES IN ADOLESCENTS AFTER GASTRIC BYPASS SURGERY*

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Nutritional deficiencies after gastric bypass surgery are well-documented in adults, but few studies have examined the impact of bariatric surgery on adolescent micronutrient status.

To describe vitamin D, ferritin, vitamin B12, and folate status in a case series of adolescents, both before and after gastric bypass surgery.

The medical records of 13 adolescents who underwent Roux-En-Y gastric bypass surgery between November 2004 and August 2007 at an academic medical center were retrospectively reviewed. Patients were prescribed a standard supplemention regimen postoperatively which included a multivamin with iron, B-complex, and ferrous sulfate, and were given additional supplementation when serum deficiencies were noted. All 25-hydroxy vitamin D (25OHD), ferritin, vitamin B12, and red blood cell (RBC) folate levels measured before and 3, 6, and 12 months post-surgery were abstracted when available, together with BMI changes. Deficiencies were defined as: 25-OHD <20 ng/ml, ferritin <12 ng/ml, vitamin B12 < 200 pg/ml and RBC folate <95 ng/ml. Borderline deficiencies were defined as: 25-OHD 20-29 ng/ml, ferritin 12-39 ng/ml, vitamin B12 200-300 pg/ml and RBC folate 95-570 ng/ml. Mixed models analyses are planned when all patients are at least 6 months post-surgery.

Subjects (4M, 9F) averaged 17.1 years (SD 1.1) and had a mean body mass index (BMI) of 54.9 (SD 11.0), with 37.5% of patients tested deficient in vitamin D, 12.5% in vitamin B12, and none deficient in ferritin or folate stores pre-operatively. In addition, 37.5% tested had insufficient levels of vitamin D for optimal bone health, while borderline status was noted in 50% for ferritin stores and 12.5% for vitamin B12, and 55.4% for folate. Patients lost a mean of 18.9% (SD 4.6) of pre-operative BMI by 3 months post-surgery, 30.3% (SD 4.1) by 6 months, and 34.5% (SD 5.4) by 1 year. Of those tested, 33.3% were deficient in vitamin D at 3 months, 57.1% at 6 months, and 50% at one year. 30% had deficient ferritin levels at 3 months, none at 6 months, and 33.3% at 12 months. No vitamin B12 or folate deficiency was noted postoperatively, although some borderline values were noted (vitamin B12 - 0% at 3 months, 33.3% at 6, 16.5% at 12; folate - 57.1% at 3 months, 80% at 6, 100% at 12).

Adolescents undergoing bariatric surgery are at risk for micronutrient deficiencies. Vitamin D and ferritin stores may be particularly vulnerable using standard supplementation protocols post-operatively.

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CLINICAL SIGNIFICANCE OF IMMUNOHISTOCHEMISTRY-DETECTED MRD IN NEUROBLASTOMA

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Neuroblastoma (NB) is the most common extracranial solid tumor in children. While many survive, some patients with tumor metastases and other disease features are at high risk for relapse and poor outcomes. An important part of clinical staging and surveillance involves evaluation of the bone marrow. Current methods for detecting minimal residual disease (MRD) in the marrow use cell morphology to identify tumor cells, but can result in high false negative rates. We investigated the value of using immunohistochemistry (IHC) to analyze marrow samples and whether MRD detected with IHC holds any clinical or prognostic significance.

A total of 325 marrow biopsies from 51 patients were identified. Of 220 biopsies which were negative for MRD by routine morphologic evaluation, synaptophysin, chromogranin, and β-catenin IHC identified isolated tumor cells (ITCs) in 9.1, 5.0 and 10.0% of biopsies, respectively. Patients with ITCs more often developed morphologic evidence of bone marrow recurrences (31%) versus patients without a history of ITCs (9%) ($p = 0.075$). Of the IHC markers evaluated, β-catenin showed the greatest sensitivity in identifying isolated tumor cells. Overall survival was not significantly different between patients with and without ITCs ($p = 0.357$).

Our preliminary conclusion is that ITCs identified using IHC may predict persistence of disease; however, they do not appear to predict significant overall survival differences. We also found that β-catenin is a sensitive immunohistochemical marker of primary and metastatic neuroblastoma. Data on long-term clinical outcomes and correlations between presence of ITCs and clinical staging is still under analysis. Given these findings, IHC markers, particularly β-catenin, may be a useful tool for monitoring patients and identifying those who have not yet attained complete remission. Larger studies on whether the presence of ITCs in marrow biopsies can predict recurrent disease or whether they have other clinical implications are needed.

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SUTURELESS MICROVASCULAR ANASTOMOSIS USING THERMOREVERSIBLE POLOXAMERS

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The ability to perform microvascular anastomosis for free tissue transfers and digital replants is tedious, time consuming, and requires a skilled microsurgeon. While a myriad of devices have simplified these complex operations, all the current devices introduce foreign materials which stimulate a foreign body reaction predisposing such anastomoses to stenosis or thrombosis. We propose a novel sutureless technique using thermoreversible poloxamers.

Rheological studies were used to engineer a formulation of P407/P188 to obtain a phase transition temperature at 40°C. Poloxamer formulations were tested on HUVECs in vitro to assess for toxicity and effects on proliferation. Anastomoses were performed on Fisher rat aortas (avg. diameter 1.18±0.02mm) using our sutureless technique (n=30) and with conventional 10-0 nylon sutures (n=30). CT angiograms, ultrasound Doppler, burst strength assays, and histology were performed at designated timepoints. Poloxamer mediated heparin delivery was assessed *in vitro* using HUVECs and tissue factor pathway inhibitor (TFPI) ELISA.

A formulation of 17% P407 and 6% P188 achieved a phase transition temperature of 40°C and was used for all subsequent experiments. Sutureless anastomoses were completed more efficiently than the hand sewn technique (8.1 ± 2.4 min vs. 47.3 ± 5.0 min, $p<0.05$) with equivalent burst strengths (>1200mm Hg, $p>0.05$). CT angiograms demonstrated equivalent patency in end-to-end anastomoses; however, end-to-side anastomoses could not be performed using traditional techniques ($p<0.001$). Doppler analysis demonstrated equivalent patency, vessel diameter, and volumetric flow (116.1mL/sec vs. 107.2 mL/sec, $p>0.05$) between sutureless and hand-sewn anastomoses. Histology demonstrated dramatically decreased inflammation and fibrosis in the sutureless group compared with the traditional technique. Application of poloxamer did not demonstrate any evidence of toxicity *in vitro* or *in vivo*. Heparinized poloxamer-induced a significant percentage increase in secretion of TFPI compared with heparin administered directly to HUVECs (231.8%, $p<0.05$) with effects lasting up to 24 hours (125.4%, $p<0.05$).

Sutureless anastomosis can be performed reliably, more efficiently, and with less intimal damage than hand-sewn anastmosis. In addition, poloxamers can also be employed as a delivery agent for anti-thrombotics simultaneously to further preserve graft patency. This technology offers a promising alternative to sutured anastomosis and may have a profound impact on the field of reconstructive microsurgery.

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QUANTITATIVE SENSORY TESTING IN YOUNG WOMEN WITH FIBROMYALGIA: AN EXAMINATION OF THERMAL SENSITIVITY AT "TENDER" AND "NON-TENDER" POINT LOCATIONS

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To measure thermal sensory neural functioning of young, female fibromyalgia patients and controls using quantitative sensory testing (QST) and to compare thermal sensory functioning at "tender" versus "non-tender" sites.

Eight female fibromyalgia patients age 18-35 with a diagnosis of at least six months duration, with no concurrent neurological or rheumatological conditions and taking no opioid pain medication were recruited through local advertisement. Fibromyalgia patients were compared with nine healthy control subjects. All study subjects were tested using heat and cold thermal QST at four specific sites: two traditional fibromyalgia "tender" point sites (lateral epicondyle and greater trochanter) and two "non-tender" sites (posterior forearm and anterior thigh).

Fibromyalgia patients demonstrated reduced heat pain tolerances and markedly reduced cold pain tolerances as compared to healthy subjects. Mean cold tolerance for fibromyalgia patients (across all sites measured) was 13.1°C (SD 12.5), as compared to 0.0°C (SD 0.1) for healthy subjects, $p < 0.005$. Mean heat tolerance for fibromyalgia patients (across all sites measured) was 43.9°C (SD 5.3), as compared to the mean heat tolerance for control subjects at 49.3°C (SD 1.0), $p < 0.01$. Intrapatient heat and cold sensory tolerance in "tender" versus "non-tender" points were not significantly different.

Fibromyalgia patients showed reduced thermal tolerance for both heat and cold at both "tender" and "non-tender" points. This alteration suggests a centrally mediated phenomenon is active in thermal tolerance in fibromyalgia patients. The lack of difference in thermal sensory tolerance between classic fibromyalgia "tender" and "non-tender" sites suggests that another mechanism may be responsible for the hyperalgesia present at fibromyalgia "tender" point locations.

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CURE OF LARGE TUMORS BY 'IMMUNOTRANSPLANT' OF ANTI-LYMPHOMA PRIMED LYMPHOCYTES INTO LYMPODEPLETED RECIPIENTS

Matthew J. Goldstein*, Joshua Brody*, Ron Levy, Stanford University (*equal contributors)

Immunotherapy for cancer can include non-specific stimulation of the immune system, active immunization with tumor-specific antigens, and adoptive immunotherapy—the transfer of tumor-specific T cells. We have developed an approach to treating lymphoma that generates a unique population of anti-tumor T cells and transfers them into lymphodepleted, recipient mice. We refer to the preparation of these cells and subsequent transfer as 'immunotransplant'. The success of immunotransplant is likely due to the elimination of immune-suppressive factors such as T regulatory cells as well as the rampant, homeostatic proliferation of transferred, tumor-specific T cells. The efficacy of this maneuver is impressive and cures large tumors in a mouse model for lymphoma.

Prior studies in this lab demonstrated the success of a vaccination maneuver in treating murine B cell lymphoma. This maneuver combines systemic chemotherapy (CTX) with intra-tumoral injection of an immuno-stimulant, CpG—a CG-enriched oligodeoxynucleotide (ODN) that ligates toll-like receptor 9 (TLR-9). An on-going clinical trial of this therapy for patients with recurrent lymphoma has demonstrated a minority of clinical responses suggesting the need for improvement.

