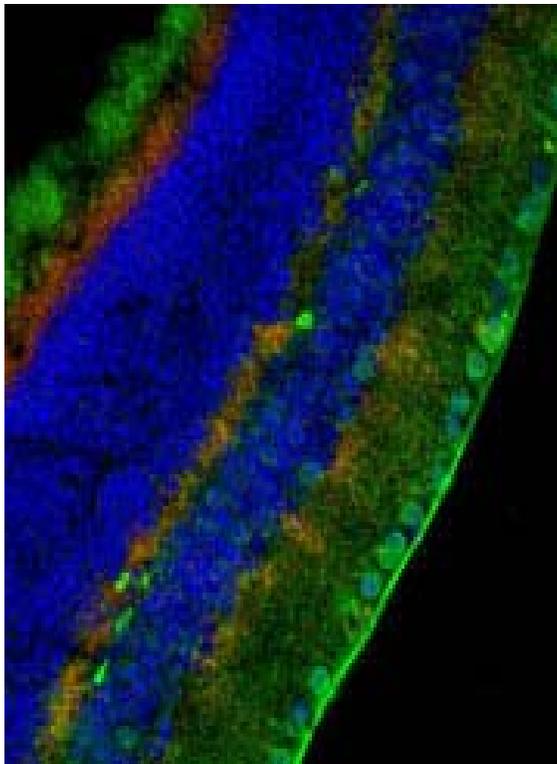




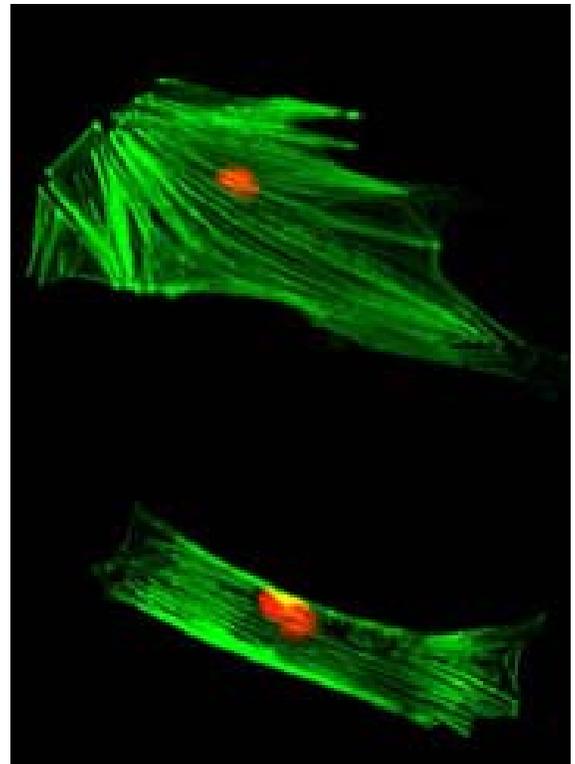
TWENTY- FOURTH ANNUAL
Stanford Medical Student Research Symposium

May 16, 2007
Fairchild Lobby

Stanford University School of Medicine



A



B

Cover References:

Figure A from: **Vazquez, Luis E.**, Beth Stevens, Navid Nouri, Gareth R Howell, Simon WM John, Ben A Barres: The role of the complement cascade in glaucoma

Confocal image, 63x, of the retina of an 11 month old glaucomatous mouse. There is evidence of synapse loss, an sign of neurodegeneration. We hypothesize that the immune complement protein C1q orchestrates the synaptic destruction, and that these events lead to Glaucoma. Nuclei in blue, synapses in red and C1q in green.

Figure B from: **Riboh, Jonathan BS**; Alphonsus Chong MD; Hung Pham BS; Michael Longaker MD, MBA; Chris Jacobs PhD; James Chang MD, FACS: Optimization of flexor tendon tissue engineering: the role of mechanical forces

Confocal image, 200x of two adipoderived stem cells. The cell (control) shown on the top grown under static conditions has a clearly radial/uniform distribution of the actin cytoskeleton. The cell shown on the bottom has been subjected to cyclic uniaxial strain, resulting in parallel orientation of the actin filaments, forming stress fibers and overall cell elongation. Green-phalloidin stain for actin, red stain-propidium iodide binds nucleic acids.

TWENTY- FOURTH ANNUAL
Stanford Medical Student Research Symposium
May 16, 2007
Fairchild Lobby
Stanford University School of Medicine

11:00 a.m.

Opening Remarks

Charles Prober, MD
Senior Associate Dean for Medical Student Education
Professor of Pediatrics and of Microbiology and Immunology

11:15 a.m.

Poster Session

Finished Research

Asya Agulnik
Beau Briese
Dora Castaneda
Trevor Chan
Richard Chiu
James Colbert
Andrea Crowell
Frederick Dewey
Hetty Eisenberg
Sepideh Gholami
Melanie Gipp
Stanley Hoang
Andrew Hsu
Kirandeep Kaur
Bradford Lee
Steven Lin
Courtney McGuire
Steven Minear
Mandar Muzumdar
Justin Odegaard
Luiz Pantalena Filho
Yannis Paulus
Sheila Ravi
Gabe Tsao
Jack Wang

Research in Progress

Emma Bakes
Nancy Benedetti
Jason Cuellar
Agnieszka Czechowicz
Vanessa Gabrovsky
Melissa Horoschak
Natalia Isaza
Bory Kea
Christle Layton
Helen Liu
Nathan Morrell
Shola Olorunnipa
Adeoti Oshinowo
Ricardo Pollitt
Benjamin Rafii
Jonathan Riboh
Louis Saddic
Jennifer Staple
Ricky Tong
Luis Vazquez
Lena Winestone

1:45pm

Closing Remarks

Philip Pizzo, MD
Dean, Stanford Medical School
Professor, Department of Pediatrics and Microbiology and Immunology

Pat Cross, PhD
Associate Dean for Medical Student Research
Professor, Department of Structural Biology

Ewen Wang, MD
Assistant Professor of Surgery (Emergency Medicine)

Awards

Norman Tong, MD
President, Alumni Association
School of Medicine - Medical Development