Immunotransplant markedly increases the potency of the primary vaccination maneuver, protects against sub-cutaneous and systemic tumor challenge, and can effectively treat large, established tumors. Both components of immunotransplant—vaccination with 'CTX + CpG' and transfer into lymphodepleted recipients—are critical to the success of this therapy. We have evaluated anti-cancer immune responses in animals treated with immunotransplant and found that immune cells from these animals recognize tumor as assessed by *in vitro* IFN-γ response assays. We observed preferential expansion of transferred tumor-specific $T_{effector}$ cells in immunotransplant recipients. Donor $CD8^+$ T cells were both necessary and sufficient for tumor protection and were enhanced by post-transplant vaccine boosting.

Our results demonstrate enhanced tumor protection and could be directly translated from the pre-clinical model into a clinical trial. We have demonstrated that immunotransplant of anti-tumor T cells induced by 'CTX + CpG' vaccination can cure large subcutaneous lymphomas and protect against tumor challenge of 10-100 times the lethal dose.

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AN ANIMAL MODEL FOR TRIGEMINAL NEURALGIA

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Trigeminal neuralgia (TN), a chronic pain syndrome of the orofacial area, is considered one of the most "agonizingly painful" medical conditions known. Current treatments available for this syndrome, including carbamazepine, baclofen, and phenytoin, are suboptimal because they produce debilitating side effects and provide only short-term, incomplete management of the patient's pain. The development of new treatment options is limited by the lack of an adequate animal model for study. The main goal of this project was to develop an animal model that closely parallels the human pathology seen in TN.

We have found that injection of a biocompatible, volume-expanding superabsorbant polymer near the trigeminal nerve root produces TN pathology in rats similar to that observed in humans. Behavior before and after compression surgery was assessed with a series of behavioral assays including VonFrey allodynia testing, air puff allodynia testing, brush allodynia testing, and spontaneous blinking analysis. We have found that our surgical intervention elicits spontaneous unilateral pain behaviors, like grimacing, and results in increased blinking in the ipsilateral eye. Trigeminal nerve root compression also lowers detection and withdrawal times to pain evoked by mechanical stimulation with brushes, VonFrey filaments, and air puffs.

A fitting model for the study of TN should produce unilateral, paroxysmal pain evoked by light touch stimulation of orofacial trigger zones. Results obtained to date are, therefore, very promising. In order to further validate this neuropathic pain model, we will continue characterizing behavior observed pre and post-operatively. We also hope to determine the efficacy of carbamazepine (first-line treatment for TN) in attenuating observed post-operative pain behaviors.

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SILENT INFARCTS IN PATIENTS WITH SICKLE CELL DISEASE AT HIGH RISK FOR CERBRAL VASCULAR ACCIDENTS ON CHRONIC RED CELL TRANSFUSION THERAPY

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Strategies to prevent silent infarcts (SI) in patients with sickle cell disease (SCD) are currently being evaluated. However, whether SI occur in SCD patients who are on chronic erythrocyte transfusions (CTX) and conversely, whether CTX is effective SI prophylaxis are unknown. We attempted to examine whether SI occur, and if so at what rate, in SCD patients who are on CTX.

We retrospectively evaluated a cohort of SCD patients at high-risk of having cerebral vascular accidents (CVA), including SCD patients with a history of CVA or who have abnormal transcranial Doppler ultrasound studies, who are cared for at Lucile Packard Children's Hospital and Children's Hospital Oakland. Subjects were prescribed and adherent to CTX, had baseline radiographic imaging of the CNS at institution of CTX, and subsequent CNS imaging. Patients with symptoms of overt stroke at the time of subsequent imaging were excluded. IRB approval for this study was obtained for the 2 participating institutions.

18 patients with Hb SS, 10 males and 8 females, with a median age of 16 years/mean 15 years (range: 6 to 23 years) met inclusion criteria. Median follow-up was 1650 days, with a mean of 2110 days (range: 270 to 6030 days), for a total of 105.5 patient-years available for analysis. 2 SIs were identified in 1 patient, and a SI rate of 2 per 100,000 patient-days was observed. 56% of patients (10 / 18) developed progressive CNS vasculopathies, with 6 patients having moyamoya.

These findings show that SI may be rare in a high-risk SCD population who are on CTX. A larger, prospective study to evaluate the efficacy of CTX for SI prophylaxis in high risk or standard risk SCD patients is warranted. In addition, routine CNS imaging for SCD patients on CTX for both primary and secondary CVA prophylaxis to detect CNS vasculopathies may be indicated.

Funding provided by the Stanford Medical Scholars Fellowship Program.

COORDINATED CELL MIGRATION IN A CONFLUENT MONOLAYER IS DRIVEN BY VISCOUS DRAG

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Endothelial cells function as the walls of blood vessels, and as such they must be able to maintain the integrity of the wall and quickly fill in any breaks that may occur. In order to more fully understand this process, we grew human primary endothelial cells (HUVECs) in a confluent monolayer *in vitro* and tracked their movements over time. We observed that these cells were not stationary but in fact migrated actively within the monolayer. Interestingly, the cells did not appear to migrate as individuals but instead moved in coordinated clusters or streams. In addition, using an siRNA screen, our lab has found a number of genes that affect the robustness of this process. Here we have attempted to use quantitative analysis and simulation to identify the biophysical mechanism underlying this streaming behavior.

Using computer simulations, we generated a number of hypothetical models that were able to reproduce streaming behavior. We then tested the various models by attempting to predict cells' future movements based on their past direction. Only a viscosity model, which models a drag force felt by cells pulling them in the direction of their neighbors' motion, was able to predict the cell movements. In addition, we were able to extract a number of quantitative parameters from the cells' motion to describe the viscous forces.

These results provide evidence for group migration as an emergent property of cell-cell adhesion, with this adhesive force creating a viscous drag within a monolayer. It seems plausible that this behavior underlies the ability of endothelial cells to maintain blood vessel integrity and close gaps that form, since they are always moving but at the same time they retain cell-cell contacts. Finally, this model may give us insights into metastasis of endothelial and epithelial cancers, where cells lose their cell-cell adhesion and migrate away from their proper location.

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NETRIN-4 ENHANCES ANGIOGENESIS AND NEUROLOGICAL OUTCOME AFTER CEREBRAL ISCHEMIA

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Functional recovery following cerebral ischemia is governed by plastic processes that result from the induction of axonal outgrowth, the restoration of synaptic architecture, and the regeneration of vascular networks. Netrin-4 has been implicated as both a synaptogenic and angiogenic factor that promotes neurite outgrowth and angiogenesis. This dual function of Netrin-4 suggests that it may be important for promoting functional recovery following cerebral ischemic injury. In this study, we investigated the expression of Netrin-4 and its putative receptors, DCC and Unc5H1, following distal middle cerebral artery occlusion (dMCAO) in mice. Netrin-4 recombinant protein was also administered via an osmotic minipump intracerebroventricularly to examine its effect on angiogenesis and behavioral recovery.

Netrin-4 protein was highly up-regulated in the ischemic core as soon as 1 day after cerebral ischemia, with subsequent down-regulation after 1 week. Its expression was limited to the area of blood-brain barrier damage, as demonstrated by the presence of Evans blue. Netrin-4 protein expression was seen in both blood vessels and astrocytic foot processes, which suggests an important role of netrin-4 in blood-brain barrier repair and angiogenesis. While there was no significant up-regulation of the putative Netrin-4 receptor Unc5H1, there was a significant increase in the expression of DCC in the ischemic penumbra. DCC protein was also found to be localized to neuronal processes, which may suggest a role in neurite sprouting. Importantly, intracerebroventricular administration of Netrin-4 into the ischemic brain increased blood vessel density and endothelial cell proliferation and improved behavioral outcome at different time points after stroke.

These findings suggest that Netrin-4 may improve post-stroke functional recovery by enhancing blood vessel proliferation and angiogenesis. It may also have an effect on neurite sprouting and synaptogenesis through its interaction with the DCC receptor. These properties make Netrin-4 an ideal candidate to improve behavioral recovery after ischemic injury.

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STOICHIOMETRY OF STIM1 AND ORAI1 IN THE SOC CHANNEL COMPLEX

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Ca^{2+} entry into non-excitable cells occurs via store-operated Ca^{2+} (SOC) channels that are activated by the depletion of Ca^{2+} from the endoplasmic reticulum (ER). Following store depletion, the ER Ca^{2+} sensor STIM1 and the SOC channel subunit Orai1 reorganize from a diffuse distribution in the ER and plasma membranes into colocalized puncta separated by a cytosolic gap of only 10-25 nm. The colocalization and close proximity of STIM1 and Orai1 at puncta raise the possibility that these proteins may interact with a defined stoichiometry to evoke SOC channel activation.

To address this question, we measured the relative amounts of STIM1 and Orai1 in puncta after store depletion in HEK 293 cells. Cells were transfected with mCherry-STIM1 and eGFP-Orai1, and treated with thapsigargin to deplete ER Ca^{2+} stores. After reaching steady-state, the mCherry:eGFP fluorescence ratio in each punctum was quantified by confocal imaging of the cell at the coverslip. By comparing this ratio to cells expressing a STIM1 variant labeled with both mCherry and eGFP, we determined the relative amounts of STIM1 and Orai1. We found that STIM1:Orai1 ratios are generally uniform across individual puncta and among all the puncta within a cell, but vary by several-fold among cells.

To test the possibility that the ratio of STIM1:Orai1 in puncta depends on relative protein expression levels, we controlled mCherry-STIM1 expression using a tetracycline-inducible cell line while transiently co-expressing eGFP-Orai1. The STIM1:Orai1 ratio was measured in cells with full ER Ca^{2+} stores, and in puncta after store depletion. We found that as the expression of Orai1 increases relative to STIM1 in the cell, the ratio in puncta reaches a limit, suggesting that the actual STIM1:Orai1 stoichiometry in the SOC channel complex is ~1:3.

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STEERABLE SHEATH FOR ENDOSCOPIC AND TRANSLUMENAL SURGERY

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The application of endoscopic tools for diagnostics and therapeutics is increasing. Intraluminal surgical procedures are enabled by technologies that provide more maneuverability and dexterity in smaller diameters. New clinical applications such as Natural Orifice Transgastric Endoscopic Surgery (NOTES) are forcing even more stringent requirements in the design of tools to navigate and perform surgical tasks. However, there are two key requirements which current technology has not addressed adequately: (1) the ability to navigate a sheath through a tortuous path without support; (2) the ability to rigidize the sheath on-demand in order to deliver and operate surgical tools.

We developed a novel technology to steer and rigidize a balloon-like sheath to reach a target in the peritoneal cavity or gastrointestinal tract. The technology is based on the ability to electrically alter the properties of a proprietary material embedded in the sheath. Selective changes to the elasticity of sheath regions cause controlled bending when internal pressure is applied. The design leaves the center of the sheath open for introduction of surgical tools.

This steerable sheath may enable previously challenging endoscopic operations by providing unprecedented maneuverability and stiffness control. It is also potentially scalable to enable the design of smaller and more dexterous endoscopic tools than what is currently available.

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FINDING HPV VACCINE CONTENT IN BLACK SOCIAL MEDIA

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The recently FDA approved human papillomavirus (HPV) vaccine has uncertain acceptance and uptake among its target market (young women and girls, ages 9 -26 years). Since successful implementation of the vaccine depends on young girls being vaccinated before the onset of sexual activity. It is important to identify the knowledge, attitudes, and beliefs of people in this group - a demographic that widely uses social media as a form of communication.

This project focuses on the use of social media (defined here as web-message boards and blogs) by young African-American women and girls to discuss and share information regarding the HPV vaccine. Our research examines methods to search for and data mine content regarding HPV vaccine knowledge attitudes and beliefs of adolescent and young African American women who use social media as a communication device.

Preliminary results indicate there is a paucity of blog sites that focus on African-American health issues. The implications of this are that more focused searches have to be created and implemented to find the appropriate data in order to find the content. Further studies will examine the content and analyze the data to draw conclusions about the knowledge attitudes and beliefs of young African American women regarding the HPV vaccine.

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DISCOVERY OF NOVEL AML TUMOR ASSOCIATED ANTIGENS USING ANTIBODY SIGNATURES AND GENE-EXPRESSION DATA

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Acute Myeloid Leukemia is a highly malignant cancer whose pathogenesis is still not well understood. It has been established that AML patients launch an adaptive immune response to their disease. Various research methods have previously been used to characterize this immune response. Our objective was to develop a bioinformatics approach to discover novel AML tumor associated antigens.

We integrated protein microarray data and publicly available gene-expression data in order to identify potential AML markers. We used protein microarrays to measure serum proteome-wide antibody levels in order to find the adaptive immune response in AML patients. The proteins with the highest antibody levels in AML patients vs. controls were considered potential AML tumor associated antigens. We further narrowed our selection of potential antigens by considering those antigens with high gene expression levels in relevant tissues or cells, such as CD34+ cells. We then validated our most promising candidates, TTK, PHLDA1 and ALK through ELISA. The ELISA results showed that TTK was differentially targeted by AML patients' humoral response ($p = 9.1e-05$), as was PHLDA1 ($p = .022$). In addition, upon further exploration of several types of leukemias, we found that both of these antigens appear to be markers for myeloid, as opposed to lymphoid leukemias.

Through integrative analysis of high-throughput data from protein microarrays and gene expression data, we found potential AML tumor associated antigens. So far, we have done an initial validation of TTK and PHLDA1 with human serum from AML patients on E.Coli expressed proteins. We plan to further validate these findings with insect expressed proteins. These findings could potentially help in generating new avenues of exploration of the pathogenesis AML, as well as offering potential diagnostics, prognostics and therapeutics. In addition, we hope to introduce a novel methodology for identifying tumor associated antigens applicable to other diseases.

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CHRONICALLY ILL CHILDREN AND THE HOSPITAL SAFETY-NET SYSTEM IN CALIFORNIA 1998-2004

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We sought to determine the degree to which chronically ill children depend on hospitals receiving Disproportionate Share Hospital (DSH) funds. To this end, we performed a retrospective analysis of a statewide discharge dataset combined with annual hospital financial data both collected by the California Office of Statewide Health Planning and Development (OSHPD). We studied children less than 18 years of age discharged from a California hospital between 1998 and 2004, observing how hospital characteristics, patient demographics, patient health status, and patient insurance status influence admission to hospitals that receive Medi-Cal DSH funds.

The number of hospitals declined from 517 to 454 between 1998 and 2004 while those receiving DSH funding increased from 106 to 128. During this time, the percentage of yearly pediatric admissions to DSH-funded, privately-owned hospitals increased from 35 to 47%, while the non-DSH-funded, privately-owned hospitals decreased from 46 to 35%. Chronically ill children with less common conditions (i.e. conditions other than asthma or psychiatric illness) reflected these trends; however, they had much higher rates of DSH-funded, private hospital use (i.e. 48 increasing to 59%), and lower rates of non-DSH-funded, private hospital use (i.e. 31 to 23%). Regression analysis showed that these chronically ill children with complex conditions are 1.8 times as likely to be admitted to a DSH-funded hospital as other children when controlling for demographic and other factors.

Children in general, and in particular chronically ill children were heavily dependent on care from hospitals receiving DSH funding. While imperfect, the DSH funding system supports institutions that care for the most vulnerable children and this must be carefully considered before radically changing this crucial funding system.

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IMPACT OF LONG TERM *IN VITRO* CULTURE UPON MITOCHONDRIAL FUNCTION IN HUMAN EMBRYONIC STEM CELLS

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In recent years, human embryonic stem cells (hESCs) have become a promising cell source for regenerative medicine. Current animal models for stem cell therapy require large numbers of hESC derivatives, often in the millions of cells. As such, it is likely that repeated passaging of hESCs will be necessary to obtain sufficient numbers for clinical applications of stem cells to be realized. Although hESCs have a theoretic potential for unlimited self-renewal, embryonic stem cells are not unlike their adult somatic counterparts in that they mis-incorporate DNA bases during replication at a rate of 1 nucleotide per 10^9. Accumulation of such mutations may affect the integrity of not only nuclear but mitochondrial DNA. In this work, we examine the impact of long term culture upon mitochondrial function as measured by ATP production, mitochondrial membrane potential, and oxygen consumption.

We assessed changes in molecular profiles associated with H9 line between young (<50 passages) and old (>100 passages) passage cells. Morphology and stem cell markers (Oct4 and SSEA4) staining showed no significant difference between young and old passages. Compared to their young counterparts, old passage hESCs preserved high telomerase activity as assessed by TRAP assay (5030±377 vs. 5014±294 unit/μg, *P*=NS). With spontaneous differentiation *in vitro*, both young and old passage cells were able to form cell types from all three germ layers as determined by semi-quantitative RT-PCR. In particular, there were no significant differences in the expression pattern of endoderm marker (AFP), mesoderm marker (Flk-1) and ectoderm marker (Ncam) between young and old passage cells after 14 days of differentiation. Similarly, implantation of both young and old passage hESCs into the subcutaneous regions of mice yielded teratomas after 4 weeks containing cellular derivatives of all 3 germ layers. Interestingly, ATP production assays revealed decreased ATP content in old passage cells as compared with their younger passage counterparts (1.33±0.04 vs. 6.38±0.24 femtomoles per cell, *P*<0.001). These results correlate with JC-1 staining tests which show that old passage cells are characterized by a higher mitochondrial membrane potential than young passage (17.2±1.9 vs. 9.8±0.5; ratio of cells exhibiting high mitochondrial uptake of JC-1 dye as compared to low uptake, *P*=0.003). Taken together, these findings suggest that prolonged culture of hESCs is associated with decreased mitochondrial function, similar to what has been observed in cellular senescence studies.

Continuous passaging of hESCs does not adversely affect cell morphology, pluripotency, or longevity. However, the potential adverse effects of long term passage upon mitochondrial function cannot be ignored as mitochondria are one of the most sensitive intracellular apparatuses responsible for preventing oxidative stress and damage. This is the first study to evaluate mitochondria integrity in human embryonic stem cells after prolonged *in-vitro* culture. Findings from this work should yield valuable insights into the optimal usage of hES cells for future clinical applications.

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A HUMAN DISEASE "ETIOME"

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It has long been known that both genetic and environmental factors contribute to human diseases. Though genetic contributions are relatively well characterized for some monogenetic diseases, there has been no effort at curating the extensive list of environmental factors.

We first identified 1,100 genes associated with 1,034 complex diseases from GAD, a database of genetic association studies. We then identified 3,342 environmental etiological factors associated with 3,159 diseases from a comprehensive search of MEDLINE articles. 863 diseases have both genetic and environmental etiological factors available. Thus, the "etiome", which is a compendium of disease etiology, including both genetic and environmental factors, is available for these 863 diseases. Clustering of all etiological factors (both genetic and environmental) puts genes in the context of environment in a quantitative manner, which may reveal novel gene functions.

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POLYMETHYLMETHACRYLATE PARTICLES ALTER P38 MAPK ACTIVITY IN MC3T3-E1 PREOSTEOBLAST CELLS UNDERGOING DIFFERENTIATION

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Department of Orthopaedic Surgery

Joint replacements may fail because of excessive release of particulate debris, causing inflammation and osteolysis. Particulate wear debris reduces bone formation by inhibiting osteogenic differentiation. However, the molecular mechanism of particle-induced inhibition of osteogenic differentiation has not been elucidated. The mitogen-activated protein kinases are important intracellular transducers of osteogenic signals that cause the differentiation of osteoprogenitor cells as well as inflammatory signals that induce cytokine production and apoptosis. In this study, we determined the effects of polymethylmethacrylate(PMMA) particles on the activity of the p38 MAPK subfamily in MC3T3-E1 preosteoblast cells undergoing differentiation.