PRESENTATIONS

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31. **Benedetti, Nancy**, Vicki Fung PhD, Mary Reed, DrPH, Laurence Baker, PhD, John Hsu, MD, MBA, MSCE: Deductible health plans and patient cost discussions with physicians
2. **Briese, Beau** and Jay Bhattacharya: Does the match decrease fellowship wages?
17. **Castaneda, Dora C.**, Heng Zhao, and Gary K. Steinberg: Examination of the protective effect of dPKC inhibitor, dV1-1, on ERK mediated pathway in focal ischemia in rat
18. **Chan, Trevor**, Frank Kuhnert, Hsiao-Ting Wang, and Calvin Kuo: Exploration of GPR124 as a novel target for antiangiogenic therapy
19. **Chiu, Richard**, Ting Ma, R. Lane Smith, Stuart B. Goodman: Osteoprogenitors are inhibited by direct exposure to polymethylmethacrylate particles and by soluble factors released from particle-activated macrophages
3. **Colbert, James A.**, Aubree Gordon, Rigoberto Roxelin, Sheyla Silva, Javier Silva, Crisanta Rocha, and Eva Harris: Stanford advisor: Dr. Julie Parsonnet: Ultrasound measurement of gallbladder wall thickening as a diagnostic test and prognostic indicator for severe dengue in pediatric patients
4. **Crowell, Andrea L.**, Jarred W Younger, Robert L Lobato, Kim M Kaplan, Ian R Carroll, and Sean C Mackey: Demographic and psychosocial predictors of disability due to pain
36. **Cuellar, J.M.**, M.I. Nemenov, M. Klyukinov, D.C. Yeomans: Nociceptor-selective activation by diode laser
37. **Czechowicz, Agnieszka D.**, Deepta Bhattacharya, Daniel Kraft, and Irving L. Weissman: Antibody-based depletion of hematopoietic stem cells empties niches for efficient transplantation
6. **Dewey, Frederick E.**, BA, James V. Freeman, MD, David Hadley, PhD, Jonathan Myers, PhD, Victor F. Froelicher, MD: Non-linear analysis of heart rate variability during recovery from treadmill testing predicts cardiovascular prognosis

7. **Dewey, Frederick E.**, BA; James V. Freeman, MD; Greg Engel, MD; Raul Oviedo, MD; Natasha Ahmed, MD; Nayana Abrol, MD; Jonathan Myers, PhD; Victor F. Froelicher, MD: Novel predictor of prognosis from exercise testing: heart rate variability response to the exercise treadmill test
8. **Dewey, Frederick E.**, BA; John R. Kapoor, MD, PhD; Ryan S. Williams, MD; Euan A. Ashley, MRCP, DPhil; David Hadley, PhD; Jonathan Myers, PhD; Victor F. Froelicher, MD: Clinical correlates and prognostic significance of exercise-associated ventricular arrhythmias in patients referred for exercise treadmill testing
5. **Eisenberg, Hetty**, and David Spiegel: Improvements in mindfulness and self-compassion are correlated with improvements in mood disturbance and health status in a population of meditators
38. **Gabrovsky, Vanessa**, Christopher L. Chavez, W.E. Jung and Michele P. Calos: Factoring in PhiC31 integrase as a cure for hemophilia A
9. **Gholami, Sepideh**, Minnie M. Sarwal, Maarten Naesens, Richard A. Barth, Hans G. Ringertz, Raymond R. Balise, and Oscar Salvatierra: Standardizing resistive indices in healthy pediatric transplant recipients of adult-sized kidneys
10. **Gipp, Melanie S.**, William Fearon, MD: Coronary physiologic measurements as predictors of clinical outcomes in cardiac transplant patients
20. **Hoang, Stanley**, Jason Liauw, Michael Choi, Matt Choi, Matt Percy, Ben Wildman-Tobriner, Cagla Eroglu, Ben Barres, Tonya Bliss, Raphael Guzman, Gary Steinberg: Endogenous Thrombospondins 1 and 2 are necessary for synaptic plasticity and spontaneous functional recovery after stroke
39. **Horoschak, Melissa**, Alice Fan, Amy Shirer, Jan van Riggelen, Jason Gotlib, Dean W. Felsher: Molecular response to targeted inactivation of BCR-ABL in chronic myeloid leukemia
21. **Hsu, Andrew R.**, Weibo Cai, Anand Veeravagu, Khalid A. Mohamedali, Kai Chen, Hannes Vogel, Lewis C. Hou, Victor Tse, Michael G. Rosenblum, and Xiaoyuan Chen: Multi-modality molecular imaging of glioblastoma growth inhibition using vascular-targeting fusion toxin VEGF121/rGel
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40. **Kea, Bory**, Robert Pesich, Lorinda Chung, Patrick Brown, and David Fiorentino: Genomic analyses identify abnormalities in lipid metabolism in dermatomyositis patients

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11. **Lee, Bradford W.**, Kuldev Singh, Parthasarathi Sathyan, Alan L. Robin: Factors associated with poor follow-up among glaucoma patients in south India
12. **Lin, Steven Y.**, Ellen T. Chang, and Samuel K. So: why we should screen all foreign-born Asian American adults for hepatitis B: a cross-sectional study of 3,163 Asians in California
41. **Liu, Helen**, David J. Wong, Howard Y. Chang: The role of CSN5 in UV-mediated DNA damage
13. **McGuire, Courtney S.**, Kristen L Cobb and Paul G Fisher: Ependymoma incidence is a function of gender and age but not time: a seer study
23. **Minear, Steven C.**, Allyson O'Donnell, Guri Giaever, Corey Nislow, Tim A. Stearns, Martha Cyert: Curcumin exposure induces G1 arrest in *saccharomyces cerevisiae* by iron starvation
42. **Morrell, Nathan**, Jae-Beom Kim, Philipp Leucht, Derk ten Berge, Roel Nusse, and Jill A. Helms: In vivo delivery of WNT proteins by liposomal packaging
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25. **Odegaard, Justin I.**, Roberto R. Ricardo-Gonzalez, Matthew H. Goforth, Christine R. Morel, Vidya Subramanian, Lata Mukundan, Alex Red Eagle, Divya Vats, Frank Brombacher, Anthony W. Ferrante, Ajay Chawla: PPAR γ controls alternative macrophage activation to ameliorate obesity-induced insulin resistance
43. **Olorunnipa, Shola B.**, Shahram Aarabi, and Geoffery C. Gurtner: The role of bone marrow-derived cells in the process of hypertrophic scar formation
29. **Oshinowo, Adeoti**, Hadiza Galadanci, MD, Mohammed Awwal, MD, Oladosu Ojengbede, MD, Lyndsay McDonough, MPH, Elizabeth Butrick, MPH, MSW, Suellen Miller, PhD, CNM: Stanford Advisor: Paul Hensleigh: Overcoming delays in childbirth due to hemorrhage: A qualitative study of the non-pneumatic anti-shock garment (NASG) in Nigeria
26. **Pantalena Filho, Luiz C.**, Catherine Guenther, Christine Ham, David Kingsley: Encoding skeletal morphology in the genome
14. **Paulus, Yannis M.**, ATul Jain, Michael W. Wiltberger, Dan E. Andersen, Phil Huie, Mark S. Blumenkranz, Daniel Palanker: Effect of pulse duration on the size and character of the lesion in retinal photocoagulation

47. **Pollit, Ricardo.** Layton, Christle. Ngyuen, Josephine, MD. Susan Swetter, MD: The effects of digital dermoscopy on skin self-examination in patients at increased risk for melanoma
33. **Raffi, Benjamin Y.**, Oscar J. Abilez, and Christopher K. Zarins: Lateral displacement is an indicator of stent-graft migration in endovascular aneurysm repair
15. **Ravi, Sheila**, Sarah Forsberg and James Lock,MD,Ph.D: Characterization of parental psychopathology in anorexia nervosa
44. **Riboh, Jonathan BS**; Alphonsus Chong MD; Hung Pham BS; Michael Longaker MD, MBA; Chris Jacobs PhD; James Chang MD, FACS: Optimization of flexor tendon tissue engineering: the role of mechanical forces
45. **Saddic, Louis**, Or Gozani, and Julien Sage: Post-translational modification of tumor suppressors and the methylation of retinoblastoma
34. **Staple, Jennifer B.**, and Dr. Peter Egbert: Assessing preoperative and postoperative visual acuity in patients receiving free cataract surgery by ophthalmologists at four eye clinics in Ghana and India
46. **Tong, Ricky T.**, Pritha Ray, and Sanjiv S. Gambhir: The mighty mouse: ubiquitous expression of tri-fusion imaging multimodality (Bioluminescence, Fluorescence, PET) reporter gene in transgenic mouse
27. **Tsao, Gabriel J.**, Jessica A. Allen, Kathryn Logronio, Judith A. Shizuru: Blood and lymphoid immune reconstitution following allogeneic hematopoietic cell transplantation in mice
35. **Vazquez, Luis E.**, Beth Stevens, Navid Nouri, Gareth R Howell, Simon WM John, Ben A Barres: The role of the complement cascade in glaucoma
28. **Wang, Jack T.**, Jeffrey L. Goldberg, and Ben A. Barres: How do CYP1B1 mutations cause glaucoma?
48. **Winestone, Lena**, Thierry Giffon, David Lewis: A novel adjuvant's immunogenicity is not mediated by plasmacytoid dendritic cells

DIAGNOSIS AND MANAGEMENT OF JAUNDICE IN HOSPITAL NO. 13: MOSCOW, RUSSIA

Asya Agulnik, Irina I Ryumina, MD² and Anthony E Burgos, MD, MPH¹ (Sponsored by Anthony E Burgos)

¹General Pediatrics, Stanford University, Palo Alto, CA, United States

²Pediatrics, Moscow Research Institute for Pediatrics and Children's Surgery, Moscow, Russian Federation

Background: The management of neonatal hyperbilirubinemia has evolved with ongoing assessment of physician practice and clinical outcomes. This process has not occurred in Russia, where despite the existence of clinical guidelines, few data have been collected regarding hyperbilirubinemia.

Objective: To assess physician practice at Hospital 13 in Moscow via 1) the correlation of physician assessment of jaundice with total serum bilirubin(TSB) and 2) the bilirubin levels at which phototherapy and or exchange transfusion occurs compared to published protocols from Russia and the United States.

Design/Methods: Cross sectional study conducted by chart review of all infants admitted to Hospital No.13 in Moscow January thru May 2005. Inclusion criteria: admitted to equivalent of Level II nursery, <28 days old at time of admission, never transferred to Level III nursery, and did not expire. Variables included birth weight, gestational age, diagnosis, TSB, physician score of jaundice level at time of TSB, and use of phototherapy or exchange transfusion.

Results: Of 825 charts reviewed, 628 were included in the study. Mean gestation 36 wks. Mean birth weight 2416 g. 87.6% had physician-diagnosed jaundice and 24.5% received phototherapy. No exchange transfusions were performed. Mean number of TSBs per subject was 4.8. TSB levels correlated poorly with documented level of jaundice ($r = 0.589$). Under two Russian protocols, providers failed to start phototherapy when indicated in infants 1500-2000 g up to 43% of the time, started phototherapy unnecessarily in infants >2500 g up to 46% of the time, and missed up to 21 exchange transfusions. Under 2004 AAP guidelines, providers failed to start phototherapy when indicated in infants 35-37 wks gestation 12% of the time, started phototherapy unnecessarily in those 38 wks 80% of the time, and missed 15 exchange transfusions.

Conclusions: Russian providers generally relied on their clinical evaluation and not TSB to determine treatment, exhibiting poor adherence to existing guidelines. These data illustrate the challenges of overcoming physician behavior in order to implement a national clinical guideline. They also reveal a true need to document bilirubin-induced neurotoxicity in order to drive effective public policy and to improve care for newborn infants with jaundice.

Funded by the Stanford Medical Scholars Research Program

PROSPECTIVE ANALYSIS OF FACTORS INVOLVING POST-SURGICAL DELIRIUM

Emma L.O. Bakes PhD (Department of Medicine), Sami M. Akram MD (Department of Cardiovascular Surgery), Jose R. Maldonado MD (Department of Psychiatry).

Delirium is defined as a sudden state of severe confusion accompanied by rapid changes in brain function, possible hallucinations and hyperactivity. Symptoms may include an inability to concentrate, plus disorganized thinking manifested by tangential or incoherent speech. Further, patients may manifest reduced levels of consciousness, sleep disturbances and drowsiness. Delirium is usually reversible and may be caused by a broad spectrum of conditions that adversely affect brain metabolism, including brain tumors, drug toxicity or withdrawal, seizures, head trauma, hypoxia, electrolyte or acid-base imbalance, hypoglycemia and hepatic or renal failure.

In short, there is only a minimal reservoir of knowledge concerning the exact pathophysiology of delirium and the proposed research will better define its specific etiology. We will do this via a prospective study examining patients' physiology before and after cardiovascular surgery, monitoring them for the development of delirium, and evaluating whether any of the parameters we are following change and correlate with the development and degree of delirium. The associated morbidity and mortality in the 27% of hospital patients developing delirium after undergoing cardiovascular surgery make its diagnosis of paramount importance on both a humanitarian and a financial level and we intend for this study to make identification of this broadly defined phenomenon more predictable and more easily quantifiable by physicians. Pre-existing frailty, coupled with a cardiac cause of delirium, and poor early recognition by treating physicians are associated with worse outcomes.

Consequently, because delirium is found in particular in the geriatric population, a population which commonly undergoes cardiovascular surgery, and because this population is on the rise due to a boost from the "Baby Boomer" generation, it is timely to perform this study and to be prepared for the incoming wave of delirium cases which will hit the medical system in the next decade. We will complete a quantitative analysis of an extensive suite of parameters proposed to influence post-surgical delirium and determine whether these parameters can be reliably monitored and measured. Identifying higher risk factors will yield preventive interventions for vulnerable populations, reduce morbidity and improve the quality of life for patients by preventing the traumatic experience of delirium related psychosis."

Funded by the Stanford Medical Scholars Research Program

DEDUCTIBLE HEALTH PLANS AND PATIENT COST DISCUSSIONS WITH PHYSICIANS

Nancy Benedetti, Vicki Fung PhD, Mary Reed, DrPH, Laurence Baker, PhD, John Hsu, MD, MBA, MSCE. Health Research and Policy Department

Background: With many new health plans, patients face increased out-of-pocket costs. There is limited information on how often patients discuss costs with their doctors when making decisions about their medical care.