MC3T3-E1 cells demonstrated activation of p38 on day 8 of growth in particle-free osteogenic medium as demonstrated by an increase in phosphorylated p38 on Western Blot. To determine the effects of PMMA particle challenge on p38 activity, MC3T3-E1 cells were grown for 8 days in osteogenic medium and then challenged with 0.15% or 0.30% v/v PMMA particles for 5, 15, or 30 minutes. All groups challenged with particles demonstrated a complete suppression of p38 activation. When cells were challenged with PMMA particles in concentrations of .075%, .15%, or .30% v/v at the same time as the addition of osteogenic medium on day 0, p38 was shown to be activated on days 1 and 4 and complete suppression of p38 activity was demonstrated on day 8. Activation of p38 on days 1 and 4 after particle challenge demonstrated a dose-dependent effect with higher concentrations of particles causing a greater activation of p38.

This study has demonstrated that challenging differentiating osteoblasts with PMMA particles causes modulation of normal p38 MAPK activation patterns. MC3T3-E1 preosteoblast cells normally demonstrate activation of p38 on day 8 of growth in osteogenic medium. Addition of particles either on day 0 or day 8 can cause complete suppression of p38 activity that normally occurs on day 8. Also, particle challenge beginning on day 0 causes activation of p38 on days 1 and 4. This particle-induced activation of p38 also appears to exhibit dose-dependency as the activation of p38 on days 1 and 4 becomes more robust with increased particle concentration. Since p38 in osteoblasts has been shown to be involved in many pathways including ones for stress, apoptosis, and differentiation, the p38 activity induced by particle challenge could be involved in a different signaling pathway from the one associated with normal p38 activation seen on day 8 of unchallenged cells. Further studies to elucidate the downstream events of this particle-induced p38 activation could lead to treatments to counteract the adverse effects of orthopaedic wear particles on osteoblastic differentiation.

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A SIMPLE DEVICE AND METHOD TO QUICKLY ALIGN, APPROXIMATE, AND ATTACH THE DEEP DERMIS AND SUPERFICIAL FASCIA SYSTEM

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Suturing is a time-consuming process, especially during surgeries requiring large incisions such as autologous breast reconstruction, abdominoplasty, soft tissue flaps, and body-contouring surgery after massive weight loss. For example, closing the deep dermis and superficial fascia system during an abdominoplasty may require 60-70 minutes based on observations and interviews of plastic surgeons. On average, one single interrupted stitch requires 34-35 seconds, with 30% of the time spent placing suture on both sides of tissue, 50% tying knots and cutting, and 20% reloading needle.

The purpose of this study was to develop a device and method to significantly reduce the amount of time required to close large incisions while at the same time maintain adequate tensile strength, tissue alignment, ease-of-use, adjustability and reversibility. The leading concept and prototype involves a combination of an elongated, barbed polymer made from monofilament suture and a controlled release mechanism to eliminate the need to tie knots and cut as well as reduce the time to reload.

Bench top testing revealed an average decrease in time for a medical student to perform 10 single interrupted stitches from 6 min 9 seconds (consistent with previously observed time of senior residents and attending surgeons in operating room of about 6 min - 6 min30sec) to 2min17 seconds, a 63% decrease in suture time ($p = 2.95*10^{-8}$). Potential benefits include a reduction in costly OR time, fewer risks of anesthesia to patients, and fewer interventions for burn patients requiring skin grafts.

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WNT-MEDIATED BONE REGENERATION

Steve Minear, Philipp Leucht, Roel Nusse, Jill Helms. Department of Surgery

The skeleton retains a lifelong capacity to re-form, whether in response to damage or when maintaining bone mass and density. One pathway responsible for bone regeneration is the Wnt pathway. Previous data suggest that Wnt signal timing is paramount: Wnt holds progenitor cells in an undifferentiated, proliferative state; later, Wnt directs their differentiation. By inactivating *Axin2*, an intracellular negative regulator of canonical Wnt, we hypothesize that cells stimulated to respond to Wnt will experience an amplified signal only at physiologically appropriate times. Consequences of this presumed selectively amplified Wnt signal are explored in this project.

A model tibial defect was implemented that regenerates over a 4-week period. The regenerating injury site was harvested at intermediate time points, and the tissue was analyzed. The injury sites of *Axin2*$^{-/-}$ mice exhibit a significant increase in new bone by post-surgical day 14 relative to wild-type controls. By post-surgical day 3, proliferation at the injury site is down-regulated, suggesting that the progenitor population is no longer expanding. Reduced proliferation suggests that either cells divided at an earlier time point in *Axin2*$^{-/-}$ mice, or that *Axin2*$^{-/-}$ mice harbor more skeletal progenitor cells than wild-type mice. We analyzed osteogenic markers present at the injury site and determined via *in situ* hybridization that cells of the injury site proceed through osteogenesis more quickly than wild-type. Moreover, we determined that osteoblast activity was up-regulated in *Axin2*$^{-/-}$ injury sites, while osteoclast activity was similar to wild-type.

Taken together, these data suggest that *Axin2*$^{-/-}$ mice have an increased ability to regenerate skeletal tissue. Whether enhanced Wnt signaling resulting from inactivation of *Axin2* also produces an animal with more progenitor cells available, or enhanced osteogenic differentiation pathway, remains to be determined.

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CHARACTERIZATION OF THE SKELETAL MUSCLE IN MICRORNA-1-2 KNOCKOUT MICE AND SCREENING MEANINGFUL MICRORNAS IN SATELLITE CELLS

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MircoRNAs are a novel form of gene regulation discovered in the last 15 years. These 18-24bp RNA sequences have multiple mRNA targets, inhibiting their translation or targeting them for destruction. MicroRNA-1-2 (miR-1-2) has recently been shown to be skeletal and cardiac muscle specific and an important regulator in cardiac myogenesis and C2C12 differentiation and proliferation. We analyzed the role of miR-1-2 in primary myoblasts behavior and skeletal muscle regeneration. In vitro, primary myoblasts from miR-1-2 KO mice show significantly increased proliferation. In vivo, miR-1-2 KO muscle fibers exhibited decreased regenerative capacity assessed as decreased muscle fiber size and accumulation of calcium deposits and fibrosis. Despite muscle regeneration defects, miR-1-2 was not found to be expressed in satellite cells.

To determine other microRNAs that would potentially have an effect on satellite cells, we collaborated with the Chen lab to identify which microRNAs were selectively expressed in satellite cells and myoblasts. These microRNAs have been put into retrovirus and lentivirus constructs. These vectors will allow me to study the effects of over-expression of various microRNAs by assessing differences in proliferation (using a competition assay) and differentiation. To date, the proliferation assay has shown a marked decrease in competitive survival of 2 specific microRNAs.

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STEREOTACTIC RADIOSURGICAL TREATMENT OF HEMANGIOBLASTOMAS IN VON HIPPEL-LINDAU DISEASE

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Stereotactic radiosurgery has been used for nearly two decades to treat hemangioblastomas, particularly those that are in surgically inaccessible locations or that are multiple, as is common in von Hippel-Lindau disease. There is a paucity of sufficiently long-term published radiosurgical treatment outcomes, particularly for spinal lesions, in a large patient population. The purpose of this study was to provide a long-term retrospective evaluation of radiosurgical hemangioblastoma treatment effectiveness, with a special emphasis on the relatively recent use of the frameless, image-guided Cyberknife in the treatment of spinal lesions.

From 1991 to 2007, 79 hemangioblastomas in 28 patients, 25 with von Hippel-Lindau disease, were treated with linear accelerator-based radiosurgery. The mean patient age was 41 years (range, 18-81 yr). The radiation dose to the tumor periphery averaged 23.4 Gy (range, 12-40 Gy). The mean tumor volume was 1.8 cm^3 (range, .058 – 65.4 cm^3). Tumor response was evaluated in serial, contrast-enhanced, computed tomographic and magnetic resonance imaging scans. The mean follow-up period was 61 months (range, 3-153 mo).

Only 3 (4%) of the treated hemangioblastomas progressed whereas 20 tumors (26%) showed radiographic regression and 54 tumors (70%) remained unchanged in size. Radiosurgery improved lesion-associated symptoms in 36 of 41 tumors with pretreatment neurological defects. During the follow-up period, 9 patients died of causes unrelated to the progression of their treated hemangioblastomas, and 5 patients suffered radiation necrosis. Stereotactic radiosurgery halted tumor progression in all but 4% of treated tumors and remains an attractive alternative to multiple surgical procedures for patients with von Hippel-Lindau disease.

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TESTING THE IMPORTANCE OF SURROUNDING RESIDUES IN POSITIONING HYDROGEN BOND DONORS IN THE OXYANION HOLE OF KETOSTEROID ISOMERASE

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Enzymes are central to biology and attractive targets for pharmaceuticals. While the chemical steps of many enzymatic reactions are known, the physical basis of enzyme rate enhancement is not yet understood. One longstanding hypothesis is that enzymes are electrostatically and geometrically complementary to substrate transition states and that the resulting transition state stabilization contributes to rate enhancement. Work with the bacterial enzyme ketosteroid isomerase (KSI), a model system in which to test this hypothesis, has suggested only a modest contribution to catalysis by charge complementarity. It is therefore of interest to test the role of shape complementarity in KSI catalysis.

In the KSI active site, residues Tyr57, Phe86, and Met116 may help position hydrogen bond donors Tyr16 and Asp103, both of which appear pre-positioned to preferentially stabilize the substrate tetrahedral transition state relative to the planar ground state. The magnitude to which mutations in Y57, F86, and M116 are able to reduce KSI catalysis could therefore suggest whether geometric complementarity plays a significant catalytic role. A series of kinetic assays that measure the conversion of substrate to product via UV absorbance spectrophotometry was used to examine the effect of each mutant on the catalytic rate constants k_{cat}, K_M, and k_{cat}/K_M.

The magnitudes of k_{cat}/K_M for single and double mutations made in Y57, F86, and M116 were at most 50-fold lower than that of wild-type. In general, effects on k_{cat} and K_M were also modest. Y57 and M116 mutations resulted in greater perturbations in k_{cat}, K_M, and k_{cat}/K_M than F86 mutations, suggesting a lesser role for F86 in KSI catalysis. The modest effects on k_{cat}/K_M observed by all mutations suggest either (1) minor roles for Y57, F86, and M116 in positioning Y16 and D103 and therefore in geometric complementarity or (2) shape complementarity makes no contribution to catalysis – e.g., mutations could cause small changes in the positioning of active site residues other than Y16 and D103. Double mutants that combine mutations at positions 16 and 103 with mutations at positions 57, 86, and 116 will better distinguish between these possibilities. NMR studies of mutants will be used to reveal changes in hydrogen bonds and therefore alterations in residue positioning and interactions in the active site. X-ray crystallography will also help demonstrate any changes in the active site environment due to mutations. Together, both present and future studies should help to clarify the role, if any, of geometric complementarity in enzymatic catalysis.