Methods: In 2006, we conducted a telephone interview study among a stratified random sample of 1500 (84% response rate) adult members of a prepaid, integrated delivery system: equal numbers with and without deductible plans (deductibles \$250-1000, median \$500), and with and without chronic diseases (asthma, diabetes, hypertension). The three deductible plans (Plans A-C) varied in the applicable services and deductible amount, with Plan A being the most generous (i.e.- fewest deductible covered services) and Plan C being the least generous. Subjects reported whether they talked about costs with a physician, and whether they changed their care-seeking behavior in response to costs for medical services. In multivariate logistic models, we adjusted for respondent characteristics (chronic disease sample, having a regular provider, age, region, self-reported health, marital status, race, gender, education and income). We weighted all analyses by sampling proportions.

Results: Overall, 11.7% of respondents with deductible plans and 7.3% with non-deductible plans reported talking with their doctor about medical costs. After adjustment, patients with less generous deductible plans (OR=2.41 for Plan B, 95% CI 1.14-5.10; OR=4.30 for Plan C, 95%CI 1.60-11.53) were more likely to talk with their doctor about costs, compared to patients with non-deductible plans. In a separate analysis, patients with the highest deductible amount (OR 3.69, 95% CI 1.54-8.82) were more likely to talk with their doctor about costs compared to patients with non-deductible plans. Overall, 31.8% with a deductible plan and 15.5% with a non-deductible plan reported delaying or avoiding office visits; and 22.2% and 4.2%, respectively, reported delaying or avoiding medical tests. Patients who reported delaying or avoiding care were more likely to report talking with their doctors than respondents who did not delay or avoid care (13.4% versus 6.3% for office visits, 16.6% versus 7.3% for medical tests).

Conclusions: Few patients reported talking with their doctor about costs, though patients facing higher costs or less generous coverage were more likely to have these discussions. Importantly, many patients also reported changing their care seeking behavior because of costs.

Implications: Patients appear to change their care seeking behavior in response to costs, but rarely discuss costs with their physicians. More research is needed to determine if these behaviors place patients at higher risk for adverse medical events or complicate the coordination of their care.

Funded by the Stanford Medical Scholars Research Program, The Commonwealth Fund, and Kaiser Family Foundation

DOES THE MATCH DECREASE FELLOWSHIP WAGES?

Beau Briese* and Jay Bhattacharya.

Medicine (Center for Primary Care and Outcomes Research)

“The Match” is an application process nearly all residencies and most fellowships require. The most common version of the match is the National Residency Matching Program (NRMP). The NRMP limits applicants to one offer of employment, a contract applicants cannot negotiate and must sign at the risk of being barred from the NRMP match for up to three years. Economic theory predicts that these anticompetitive restrictions incentivize employers to reduce wages. We conducted the first parametric and nonparametric multidimensional statistical analysis contrasting the wages of subspecialties that use a match to the wages of subspecialties that do not within a given specialty; we examined AMA data from 75.6% (n=1447) of American pediatric and internal medicine first-year fellowship programs in survey years 2001-2002, 2003-2004, and 2004-2005.

Median economic wages of matched subspecialties were 28.6% lower in internal medicine and 23.6% lower in pediatrics than wages of nonmatched subspecialties in 2004-2005 ($p < .001$). Matched and nonmatched fellows earn the same salaries. Matched fellows have lower wages because they are required to work more hours to earn that salary (240 additional hours annually in internal medicine and 205 in pediatrics; $p < .001$). The extra hours cost fellows opportunities to moonlight. In pediatrics, median three-year wage growth was 5.43% lower in matched subspecialties ($p < .001$). At the 25th percentile, wage growth even more depressed – 15.4% lower ($p < .001$). Internal medicine harbored no relevant differences in wage growth. Findings were generally consistent at the mean, 25th, 50th, and 75th percentile of wages over the three years studied.

Our findings follow the predicted patterns of an anticompetitive market: NRMP restrictions appear to reduce fellowship wages by increasing work hours. The economic cost to residents and fellows exceeds \$250 million/year. Where applicants have the freedom to receive multiple offers and negotiate, markets provide greater wages while requiring fewer work hours. Future research will reveal how we can transition match residencies and fellowships to fair, organized, and stable markets that will increase the wages of young MDs.

Funded by the Stanford Medical Scholars Research Program

EXAMINATION OF THE PROTECTIVE EFFECT OF DPKC INHIBITOR, DV1-1, ON ERK MEDIATED PATHWAY IN FOCAL ISCHEMIA IN RAT

Dora C. Castaneda* Heng Zhao, and Gary K. Steinberg.
Department of Neurosurgery

Both δ PKC and Erk1/2 activity may contribute to ischemic damage after stroke. Some in vitro reports in non-neuronal systems suggest cross talk between these two pathways, e.g., δ PKC activity up regulates the level of phosphorylated Erk1/2. Whether the δ PKC and Erk pathways are coordinated to mediate ischemic damage after stroke is not known.

Therefore we investigated the relationship between these pathways in a model of focal ischemia by observing and modifying the activation state of each pathway alone and in combination with the other. Inhibitors of Erk1/2 pathway, U0126, the δ PKC inhibitor, δ V1-1, and the δ PKC activator, ψ -RACK, were employed in a middle cerebral artery occlusion (MCAO) model.

Here we report that inhibiting both the ERK1/2 and the δ -PKC pathway offers greater protection than either alone, suggesting that they may act in parallel. In addition, the δ PKC agonist ψ -RACK partially abolishes the protection afforded by the Erk1/2 inhibitor U0126. Furthermore, we found that U0126 delivered at the onset of ischemia was neuroprotective but not when delivered at reperfusion. Finally, we detected levels of phosphorylated ERK1/2 (P-Erk1/2) at various time points during and after ischemia. P-Erk1/2 transiently increased during ischemia and after reperfusion. As expected, the Erk1/2 inhibitor U0126 reduced the level of P-Erk1/2. However, the δ PKC agonist, δ V1-1, and δ PKC inhibitor, ψ -RACK, enhanced and blocked protein levels of p-Erk1/2, respectively.

Funded by PO1 NS037520-07S1 (GKS & DCC) and Stanford Medical Scholars Research Program (DCC)

EXPLORATION OF GPR124 AS A NOVEL TARGET FOR ANTIANGIOGENIC THERAPY

Trevor Chan, Frank Kuhnert, Hsiao-Ting Wang, and Calvin Kuo.

Stanford University School of Medicine, Department of Medicine, Division of Hematology

Cancer therapy targeting tumor blood vessels has an attractive simplicity as tumors clearly require a blood supply for nutrition and growth. Accordingly, antiangiogenic therapy represents a promising new modality for the treatment of brain tumors for which systemic chemotherapy has encountered unique obstacles such as the blood-brain barrier.

The Kuo Laboratory has demonstrated that an orphan G-protein coupled receptor, GPR124, is involved in the development of the central nervous system vasculature. Within this context, we explored GPR124 as a novel target for antiangiogenic therapy. Mice with preestablished subcutaneous fibrosarcomas received single i.v. tail vein injections of an adenovirus expressing GPR124 ectodomain capable of sequestering GPR124 ligand, and tumor growth was followed to reveal a significant reduction in tumor volume and decreased vascular density compared to controls. Preliminary results revealed that tumor-bearing mice treated with GPR124 ectodomain had a 45% reduction in tumor volume compared to the controls. Further analysis of harvested tumors revealed a 41% reduction in pericyte content in tumors treated with GPR124 ectodomain. GPR124 blockade did not significantly affect endothelial cell content compared to controls. These initial results provide further evidence of GPR124 function in vascular development.

The development of tumor vasculature is fully recognized to precede and to be necessary for the development of frank tumorigenicity. This study utilized an adenoviral expression platform to systemically inhibit GPR124 signaling within tumor vasculature resulting in the inhibition of tumor growth. Such results may help validate GPR124 as a vascular specific determinant that may be exploited as a therapeutic target for antiangiogenic therapy.

Funded by the Stanford Medical Scholars Research Program

OSTEOPROGENITORS ARE INHIBITED BY DIRECT EXPOSURE TO POLYMETHYLMETHACRYLATE PARTICLES AND BY SOLUBLE FACTORS RELEASED FROM PARTICLE-ACTIVATED MACROPHAGES

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

Implant loosening of total joint arthroplasty is a combined effect of bone destruction and reduced bone formation resulting from the activity of cells exposed to wear debris particles. The inhibition of osteogenesis by orthopedic wear debris may be due to a direct effect of particles on osteoprogenitors or an indirect effect of inhibitory factors released from particle-activated cells. This study determined whether the direct exposure of osteoprogenitors to particulate materials of polymethylmethacrylate (PMMA) bone cement inhibits the ability of these cells to differentiate into osteoblasts, and whether macrophages, marrow stromal cells, and marrow stromal-derived osteoblasts exposed to PMMA particles produce soluble factors that can indirectly inhibit osteogenesis.

Osteogenesis was induced by growing primary murine marrow stromal cells (MSCs) and MC3T3-E1 preosteoblasts in osteogenic medium containing 50 µg/ml ascorbic acid and 10 mM β-glycerophosphate (medium for MSCs also contained 0.1 µM dexamethasone). MSCs and MC3T3-E1 cells were treated with PMMA particles (0.30% v/v) throughout the osteogenic culture period. Additional cultures of MSCs were incubated in conditioned medium collected from cultures of murine Raw264.7 macrophages, marrow stromal-derived osteoblasts, and MSCs that had been challenged with PMMA particles (0.30% v/v). All cultures were assessed for the quantity of mineralized nodules/matrix and alkaline phosphatase-positive colonies at the end of a 15-day culture period.

MSCs and MC3T3-E1 cells directly treated with PMMA particles showed a ≥ 95% reduction in the quantity of mineralized matrix/nodules and alkaline phosphatase-positive colonies. MSCs grown in conditioned medium from particle-treated macrophage cultures showed a significant 84% reduction in mineralization but a non-significant change in the quantity of alkaline phosphatase-positive colonies. MSCs grown in conditioned media from particle-treated cultures of osteoblasts and MSCs showed non-significant changes in both outcome parameters. These results demonstrate that the direct exposure of osteoprogenitor cells to PMMA particles causes complete suppression of osteogenesis, and that macrophages exposed to PMMA particles release soluble factors that inhibit mineralization. Marrow stromal cells and osteoblasts, however, do not release detrimental factors that inhibit osteogenesis. The suppression of osteoprogenitor differentiation therefore appears to be a combined effect of direct exposure to PMMA particles and inhibitory factors released from particle-activated macrophages.

Funded by the Stanford Orthopedic Research Fund, Zimmer Inc., and the Stanford Medical Scholars Research Program

ULTRASOUND MEASUREMENT OF GALLBLADDER WALL THICKENING AS A DIAGNOSTIC TEST AND PROGNOSTIC INDICATOR FOR SEVERE DENGUE IN PEDIATRIC PATIENTS

James A. Colbert¹, Aubree Gordon², Rigoberto Roxelin³, Sheyla Silva³, Javier Silva³, Crisanta Rocha³, and Eva Harris²

¹Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA

²Divisions of Infectious Diseases and Epidemiology, School of Public Health, University of California, Berkeley, 140 Warren Hall, Berkeley, CA, 94720-7360

³Infectious Diseases Unit, Hospital Infantil Manuel de Jesús Rivera, Managua, Nicaragua

Stanford advisor: Dr. Julie Parsonnet, Division of Infectious Diseases and Epidemiology, Stanford University School of Medicine

Background: Dengue is a major cause of morbidity in tropical regions worldwide. This study examines the clinical utility of gallbladder wall thickening (GBWT) measured by ultrasound as an indicator of plasma leakage and disease severity in children infected with dengue virus.

Methods: The study included 73 children (<15 years old) who presented to the national pediatric reference hospital in Nicaragua, between August 2005 and February 2006 with clinical symptoms consistent with dengue fever. Patients were divided into three categories: 18 (25%) other febrile illness (OFI), 44 (60%) dengue fever (DF), and 11 (15%) dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Patients received 1-5 (mean 2.34) ultrasounds during their illness.

Results: The lowest mean GBWT (2.00mm) was obtained from patients with OFI, while DF patients had a mean GBWT of 3.31mm, and patients with DHF/DSS displayed a mean GBWT of 6.21mm. Differences in GBWT between the three patient groups was significant 3-4 days as well as 5-6 days post-onset of symptoms ($p < 0.01$, MANOVA), and GBWT was significantly correlated with the hallmark features of DHF/DSS, thrombocytopenia and elevated hematocrit/hemoconcentration ($p < 0.01$, t-test). Receiver operating characteristic (ROC) analysis was performed to determine the optimal cutoff values for GBWT; 4mm and 5mm cutoffs resulted in the highest sensitivity and specificity on both days 3-4 and 5-6 post-symptom onset.

Conclusions: GBWT can serve as a clinically relevant diagnostic test and prognostic indicator of severe dengue in pediatric populations. Furthermore, GBWT cutoff values of 4mm or 5mm are highly associated with disease severity.

Funded by the Stanford Medical Scholars Research Program

DEMOGRAPHIC AND PSYCHOSOCIAL PREDICTORS OF DISABILITY DUE TO PAIN

Andrea L Crowell, Jarred W Younger, Robert L Lobato, Kim M Kaplan, Ian R Carroll, and Sean C Mackey
Department of Anesthesia, Stanford School of Medicine

Pain is one of the most common reasons that patients present to their health care providers. Persistent pain lasting six months or more, has been reported in 22% of primary care patients in the most recent multinational study (Gureje, et. al., JAMA, 1998). Interestingly, the degree to which a person is in pain is not the strictly correlated with the degree of disability the person experiences. Chronic pain patients exhibit considerable variability in the degree to which they are impaired by their condition. Here, we take an epidemiological approach to determine predictors of moderate versus severe pain disability. Participants included more than 5000 patients seen at the Stanford Pain Center from January 2001 to September 2006. The participants were administered the Treatment Outcomes in Pain Survey (TOPS), a global assessment of health and wellbeing that is based on the Short-Form 36 Health Survey (SF-36). The measure assesses bodily pain, physical functioning, mental health, and role limitations due to physical and emotional problems.