Funding provided by the Stanford Medical Scholars Fellowship Program

USING SYNTHETIC LETHALITY AS A NEW APPROACH TO TREAT RCC: A NOVEL MOLECULE INDUCES VHL-DEFICIENT CELL DEATH THROUGH INDUCING METABOLIC DERANGEMENT.

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Renal cell carcinoma (RCC) is the most lethal cancer among the common urologic cancers, claiming the lives of nearly 13,000 people a year in the United States, and growing. Advanced RCC is resistant to standard radiation and chemotherapy. Therefore, there is a great need to find drugs to treat this disease. The discovery of the regulatory proteins, VHL and downstream HIF, as critical components of RCC tumorigenesis has accelerated the search for these drugs. The research community has been focusing on finding inhibitors of HIF and downstream HIF targets, without discovering a clearly effective compound. There has been, however, little use of synthetic lethality as a way to search for anti-RCC drugs. Thus, our lab has developed a fluorescent high-through-put assay and screened 64,000 compounds for synthetic lethality with VHL-deficient cells, cells that are critical for RCC development. Several families of compounds were identified to have selective toxicity against VHL-deficient cells. We have begun to characterize these molecules.

Recently, we evaluated one of these compounds, 30408, with standard cell survival assays. We confirmed that 30408 exhibits selective toxicity against VHL-deficient cells. We also found that 30408 acts in a HIF-dependent manner, leading to cell death, and its effects are reversible only at the early stages of treatment. In the search for the target of 30408, we found that it causes a deranged state of cellular metabolism. In response to 30408, VHL-deficient cells showed decreased ATP levels, decreased mitochondrial mass, and decreased glucose transport. These effects were not seen in a genetically matched RCC cell line with wild-type VHL, suggesting that this compound may be a RCC-specific cytotoxin.

We are still investigating the relationship between the changed ATP level, mitochondrial level, and glucose transport seen after 30804 treatment, with the hypothesis that the drug acts at the glucose transporter level.

HERMES C: RF WIRELESS LOW-POWER NEURAL RECORDING SYSTEM FOR FREELY BEHAVING PRIMATES

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Neural prosthetics for motor systems is a rapidly growing field with the potential to provide treatment for amputees or patients suffering from neurological injury and disease. To determine whether a physically active patient such as an amputee can take advantage of these systems, we seek to develop an animal model of freely moving humans.

Therefore, we have developed and tested HermesC, a system for recording neural activity from electrode arrays implanted in rhesus monkeys and transmitting this data wirelessly. This system is based on the integrated neural interface (INI) microchip, which amplifies, digitizes, and transmits neural data across a ~900 MHz wireless channel. The wireless transmission has a range of ~4 m in free space. All together, this device consumes 11.7 mA from a 4.0 V lithium ion battery pack for a total of 46.8 mW. To test the performance, the device was used to record and telemeter one channel of broadband neural data at 15.7 kSps from one monkey doing various physical activities in a home cage, such as eating, climbing and swinging. The in-band noise of the recorded neural signal is 34 μVrms, which is low enough to allow the detection of neural units on an active electrode. This system can be readily upgraded to use future generations of the INI chip, with circuits providing 96 channels of programmable threshold crossing event data.

This wireless system attempts to open up a new class of experiments in systems neuroscience. If future versions of this system are successful, such studies will dramatically advance our understanding of cortical motor control.

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SEQUELAE AND PATIENT FOLLOW-UP AFTER MINOR HEAD INJURY

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This study examines the rates of post-concussive symptoms and clinical follow-up in patients treated for minor head injuries by the trauma service at Santa Clara Valley Medical Center (SCVMC). Previous research estimates that approximately half of patients who sustain minor head injuries experience persistent symptoms from the injury. Studies also suggest that follow-up care and rehabilitation for such patients can reduce severity of symptoms. We performed a retrospective analysis on patients identified using the SCVMC Trauma Registry as having been treated for a minor head injury (MHI) during the two-year period spanning May 2004 to April 2006. After applying exclusion criteria, we collected clinical and demographic information via chart review. We then attempted to contact each of these patients by telephone, and ultimately conducted 122 phone interviews. Interviewers asked directed questions about post-concussive symptoms using a modified version of the Rivermead Post-Concussive Symptoms Questionnaire. Interviews also collected data on clinical follow-up for head injury.

We initially identified 1,147 patients using the registry. We excluded 274 patients, 214 of which were excluded due to positive intracranial findings on CT scan. We performed chart review on the remaining 873 MHI patients, and successfully interviewed 122 of these (14%). 86 interviewed patients (70.5%) reported experiencing at least one symptom on the 15-symptom PCS survey. 59 interviewed patients (48%) reported receiving some follow-up care. Of the patients who reported at least one PCS symptom, 39 (45%) did not receive follow-up care. Overall, most patients did not seek follow-up care because they “felt fine;” however, a minority report that a lack of ability to pay prevented them from seeking care. Patients with private insurance were more likely to receive follow-up care than self-pay, Medicaid, and Medicare patients.

We conclude that a significant number of patients who experience enduring symptoms after MHI do not receive follow-up care. If follow-up care improves outcomes in such patients, this suggests an opportunity to achieve better results through increased interventions after MHI.

Funding provided by the Stanford Medical Scholars Fellowship Program and the SCVMC Department of Surgery – Trauma Service.

DESCRIBING THE UNDERLYING CAUSES OF CHILDHOOD OBESITY IN INDIA: A CASE-CONTROL PILOT STUDY

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The prevalence of childhood obesity is quickly becoming a global health concern as the number of individuals with unhealthy body mass and fat content grows. Diet and exercise-related risk factors for obesity, as well as its physiological correlates are becoming increasingly well-defined among U.S. and European populations. In contrast, there is a paucity of information regarding the existence of such relationships in developing countries, including India. Given the differences between cultural practices, infrastructure and environmental exposures in developed versus developing countries, it is important to characterize physiological, nutritional and physical activity measures in obese/overweight versus healthy weight children in developing nations. Our study addressed this by collecting diet and nutrition information, anthropometric measures, and physiological variables in a population of children between 9-19 years of age in Mysore, India. In doing so, we have identified potential risk factors in the development of childhood obesity in India, and some promising leads towards targeting interventions that may be instrumental in preventing the development of metabolic diseases downstream of obesity.

Using nutrition and physical activity questionnaires validated in the U.S., our data reveal an overall lack of nutritional education within the school system and from the medical community, as well as an overall lack of physical activity- two factors that may have direct implications on the development of childhood obesity. With respect to our physical activity survey, we were unable to validate it in an Indian population. Regarding physiological measures, we found that overweight/obese weight status was associated with increased waist circumference, systolic and diastolic blood pressure, VLDL, and triglycerides. Additionally, free insulin levels were significantly increased in overweight/obese individuals along with HOMA-IR scores, a screening tool for insulin resistance, while fasting blood glucose remained unchanged. This indicates not only that these children have impaired glucose metabolism compared to their healthy weight counterparts, but also that they are not yet decompensated with respect to insulin sensitivity, suggesting that the process may be reversible with proper glucose management. In summary, we believe that we have identified several areas of opportunity for improvement in the prevention of childhood obesity and some evidence that, in the Indian population, childhood obesity presents a challenging problem whose sequelae are serious but not completely irreversible.

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EFFECT OF GINGKOLIDE A ON BEHAVIORAL AND BIOCHEMICAL MARKERS OF LEARNING AND MEMORY IN TS1CJE, A MOUSE MODEL OF DOWN SYNDROME

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Recent studies exploring the molecular basis of deficits in learning and memory in Down Syndrome (DS) suggest a role for altered synaptic plasticity in the hippocampus. In mouse models of DS, it has been suggested that enhanced γ-aminobutyric acid (GABA)-ergic inhibition in the dentate gyrus is responsible for a reduction in long-term potentiation. The GABA-A receptor antagonist picrotoxin relieves this effect, implicating GABA-A receptor antagonists as a putative pharmacological treatment for DS. However, picrotoxin's epileptogenic side effects prevent its use as a treatment of cognitive impairment in DS patients. Terpene trilactones, active constituents of ginkgo biloba, are known to antagonize the GABA-A receptor. Several behavioral studies have demonstrated positive effects of terpene trilactones on learning and memory in both humans and animal models. Here, we administered the terpene trilactone, Ginkgolide A to a mouse model of DS in acute and chronic time courses.

We then measured behavioral manifestations of learning and memory using a T-maze and, after chronic administration, measured expression of subunits of the GABA A and B Receptors using a Western Blot.

Neither Ts1Cje nor 2N mice treated with Ginkgolide A showed improvement in memory wither either acute or chronic administration. After chronic administration of Ginkgolide A, no difference was found in GABA-A or B Receptor subunit expression in the hippocampi of drug treated or control animals.

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AN ANALYSIS OF HEALING FOLLOWING RETINAL PHOTOCOAGULATION

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Photocoagulation is the established treatment for numerous ocular diseases, yet it results in permanent retinal scarring, decreased vision, and blind spots. In order to develop a minimally traumatic retinal treatment, we assess changes in retinal morphology over time following short duration retinal photocoagulation. We studied the healing of retinal lesions in rabbits through clinical appearance, histology, and fluorescein angiography and in humans through high-resolution OCT. Eighteen Dutch belted rabbits underwent photocoagulation with a 532nm Nd:YAG laser using pulse durations of 5-100 ms. The lesion clinical appearance ranged from subthreshold to intense. The animals were sacrificed at 1 hour, 1 day, 1 week, 1 month, 2 months, and 4 months post treatment, and fixed eyes were stained with toluidine blue. High-resolution OCT examinations were performed using the Zeiss Cirrhus and Heidelberg Units in patients with photocoagulation lesions.