K-means cluster analysis was performed using the eight subscales of the SF-36. Two groups were formed, representing moderate and severe disability. Patients in the Moderate Cluster reported physical health subscale scores that were roughly 1.5 standard deviations below U.S. population norms. Patients in the Severe Cluster reported marked deficiencies in both physical and mental health, with averages from each of the eight subscales falling roughly 2 standard deviations below U.S. norms. A multiple logistic regression was then performed to identify significant predictors of symptoms. Predictors included: age, sex, income, coping strategies, and perception of control over pain.

Among chronic pain patients, pain severity and resulting disability may be significantly influenced by demographic and trait psychosocial factors. Recently, additional psychological questionnaires have begun to be administered to this pain population, including the Minnesota Multiphasic Personality Inventory. As this data is collected, we plan to further elucidate how personality and other psychological factors influence disability risk or resistance among chronic pain patients.

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NOCICEPTOR-SELECTIVE ACTIVATION BY DIODE LASER

J.M. Cuellar^{1,2}, M.I. Nemenov^{1,2,3}, M. Klyukinov^{1,2}, D.C. Yeomans^{1,2}

1. Anesthesia, Stanford University,
2. Pain Research, Stanford University,
3. R&D, Lasmed, LLC, Mountain View, CA

Selective activation of A or C nociceptors may enable focused study of changes occurring in various pain states.

Methods: Infrared diode laser irradiation uniformly heats skin and was used for 2 types of stimuli: short duration (50-200ms, 3 mm²)-high intensity (high heating rate) pulses, which produce a pricking pain sensation in humans and reflex withdrawal in rats, or long-duration (1.5-20s, 40 mm²)-low-intensity (low heating rate) pulses, which produce a burning pain sensation in human and withdrawal in rats. The following experiments were performed in adult male SD, isoflurane-anesthetized rats: 1) In vivo, extracellular single-unit, spinal dorsal horn neuron recordings during laser stimulation of the hindpaw; 2) In vivo, intact-brain, extracellular single-unit trigeminal ganglion neuron recordings during laser stimulation of the face and 3) In vivo, teased-fiber saphenous nerve recordings (silver electrode) during laser stimulation of the leg.

Results: 1) DHN's: intensity-dependent activation of WDR neurons at latencies consistent with C-fiber and A-delta fiber input were observed during low rate (1.5 – 20s) and high rate (200ms) laser stimulation, respectively. A significant proportion of neurons displayed tachyphylaxis to repeated, constant-intensity low rate laser stimulation at ISI of up to 5 min; 2) TGN's: responded to low rate (10 or 15s) laser stimulation of the face in a graded, intensity-dependent manner. Nerve conduction velocity was approximately 1.5 m/s. These units did not respond to short-pulse (up to 200 ms) laser stimulation of any intensity, providing evidence for selective nociceptor activation. Nociceptive units responding to high rate laser stimulation or conducting at velocities corresponding to fast A nociceptors have not yet been observed during this preliminary study; 3) Saphenous nerve: limited preliminary data have shown neurons with conduction velocity of ~ 1m/s which respond in a intensity-dependent manner to low rate (10s) laser stimulation but not high rate laser stimulation, demonstrating selective activation by the laser.

Conclusions: these combined experiments are consistent with human psychophysics and previously published behavioral pharmacologic results, and provide evidence that low heating rate laser stimulation selectively activates C thermonociceptors and that high rate stimuli activates only A nociceptors.

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ANTIBODY-BASED DEPLETION OF HEMATOPOIETIC STEM CELLS EMPTIES NICHES FOR EFFICIENT TRANSPLANTATION

Agnieszka D. Czechowicz, Deepta Bhattacharya, Daniel Kraft, and Irving L. Weissman.

Institute of Cancer and Stem Cell Biology and Medicine
Department of Pathology
Stanford University School of Medicine

Hematopoietic stem cells (HSCs) are used therapeutically in bone marrow/hematopoietic stem cell transplantation (BMT/HSCT) to correct hematolymphoid abnormalities. Upon intravenous transplantation, HSCs can home to specialized bone marrow niches, self-renew and differentiate and thus generate a new, complete, disease-free hematolymphoid system. Unfortunately the use of BMT has been limited to fatal disorders, due to the risks associated with the toxic conditioning regimens necessary for HSC engraftment. It is not fully understood why these regimens are necessary. Understanding the barriers to HSC engraftment could lead to the creation of more specific conditioning regimens, which would decrease toxicity by lowering side-effects, and thus would result in more widespread applications of BMT.

To determine the barriers to HSC engraftment, we tested individual properties associated with the current conditioning regimens. We examined HSC engraftment in immunodeficient recipients, and found that HSC engraftment levels do not exceed ~0.5% following transplantation without conditioning, regardless of the number of HSCs transplanted. We next attempted to reduce the number of host HSCs in order to determine whether they played a role in limiting donor HSC engraftment. Administration of ACK2, an antibody that antagonizes c-kit function, led to the rapid and transient removal of >98% of endogenous HSCs thus resulting in available niches for engraftment. Following ACK2 clearance from serum, transplantation of these animals with donor HSCs led to chimerism levels of up to 90%, representing a 180-fold increase as compared to unconditioned animals. This non-myeloablative conditioning regimen had few side effects, other than temporary loss of coat color. Even in untransplanted animals, the HSCs rapidly recovered and animals remained healthy and fertile.

Thus the immune system is not the only barrier to HSC engraftment. Donor HSC engraftment is also restricted by occupancy of appropriate niches by host HSCs. As we have shown eliminating host HSCs prior to BMT can lead to therapeutic levels of donor engraftment without toxicity. Extrapolation of these methods to humans may enable efficient yet mild conditioning regimens for transplantation, thus expanding the potential applications of BMT to include multiple sclerosis, type 1 diabetes and tolerance in organ transplantation.

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NON-LINEAR ANALYSIS OF HEART RATE VARIABILITY DURING RECOVERY FROM TREADMILL TESTING PREDICTS CARDIOVASCULAR PROGNOSIS

Frederick E. Dewey, BA, James V. Freeman, MD, David Hadley, PhD, Jonathan Myers, PhD, Victor F. Froelicher, MD, Stanford University School of Medicine, Palo Alto, CA; Stanford University Division of Cardiology, Palo Alto, CA; Cardiac Science, Bothell, WA; VA Palo Alto Health Care System, Palo Alto, CA

Background We have recently shown that greater short-term (rMSSD) and high frequency heart rate variability (HF HRV) during recovery from treadmill testing are associated with increased cardiovascular mortality. At rest, very high rMSSD and HF HRV reflect non-respiratory sinus arrhythmia (NRSA), which is manifested in greater values of the non-linear heart rate variability parameter SD1/SD2. NRSA is associated with pharmacologically induced sympathovagal antagonism, which is also present during initial recovery from exercise. We aimed to evaluate the prognostic power of SD1/SD2 and its correlation with rMSSD and HF HRV during recovery from exercise.