In rabbits, all pulse durations displayed RPE continuity by one week. The width of the retinal lesions decreased over time, with “light and greater intensities” (pulse durations of 10 to 100 ms) showing stabilization of lesion diameter by one month at approximately 35% of the initial lesion diameter. Lighter lesions characterized as “barely visible or invisible” (pulse durations of 7 and 5 ms, respectively) exhibited initial photocoagulation of the photoreceptor layer, but later showed complete restoration of the continuity of the photoreceptors layer with no residual gaps. In humans with burn lesions of lower initial intensity yet still visible ophthalmoscopically, there also appeared to be a similar phenomenon occurring as displayed by continuous photoreceptor and RPE layers observed on OCT. Such relative sparing of the inner retinal layers and restoration of photoreceptor continuity is in striking contrast with conventional thermal burns of higher intensity and duration, in which virtually all retinal elements are typically replaced by glial scars.

The decreasing width of the retinal damage zone seen both in rabbits histologically, and in humans by high resolution OCT, suggest migration of photoreceptors from surrounding unaffected areas in less intense burns. In conjunction with the fact that laser photocoagulation parameters can be optimized through shorter pulse duration to avoid damage to the middle and inner retinal layers acutely, it now appears possible to prevent permanent scarring in the photoreceptor layer as well. These results suggest that it may now be possible using pulses in the millisecond range to produce mild lesions with temporary outer retinal damage, which heals over time. It remains to be seen whether the positive therapeutic benefits of photocoagulation require some degree of permanent damage to the photoreceptors and pigment epithelium. The findings have potentially significant implications with regard to choosing optimal laser settings for therapeutic applications in humans.

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ROLE OF THE TOLL-LIKE RECEPTOR PATHWAY IN RECOGNITION OF WEAR PARTICLES

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Aseptic loosening of joint replacements is the most common cause of revision surgery. The etiology is related to wear particles from the implant, which produce chronic inflammation resulting in periprosthetic osteolysis. Macrophage activation is a key component of this inflammatory response. One pathway for macrophage activation involves the innate immune system via activation of Toll-Like Receptors (TLR). Many of the effects of TLR's are due to interaction with Myeloid Differentiation primary response gene 88 (MyD88), an adapter protein which couples the TLR to downstream signaling kinases, eventually culminating in activation of the transcription factor NFKB. Wear debris-induced inflammatory osteoclastogenesis requires cytokine production and osteoclast differentiation via the NFKB pathway. The current study investigates whether MyD88 plays a role in the macrophage inflammatory response to wear-debris recognition.

No previous experiments have been conducted in this area. To assess the role of MyD88 in wear-debris particle recognition, we used both a cell line and primary bone marrow derived macrophages. For experiments utilizing the murine macrophage cell line, cells were incubated with media containing PMMA particles and one of the following treatments: 1) MyD88 inhibitory peptide, which binds the MyD88 monomer, blocking MyD88 activation; 2) Control peptide, which crosses the cell membrane but does not interact with MyD88; 3) no peptide. For experiments utilizing primary cells, we isolated bone marrow derived macrophages from the femurs of wild type (WT) and MyD88-/- knockout mice. In both experiments, samples from the culture media were collected at 1, 4, and 12 hours post particle challenge and TNF-α levels were quantified using ELISA kits.

In both the macrophage cell line and primary bone marrow derived femoral macrophages, the response to PMMA particles is largely dependent upon the adapter molecule MyD88, presumably as part of Toll-Like Receptor signaling. The particle induced increase in TNF-α production was decreased by approximately 50% when MyD88 signaling was disrupted by either an inhibitory peptide which blocks MyD88 activation or by disruption of the MyD88 gene. Future studies will examine the roles of specific TLR's and of TLR signaling through MyD88-independent pathways such as TRIF. The TLR pathway of the innate immune system may represent a novel therapeutic target for prevention and treatment of particle associated periprosthetic osteolysis.

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THE EFFECT OF GASTRIC BYPASS ON COGNITION

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The increasing prevalence of morbid obesity and its comorbidities such as diabetes makes studying the effects vital. Higher glucose levels are associated with cognitive impairment and dementia. Currently, gastric bypass (GB) surgery is one of the few effective methods for long-term weight loss and glycemic control.

Previous studies have demonstrated that obesity and poor glycemic control are associated with neuronal loss, cognitive decline and dementia. A study of eight diabetic bariatric patients showed increases in cognition with weight loss. This suggests that improving glycemic markers would improve cognitive function and reverse impairment. We aim to improve previous studies by increasing sample size and heterogeneity. We hypothesize GB will increase glycemic control and cognition scores for both diabetic and non-diabetic patients.

Cognition scores and glycemic markers of a cohort of 30 obese patients undergoing GB were assessed preoperatively and at three and six months postoperatively. The battery of tests covered six broad areas of cognition: verbal memory and learning, attention, visuospatial performance, processing speed and executive function. Preliminary results suggest GB is associated with significant improvement in: attention and memory (p-value < 0.002), processing speed ($p<0.03$) and verbal learning ($p<0.04$) after 6 months in both patient populations. In addition, other tests involving executive functioning and verbal fluency show trends toward significance and will be interesting to follow.
These results demonstrate an additional benefit of GB and enhance our knowledge of the relationship between obesity, diabetes and cognition and may provide insight into other cognitive diseases such as dementia.

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ENGINEERING A WNT HYDROGEL DELIVERY SYSTEM FOR TISSUE REGENERATION

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Wnt lipoproteins are important signaling molecules involved in initiating and maintaining self-renewal and proliferation in multiple progenitor or stem cell populations in the human body. This signaling molecule has the potential to promote healing of many clinically important injuries in bone, eye, skin, and multiple other tissues. The major limiting factor of *in vivo* Wnt protein delivery is its profound lability. To realize the potential of this protein, an efficient, effective, and controlled Wnt delivery system is necessary.

Our laboratory has employed liposomes as a delivery vehicle for the Wnt protein which in addition protects the protein from proteases at the injury site. These liposomes can be injected *in vivo* in order to induce proliferation of stem cells that can regenerate injured tissues. Although the Wnt liposomes are effective at recruiting the body's stem cells, a method of directing their efforts specifically to injured tissue is necessary to optimize timing of Wnt activity and to minimize side effects. The purpose of this study is to assess the feasibility of integrating Wnt liposomes into hydrogels, which would serve as both a scaffold for the regenerating tissue and a vector to deliver and further stabilize the Wnt protein. In order to determine the effectiveness of hydrogels for this purpose, it is necessary to determine the level and duration of Wnt activity from the hydrogels relative to purified protein and liposome-stabilized protein. *In vitro* studies to date have indicated that alginate hydrogels can successfully encapsulate Wnt liposomes and provide additional stability.

Initial results indicate that Wnt liposomes can be integrated into alginate hydrogels, but have reduced signaling activity compared to pure Wnt liposomes due to their prolonged encapsulation. Future studies will modify the hydrogel degradation properties to improve Wnt liposomal signaling activity and subsequently will move to *in vivo* mouse studies.

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COMPARISON OF ROUNDING ACTIVITIES AND PERCEPTIONS BETWEEN HOSPITALS AND SERVICES

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Making rounds is the primary activity for both patient care and learning the practice of medicine on most in-patient services in the United States. However, much of the key observational data on rounds are 15 years old, are limited to one institution or one inpatient service, and do not include pediatrics. Duty hours restrictions now limit the in-hospital time available for both patient care and educational activities, therefore we sought to measure the effect that duty hours and patient load upon the location and content of rounds.

Direct observations of physician teams are ongoing to record the location where rounds occurs and the activities performed on rounds for two medical specialties, pediatrics and internal medicine, at two academic medical centers.

Preliminary data from 32 observations (24 pediatrics, 8 medicine) suggest more patient interactions occur on internal medicine services (5.3 minutes/patient vs. 2.9 minutes/patient), and more time devoted to teaching on internal medicine services (11.9% (16.4 minutes) vs. 6.8% (9.2 minutes)). Additionally a greater proportion of rounds occurred at the bedside or in a conference room on internal medicine (36% bedside, 23% conference room, 40% hallways) than on pediatrics (13% bedside, 9% conference room, 76% hallways). Over both specialties, the patient census is inversely associated with time spent on patient interactions ($r = -.44$) but appears unrelated to minutes spent teaching ($r = .02$). This abstract describes a study currently in progress thus it is not yet appropriate to draw conclusions or perform estimations of statistical significance. Detailed analysis, linear modeling, and appropriate statistical tests will be performed following the completion of data collection by May 1st 2008. Qualitative data-collection (interviews and written feedback) to further contextualize our findings is also ongoing.

QUANTIFICATION OF SOFT ATHEROSCLEROTIC PLAQUE IN THE SYSTEMIC ARTERIES USING CT ANGIOGRAPHY

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Atherosclerosis results in the deposition of “plaques,” which can reduce or interrupt blood flow to distal tissue, which, in turns, causes loss of function of the affected tissue. Research into the quantification of atherosclerosis usually focuses on the coronary arteries. Atherosclerosis in the extra-coronary arteries stays largely asymptomatic until serious degeneration has occurred, causing an aneurysm or dissection after many years. We intend to quantify the atherosclerotic burden in the extra-coronary arteries, starting with the aorta. Further, we intend to characterize atherosclerotic plaque by measuring soft (thought to be unstable) and well as calcified (thought to be stable) plaque.

Our algorithm first uses established methods to determine the inner (luminal) wall of the contrast-enhanced artery, thereby defining the border between the lumen and the soft and calcified plaque. The outer wall of the aorta is sometimes well defined when there is a thick enough layer of adjacent low density fat or high density bone. However, in many places along the length of the aorta, the outer wall may be indistinct or almost invisible. Using the well-defined segments of the outer wall as anchors, a shortest path algorithm is then used to find the outer wall, including its indistinct segments. The calculated path minimizes path length and tortuosity and tries to follow the indistinct layer of fat while avoiding high density structures. Preliminary results indicate a very good delineation of the outer wall in degenerate and normal aortas (Fig 1). Some heuristic methods were needed to avoid very low-density structures such as the lung.

There are two aspects of our algorithm that need to be validated. The first is the accuracy of the algorithm in-vivo, in order to verify that the wall thickness calculated is a reliable reflection of the actual wall thickness. In addition, an in-vivo proof-of-concept study is required in order to validate the data gathering and analysis aspects of the algorithm as well as its stability in a large series of patients.

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HIGH INTENSITY RESISTANCE TRAINING INDUCES LEFT VENTRICULAR HYPERTROPHY WITHOUT HEMODYNAMIC BENEFITS IN YOUNG HEALTHY MALES

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We performed a randomized controlled trial to determine the physiological consequences of HIRT on left ventricular end diastolic diameter (LVD), left ventricular posterior wall thickness (LVPWT), left ventricular mass (LVM), blood pressure and heart rate.

From LVD and LVPWT, measured via M-mode echocardiography, we estimated changes in left ventricular mass by using a derivation of the Troy equation, substituting LVPWT for interventricular septum thickness in diastole. The 3 week study included 26 young and healthy male subjects, n=17 exercise (EX) and n=9 control (CT). The intervention consisted of a 3 day per week regimen. Each workout session involved 8 power lifting exercises targeting major muscle groups and lasted 2 hours. Five sets of each exercise were executed; all were maximum lifts to fatigue.

The EX group demonstrated a significant increase compared to the CT group in LVD ($p=0.0004$) with an average increase of 0.15 cm, from 5.03 cm to 5.18 cm. The CT group had an average decrease of 0.01 cm of LVD from 4.97 cm to 4.96 cm. The EX group increased left ventricular mass by 11.6 g from 273.3 g to 284.9 g, while the CT group decreased left ventricular mass by 3.06 g from 251.3 g to 248.3 g. No significant changes in LVPWT ($p=0.41$), resting heart rate ($p=0.68$), or blood pressure (systolic $p=0.68$ and diastolic $p=0.77$) were observed.

A rapid and significant change in left ventricular mass and diameter occurred as a result of three weeks of HIRT. These data suggest that a vigorous workload (87%-100% of 1 repetition maximum lift) as a part of an exercise program elicits left ventricular dilation and left ventricular mass increase without hemodynamic benefits in young healthy men.

HIRT alone does not decrease blood pressure or heart rate. The significance of left ventricular diameter and mass changes are not understood.

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PPARδ DRIVES ALTERNATIVE (M2) ACTIVATION OF KUPFFER CELLS TO AMELIORATE OBESITY-INDUCED INSULIN RESISTANCE

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Macrophage infiltration and activation in metabolic tissues underlie obesity-induced insulin resistance and type 2 diabetes. While inflammatory activation of resident hepatic macrophages potentiates insulin resistance, the functions of alternatively activated Kupffer cells in metabolic disease remain unknown. Here we show that, in response to the T helper type 2 (Th2) cytokine interleukin-4 (IL-4), peroxisome proliferator activated receptor δ (PPARδ) directs expression of the alternative phenotype in Kupffer cells of lean and obese mice. Importantly, adoptive transfer of PPARδ null bone marrow into wild type mice diminishes alternative activation of hepatic macrophages, causing hepatic dysfunction and insulin resistance. Suppression of hepatic oxidative metabolism is recapitulated by treatment of primary hepatocytes with conditioned media from PPARδ null macrophages, indicating direct involvement of Kupffer cells in controlling liver lipid metabolism. Together, these data suggest an unexpected beneficial role for alternatively activated Kupffer cells in metabolic syndrome, and identify a new cellular target for treating insulin resistance.

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TH2 CYTOKINES AND STAT6 REGULATE HEPATIC FUEL SELECTION AND ENHANCE INSULIN ACTION

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Obesity promotes inflammatory activation of the innate immune system, leading to insulin resistance and type 2 diabetes. We show, in contrast, that the T helper type 2 (Th2) adaptive immune responses protect mice from the detrimental effects of diet-induced obesity. Administration of the Th2 cytokine interleukin-4 (IL-4) improved glucose tolerance and insulin sensitivity in obese mice, whereas genetic disruption of signal transducer and activator of transcription 6 (STAT6), the mediator of Th2-type immune responses, inhibited insulin action. The anti-diabetic effects of the IL-4/STAT6 axis are, in part, mediated by regulation of hepatic fuel selection via inhibition of peroxisome proliferator activated receptor α (PPARα). These findings thus have identified a new biological function for Th2 immunity in the regulation of glucose homeostasis and insulin sensitivity.

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KNOWLEDGE OF MEDICAL ERRORS: A SIGNIFICANT DETERMINANT OF RISK PERCEPTION

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Recent studies have shown there is a consensus among the public that health care is not as safe and reliable as it should and could be. Confidence in healthcare systems and health care professionals is on the decline. Too many medical errors are occurring, resulting in the deaths of hundreds of thousands of citizens annually worldwide. Because one of the main objectives of a health care system is to meet the needs of its citizenry, it is important for health policy makers to understand the public's perception of their health care system as well as the determinants that help form the public's perception of how safe their system is. A good measure for this is looking at the public's risk perception of medical errors.

This paper argues that knowledge of medical errors is one important key determinant, with an increasing knowledge of medical errors being linked with an increase in an individual's risk perception of medical errors occurring. This study empirically examines the effect of increased knowledge of medical errors on an individual's risk perception. The 2006 Eurobarometer 64.1 survey is employed and the study undertakes multivariate analysis.

The results provide evidence of an increased knowledge of medical errors being linked with an increase in an individual's risk perception of medical errors occurring. This is the first study to our knowledge that has analyzed the impact of information acquisition/knowledge of medical errors on individuals' risk perceptions of medical errors occurring to them.

INTEGRATIVE ANALYSIS OF GENOMIC AND TRANSCRIPTIONAL PROFILES IN COLORECTAL CANCER

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Genomic instability catalyzes neoplastic development in colorectal cancer. While a smaller proportion (~15%) of cancers harbor mutations in the DNA mismatch repair pathway, thereby exhibiting microsatellite instability (MSI), the predominant instability phenotype observed in ~85% of cancers is chromosomal instability (CIN), characterized by widespread aneuploidy, chromosomal aberrations and loss of heterozygosity events.

To determine the differences in the genomic and transcriptional profiles between each genomic instability phenotype, we performed array-based comparative genomic hybridization (aCGH) and gene expression profiling on a collection of 29 commonly used colorectal cancer cell lines exhibiting both instability phenotypes. Interestingly, unsupervised cluster analysis of DNA copy number aberrations (CNAs) revealed three classes of cell lines: one comprised of the MSI cell lines, while the CIN cell lines were divided into two distinct classes. Between the two CIN clusters, significant differences in DNA copy number were observed on chromosome arms 13q and 18q. Notably, cluster analysis across three publicly available aCGH datasets of primary colorectal tumors (n=221) similarly revealed two distinct classes of CIN tumors while all MSI tumors clustered together. For integration of the two microarray data types, we developed a software tool, DR-Integrator (DNA/RNA-Integrator), to identify CNAs underlying changes in gene expression. Overall, 6% of genes (961 of 15274) had CNAs that significantly correlated with gene expression changes ($r > 0.51$; FDR < 0.05). Several regions harboring known cancer genes were found to have correlated DNA copy number and gene expression values (e.g., *SMAD4* on 18q21.1). Further, DR-Integrator analysis also uncovered a number of genes with significant alterations in both DNA copy number and gene expression (e.g., amplified and overexpressed) between the three cell line subgroups, thereby suggesting candidate subtype-specific cancer genes.

Taken together, these findings suggest that unlike MSI, the CIN phenotype may be far more genetically heterogeneous, comprising at least two subtypes, which may have prognostic and therapeutic implications. Finally, our development of DR-Integrator provides a broadly useful analysis tool to integrate aCGH and gene expression microarray data for the identification of candidate cancer genes.

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ASYMMETRIC DIMETHYLARGININE PREDICTS MORTALITY AND MAJOR ADVERSE CARDIOVASCULAR EVENTS IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

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Patients with peripheral arterial disease (PAD) are at high risk for future cardiovascular disease events. Several parameters of vascular function are abnormal in PAD patients, including ankle-brachial index, claudication time, flow-mediated vasodilation and vascular compliance. In addition, PAD patients have elevated plasma levels of asymmetric dimethylarginine (ADMA), the endogenous inhibitor of nitric oxide synthase, which is associated with impaired endothelial vasodilator function. We hypothesized that one or more of these vascular tests would add predictive value to the traditional predictors of major adverse cardiovascular events (MACE).

We acquired baseline measurements of the above vascular tests as well as traditional cardiovascular risk markers in 133 PAD patients enrolled in the NO-PAIN study (mean age 73 years, 101 males), which was a randomized, placebo-controlled trial of long-term L-arginine supplementation. MACE was predefined as myocardial infarction, stroke, revascularization and all-cause mortality. Detailed telephone follow-up was obtained in 125 subjects (mean follow-up 35 months) using a structured questionnaire.

At least 1 MACE was observed in 49 of 125 patients (39%). Cox regression analysis showed that age (P=0.023) and ADMA (P=0.028) were the only significant predictors of death, independent of vascular function measures and traditional cardiovascular risk factors such as lipids, glucose, blood pressure and smoking history. In addition, subjects with ADMA levels in the highest quartile (>0.84 μmol/L) showed significantly greater occurrence of MACE compared to those with their ADMA levels in the lower 3 quartiles (P=0.001, log-rank test).

Our investigation indicates that the plasma ADMA concentration is an independent marker of cardiovascular risk in patients with PAD. Indeed, in this population of patients, it is a more powerful predictor of MACE than traditional cardiovascular risk factors and other non-invasive vascular tests. More studies are warranted to confirm that ADMA is a useful prognostic indicator in PAD.

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QUANTITATIVE HISTOLOGY OF THE MITRAL VALVE IN AN OVINE MODEL

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Department of Cardiothoracic Surgery

Recent literature shows that the mitral valve leaflets are not passive sheets of tissue that merely flapped together with each heart beat, but rather, are heterogeneous and dynamic with a readily adapting tissue architecture. Yet, few studies have quantified their histological characteristics of fiber orientation, thickness, and cellular distribution. By understanding the structure of the mitral valve, we can begin to better understand its function. This in turn will lead to the advancement of surgical techniques and devices..