Methods and Results We evaluated 1335 subjects (95% male, mean age 58) who were referred for exercise treadmill testing between 1997 and 2004 in the VA Palo Alto Health Care System. The SD1/SD2 ratio was quantified by Poincare plot of R-R intervals for the first two minutes of recovery from exercise. Multivariable Cox survival analysis was used to evaluate the prognostic power of SD1/SD2 after adjusting for potential confounders (Duke Treadmill Score, heart rate reserve, heart rate recovery, and clinical risk factors). During the 5.0 year mean follow-up, 133 subjects died and 53 of these deaths were due to cardiovascular (CV) causes. The SD1/SD2 ratio was significantly higher in non-survivors than in survivors (median 0.58 vs. 0.27, respectively, $p < 0.001$). After adjusting for confounders, $SD1/SD2 > 0.59$ (top quintile) was associated with a hazard ratio of 1.9 (95% confidence interval 1.3-2.8) for all-cause mortality and 2.6 for CV mortality (95% confidence interval 1.4-4.6). The SD1/SD2 ratio was highly correlated with rMSSD and HF HRV during recovery ($r = 0.89$ and $r = 0.67$, respectively, $p < 0.001$).

Conclusions Increased SD1/SD2 ratios during recovery from clinical treadmill testing are significantly associated with both increased CV mortality and greater short-term and HF HRV. Sinus arrhythmia not due to respiration may be implicated in the development of unstable rhythms and CV death in periods of increased sympathovagal antagonism.

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NOVEL PREDICTOR OF PROGNOSIS FROM EXERCISE TESTING: HEART RATE VARIABILITY RESPONSE TO THE EXERCISE TREADMILL TEST

Frederick E. Dewey, BA; James V. Freeman, MD; Greg Engel, MD; Raul Oviedo, MD; Natasha Ahmed, MD; Nayana Abrol, MD; Jonathan Myers, PhD; Victor F. Froelicher, MD, Stanford University School of Medicine, Palo Alto, CA; Stanford University Division of Cardiology, Palo Alto, CA; VA Palo Alto Health Care System, Palo Alto, CA

Background Heart rate variability (HRV) at rest reflects cardiovascular responses to sympathetic and parasympathetic nervous system activity. Decreased heart rate variability during resting short term and ambulatory 24 hour recordings has been demonstrated to be associated with unfavorable prognosis. However, the prognostic potential of exercise induced HRV (EI-HRV) has not been investigated. We aimed to evaluate the prognostic power of EI-HRV during and after standard clinical exercise testing.

Methods and Results Time- and frequency-domain HRV analysis was performed on R-R interval data taken from 1335 subjects (95% male, mean age 58) during the first and last two minutes of exercise treadmill testing and the first two minutes of recovery. Cox survival analysis was performed for 53 cardiovascular and 133 all-cause mortality endpoints that accrued during the 5.0 year mean follow-up. After adjusting for potential confounders, greater root mean square successive difference in RR interval (rMSSD) during peak exercise and recovery, greater high frequency (HF) power and percentage of HF power, lower percentage of low-frequency (LF) power, and lower LF/HF ratio during recovery were significantly associated with increased risks for all-cause and cardiovascular death. Of all time domain variables considered, log rMSSD during recovery was the strongest predictor of cardiovascular mortality (adjusted hazard ratio 5.0, 95% confidence interval 1.5-17.0 for the top quintile compared to the lowest quintile). Log HF power during recovery was the strongest predictor of cardiovascular mortality in the frequency domain (adjusted hazard ratio 5.9, 95% confidence interval 1.3-25.8 for the top quintile compared to the lowest quintile).

Conclusions EI-HRV variables during and after clinical exercise testing strongly predict both cardiovascular and all-cause mortality independent of clinical factors and exercise responses in our study population. These results contrast to results of studies performed at rest and invite new explanations for the relationship between HRV parameters and autonomic modulation of heart rate during and after exercise.

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CLINICAL CORRELATES AND PROGNOSTIC SIGNIFICANCE OF EXERCISE-ASSOCIATED VENTRICULAR ARRHYTHMIAS IN PATIENTS REFERRED FOR EXERCISE TREADMILL TESTING

Frederick E. Dewey, BA; John R. Kapoor, MD, PhD; Ryan S. Williams, MD; Euan A. Ashley, MRCP, DPhil; David Hadley, PhD; Jonathan Myers, PhD; Victor F. Froelicher, MD, Stanford University School of Medicine, Palo Alto, CA; Stanford University Division of Cardiology, Palo Alto, CA; Cardiac Science, Bothell, WA; VA Palo Alto Health Care System, Palo Alto, CA

Background The prognostic significance of PVCs associated with clinical exercise testing remains controversial. The clinical correlates of exercise test-induced PVCs are also unclear. We aimed to evaluate the clinical correlates and prognostic significance of exercise test associated premature ventricular complexes (PVCs).

Methods and Results We studied 1847 heart-failure-free patients who underwent clinical treadmill testing between 1997 and 2004 in the Veterans Affairs Palo Alto Health Care System. Logistic regression was used to evaluate the clinical and exercise test associations of exercise and recovery PVCs. Multivariable and propensity-score adjusted Cox survival analyses were used to evaluate the prognostic significance of exercise associated PVCs. There were 850 subjects (47%, median rate 0.43 per minute) who developed exercise PVCs and 620 subjects (34%, median rate 0.60 per minute) had recovery PVCs. Resting PVCs and greater age, height, and systolic blood pressure were key predictors of both exercise and recovery PVCs. Whereas exercise PVCs were related to the heart rate increase with exercise, however, recovery PVCs were related to coronary disease and ST segment depression. During a 5.4 year mean follow-up, 161 (9%) subjects died and 53 (33%) of these deaths were due to cardiovascular causes. Recovery PVCs, but not exercise PVCs, were associated with 66 - 86% greater propensity-adjusted mortality rates (hazard ratio 1.86, 95% confidence interval 1.25-2.78, for infrequent PVCs and hazard ratio 1.66, 95% confidence interval 1.04-2.66, for frequent PVCs compared to subjects without PVCs), but the additional predictive accuracy provided was limited (C indexes 0.75 for recovery PVCs and established risk factors vs. 0.74 for established risk factors).

Conclusions In patients without heart failure, exercise associated PVCs provide limited additional prognostic information beyond that provided by established risk factors. Exercise period PVCs are related to sympathetic nervous system activity while PVCs occurring with the return of vagal tone in recovery are related to ischemia and cardiac pathology.