Hence, the goal of this study was to develop and apply qualitative and quantitative histologic techniques to assess regional thickness, composition, and structural orientations of mitral valve leaflets taken from an ovine model. This comprehensive approach to characterizing the mitral valve employed polarized microscopy, histological staining, and digital measurement.

Using polarized microscopy, a qualitative view of fiber orientation was taken showing that it varies not only across the surface, but also with depth. The valves were then sectioned and stained with Gomori Trichrome. Measurements were taken with a digital microscope, and plotted using large order Fourier series to create a surface contour. A highly nonlinear surface was found predominantly on the ventricular side of the mitral valve from the annulus to the leaflet edge. The ratio of myocyte to collagen content was also calculated overall for each section using a digital microscope and Matlab 7.0. Though the ends carried the highest proportion of muscle, the composition unsteadily oscillates from anterior commissure to posterior commissure, further proving that the leaflet is a complicated, inhomogeneous structure.

This study establishes techniques from which the resulting characteristic data is now used to add needed detail to the laboratory's mitral valve finite element analyses. By gaining knowledge of structure, we have improved our prediction of function.

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OVERDIAGNOSIS IN LUNG CANCER SCREENING AND ITS IMPACT ON MORTALITY: SIMULATION BASED SENSITIVITY ANALYSIS

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Lung cancer screening has been controversial for the past few decades, with the major issue being whether the observed stage shift due to screening truly reduces lung cancer mortality through the early detection of tumors or actually overdiagnoses early-stage cases that are not life-threatening. Overdiagnosis has important health and economic consequences because it can lead to excessive follow-up procedures, such as additional scans, biopsies and unnecessary surgeries. The purpose of this project was to analyze the effect of overdiagnosis on the efficacy of computed tomography (CT) screening for lung cancer through the use of a stochastic simulation model of lung cancer screening trials.

Overdiagnosed tumors were added to the Incidence Component of the model, and all overdiagnosed tumors were counted in the first round of screening to simulate an extremely long doubling time. The model was validated using data from the Mayo CT trial on observed screen-detected cases. Calibration of the initial overdiagnosis component to the incidence data from existing lung cancer databases yielded an overdiagnosis parameter value of 0.0383, which suggests that detected tumors have a 3.83% likelihood of being an overdiagnosed tumor. The risk of mortality due to overdiagnosis was estimated based on the risk of death due to surgery, and as the risk of mortality due to overdiagnosis was incrementally increased from 0% to 10%, the effect on the reduction of mortality was observed. The reduction in mortality dropped from 18% without overdiagnosis to 12% with the upper extreme of 10% risk of mortality due to overdiagnosis.

Even with overdiagnosis, screening does seem to confer a reasonable mortality reduction benefit that could justify LDCT screening for lung cancer. Overdiagnosis is still not well understood and not easily quantified, but the use of a statistical model allows for assessment of the efficiency of screening under various hypothetical scenarios. The model can be extended to predict health and economic outcomes of alternative CT screening protocols that would not be feasible to evaluate without additional clinical trials. If our hypothesis that CT screening has a positive effect on lung cancer mortality continues to hold true according to the simulations, this project will also prepare a basis for the cost-effectiveness analysis of CT screening for lung cancer.

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MAGNETIC RESONANCE ANALYSIS OF THE HIPPOCAMPAL VOLUMES IN CHILDREN WITH SEIZURES

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Hippocampal volumetry is an essential research tool that has been used to track the progression of hippocampal sclerosis (HS) and temporal lobe epilepsy (TLE) in adults. It also aids in pre-operative assessment of candidates for epilepsy surgery. However, this very important tool is not widely available for children. Asymmetries in the hippocampal size and shape are frequently noted on the brain MR's (magnetic resonance) of children, yet very little data is available to determine the significance of such findings.

Defining the normal range of hippocampal asymmetry would aid in determining potential correlations of hippocampal abnormalities with outcomes in pediatric cases of TLE. However, there are many obstacles to obtaining such a data set in normal children. In this study we analyzed the hippocampal volumes of children who received a brain MRI to evaluate various types of seizures. 41 children age 1-17 years old who received a brain MRI in 2005-2006 were included in the study. In this group we found the mean left hippocampal volume to be 2.21 (SD 0.42) and mean right hippocampal volume to be 2.28 (SD 0.42) both consistent with measurements previously reported in the literature.

No significant hippocampal asymmetry was found in this group of children. We are analyzing correlations between quantitative measurements of hippocampal volume (using automated 3D reconstruction from area tracings of each MRI slice; intra-observer variability reported) and the qualitative measurements, which are the current standard of every day practice, performed by an expert pediatric neuroradiologist The ultimate value of this study would be to establish potential correlations of hippocampal asymmetry with EEG findings suggestive of epilepsy. The data analysis to establish such a correlation is ongoing.

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IMPROVING SAFETY IN THE OPERATING ROOM: A SYSTEMATIC LITERATURE REVIEW OF RETAINED SURGICAL SPONGES

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The Agency for Healthcare Research and Quality uses hospital administrative data to assess operating room safety. One of these Patient Safety Indicators (#5) is foreign body retained during surgical procedures. Retained surgical sponges, also known as gossypibomas, are the most commonly retained foreign bodies. The purpose of this study was to systematically review the literature on retained surgical sponges to characterize patient and case attributes and risk factors.

We searched the National Library of Medicine's Medline and the Cochrane Library databases (final search performed August 14, 2007) for English language articles. This search identified 160 abstracts to be screened, 51 of which were disqualified. This yielded 109 reports of 200 cases from 1963-2007 in 25 countries, with 56% of cases published after 2000. Two authors independently abstracted data from these meta-analyses, review articles, case reports and associated bibliographies. We then identified frequency of various risk factors in reported cases.

Gossypibomas in patients (mean 49 yrs, range 6-85 yrs) were most commonly reported to be found in the abdomen (56% of published studies), pelvis (19%), and thorax (11%). Head/neck, limb, and spine cases represent another 15% of cases. Average time to discovery is 6.5 years (SD 10 years, range less than three months to 40+ years). Excluding physical exam, the most commonly used methods of detection are CT (57%), radiography (39%), and ultrasound (33%) although more than one imaging method may be used in one case. Pain/irritation, palpable mass, and fever appear to be the leading clinical signs and symptoms (56% exhibit more than one symptom), but 6% of cases are asymptomatic. Commonly reported risk factors include both case characteristics (e.g., emergency procedures and use of non-radiopaque sponges), as well as human factors (e.g., inconsistent adherence to policies and poor communication).

The patterns of factors that lead to retained foreign sponges after surgery are becoming increasingly well understood, and multidisciplinary solutions to further reduce this low frequency event are necessary. However, given the complexity of both the care and the surgical suite environment, reducing the incidence of this patient safety indicator to zero may prove elusive.

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AIDING THE DETECTION OF VASCULAR TRAUMA WITH IMAGE-BASED MODELS OF BLOOD FLOW

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We are helping to develop a portable cuff that can be brought to an injured patient in the field and automatically detect and treat internal bleeding. The detection will be done by diagnostic ultrasound and the treatment by high intensity focused ultrasound.

In the hands of an experienced sonographer, a currently available portable ultrasound allows for a quick and inexpensive diagnosis of vascular trauma in the field. However, trained sonographers are usually not available at trauma settings, such as in the battlefield. Recent studies by Luo 2007 quantified ultrasound signatures of bleeding at the sites of vessel puncture, which can be used in automated algorithms for ultrasound diagnosis of suspected bleeding sites. However, trauma patients often have large areas of injury requiring extensive scanning to find the bleeding site. An algorithm that can first assess a vascular tree and localize suspicious branches for further examination or therapy would be useful.

Our approach to algorithm development was to 1) Demonstrate a physics-based model to characterize blood flow in normal humans subjects, and 2) Evaluate algorithm's ability to detect vascular abnormalities by looking for flows that deviate from the model with: a) 3D trauma simulations b) In vivo trauma animal models. This study was focused on the vascular tree of the upper extremities. Normal arm blood flow, regardless of physiological state, was well characterized with vessel diameters using the Power Law. We were then able to define a metrics that quantified the flow deviations from the law due to abnormalities. Simulations showed that this metrics could potentially detect a bleed only 1/5 size of a vessel.

In vivo models of acute bleeding are still needed to determine the true sensitivity of our bleed detection metrics since there are many autoregulatory processes which occur in the setting of acute bleeding which are not captured in the simulation. However, initial evaluations showed that this metrics can potentially complement other methods in a comprehensive automatic bleed detection algorithm for the device.

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RENAL CELL CARCINOMA: MOLECULAR MARKERS OF HISTOLOGIC SUBTYPE ASSESSED BY TISSUE MICROARRAY

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The three subtypes of renal cell carcinoma (RCC), clear cell, papillary renal cell, and chromophobe cell, have distinguishing histological features and genetic markers. However, the only method of RCC classification currently available in clinical pathology laboratories is examination of sections stained with hematoxylin and eosin (H and E), which is often insufficient for distinguishing between clear and chromophobe cell carcinomas. This is problematic, as accurate determination of subtype is crucial for risk assessment and treatment of RCCs.

We used previously generated cDNA microarray expression profiles of 41 renal neoplasms to identify 685 potential markers of clear or chromophobe cell carcinomas (Higgins et al, Am J Pathol, 2003). Immunohistochemical staining for a subset of these markers was performed using a tissue microarray (TMA) containing 244 clear and 27 chromophobe cell carcinomas and the degree and pattern of staining of TMA cores was assessed. Of the 39 potential subtype markers tested, vimentin and CD9 staining best distinguished between clear and chromophobe cell carcinomas, with the combination of vimentin negativity and strong CD9 positivity distinguishing chromophobe from clear cell carcinomas with a sensitivity of 88.4% and a specificity of 99.1%.

These results suggest that vimentin and CD9 staining may provide a simple immunohistochemical means of distinguishing between clear and chromophobe cell carcinomas when H and E staining fails and thus could be a useful adjunct in the clinical diagnosis of RCC. Further studies should be performed to confirm these findings.

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