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IMPROVEMENTS IN MINDFULNESS AND SELF-COMPASSION ARE CORRELATED WITH IMPROVEMENTS IN MOOD DISTURBANCE AND HEALTH STATUS IN A POPULATION OF MEDITATORS

Hetty Eisenberg and David Spiegel. Department of Psychiatry; Independent Concentration in Integrative Medicine

This study applies an Eastern meditation concept in a Western hospital setting to analyze the impact of the mind on mental and physical illness, yielding new clinical significance. Mahayana Buddhist phenomenology of dualistic consciousness inspires the analysis of a population of 129 participants in the Mindfulness-Based Stress Reduction Program course, a meditation course taught in hospitals around the world. Participants in the study filled out four questionnaires that quantified levels of mood disturbance (POMS), general health status (SF-36), mindfulness (MAAS), and self-compassion (SCS) at both weeks 1 and weeks 8 of the course and T-test and correlation analyses were performed.

Participants' scores improved significantly on all measures over the course of the eight weeks. Low levels of mindfulness and self-compassion were correlated with high levels of mood disturbance and poor general health status at week 1. Participants with lower levels of mindfulness and self-compassion at week 1 experienced greater improvement in mood disturbance and general health status by week 8. Levels of mood disturbance and general health status were not correlated with greater improvement in mindfulness and self-compassion. Greater changes in mindfulness and self-compassion were correlated with greater changes in mood disturbance and general health status.

The results of this study indicate that improving patients' levels of mindfulness and self-compassion can significantly benefit their mental and physical health status and argues for the introduction of mindfulness meditation into general medical practice. Moreover, the results of this study indicate that patients' baseline mental and physical health status does not limit the degree to which they are able to cultivate mindfulness and self-compassion. This study demonstrates that an interpretation of a non-Western understanding of illness can productively inform Western medical analysis. Future studies will be useful to define which populations benefit most from mindfulness meditation, as well as to investigate the feasibility of introducing the practice into the general medical population.

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FACTORING IN PHIC31 INTEGRASE AS A CURE FOR HEMOPHILIA A

Vanessa Gabrovsky, Christopher L. Chavez, W.E. Jung and Michele P. Calos
Department of Genetics, Stanford University School of Medicine

The extent to which current models of gene therapy will be successfully translated into common clinical applications is limited by issues such as achieving stable levels of expression, immunogenicity problems, size constraints, and insertional mutagenesis. The phiC31 integrase system offers a novel non-viral approach to gene therapy that to date has successfully addressed each of the above concerns. A plasmid carrying the therapeutic gene and an *attB* site is co-introduced with a plasmid encoding phiC31 integrase, resulting in integration at endogenous “pseudo” *attP* sites in mammalian chromosomes. The effectiveness of the phiC31 system as a tool for gene therapy has been demonstrated by many studies both *in vitro* and *in vivo*.

One of our goals is to develop clinically-acceptable methods to administer a site-specific integrase along with a factor VIII-*attB* plasmid to hemophilia A patients. As a result of successful results in tissue culture, we plan to deliver a vector carrying the factor VIII gene and an *attB* site, together with a vector encoding phiC31 integrase, via high-pressure tail vein injection in mouse models of hemophilia A. Our current data suggests this approach results in therapeutically-relevant levels of biologically active hFVIII that will result in phenotypic correction of the disease.

The second phase of this project will develop a catheter-based hydrodynamic technique to administer the factor VIII gene and phiC31 integrase to the livers of cynomolgus monkeys. The high level of sequence identity between the monkey and human genomes means that this work should predict which integration sites are preferred in human liver *in vivo*. Furthermore, the degree of anatomical similarity will enable us to establish a viable method for non-viral gene therapy in humans. This latter part of our work is crucial to developing not only a legitimate vehicle for delivering plasmid DNA on a larger scale, but also in evaluating the long-term correction of the disease *in vivo*.

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STANDARDIZING RESISTIVE INDICES IN HEALTHY PEDIATRIC TRANSPLANT RECIPIENTS OF ADULT-SIZED KIDNEYS

Sepideh Gholami, Minnie M. Sarwal, Maarten Naesens, Richard A. Barth, Hans G. Ringertz, Raymond R. Balise, and Oscar Salvatierra
Department of Transplant Surgery and Pediatric Radiology

Resistive Indices (RI) measured by Doppler Ultrasonography (DUS) are used to evaluate transplanted kidney function. Although increased RI is an early indirect marker for decreased graft function, normative data for RI have not been generated for pediatric recipients of size discrepant adult sized kidney (ASK) transplants. The objective of this study was to investigate the normal intrarenal vascular RI and their distribution for normally functioning ASK transplants in pediatric recipients across different groups based on body surface area (BSA).

47 healthy pediatric kidney transplant recipients of ASKs were prospectively followed for a minimum of six months post transplant. Inclusion criteria included normal renal biopsy within a month of DUS and/or stable serum creatinine level one month pre and post DUS. A total of 205 DUS were performed on patients in different BSA groups; 68 in the group of recipients with a BSA < 0.75 (group 1), 84 with a BSA between 0.75 and 1.5 (group 2), and 53 with a BSA \geq 1.5 (group 3). RI were measured in segmental arteries at the upper, middle, and lower poles of the transplanted kidney and average RI were utilized for statistical analysis. Renal volumes were measured one week and 6 months after transplantation to calculate the change in volume post transplant. Mean RI \pm S.D. for DUS values performed during the first six months post-transplant were calculated to be 0.68 ± 0.07 , 0.64 ± 0.07 , and 0.61 ± 0.07 for groups 1, 2, and 3 respectively. Statistically significant differences were observed between groups 1 and 2 ($p < 0.004$) as well as groups 1 and 3 ($p < 0.0001$). No significant difference was found between groups 2 and 3 ($p = 0.07$). RI increased during the first six months in group 1 but not groups 2 and 3. All three BSA groups showed a significant reduction in mean renal volume from 1 week to six months post transplantation with the greatest (31%) occurring in group 1 (BSA of < 0.75).

A wide normal range for RI was observed in stable transplants, but renal transplant RI reflect recipient BSA dependency. Mean RI were demonstrated to be significantly higher in smaller versus older pediatric transplant recipients of adult sized kidneys. The higher resistance to intra-renal vascular flow and significant decrease in mean renal volume in the smallest group likely reflects autoregulatory vasoconstrictive mechanisms to accommodate the size discrepant transplanted adult sized kidney to the smaller pediatric recipient vasculature and associated lower renal artery flow. Application of the generated RI/BSA plots in this study provides a reference resource for clinical interpretation of transplant dysfunction. Further studies will correlate RI and biopsy findings to predict kidney function for pediatric transplants.

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CORONARY PHYSIOLOGIC MEASUREMENTS AS PREDICTORS OF CLINICAL OUTCOMES IN CARDIAC TRANSPLANT PATIENTS

Melanie S. Gipp, William Fearon, M.D. Department of Cardiovascular Medicine, Stanford University School of Medicine

Background: A leading cause of graft loss and late death after transplantation is cardiac allograft vasculopathy (CAV), an accelerated form of diffuse and obliterative arteriosclerosis. Current techniques for diagnosing CAV are based on anatomic changes, occurring in the larger epicardial coronary arteries. CAV likely affects both the epicardial vessels and the coronary microcirculation, and because of its diffuse nature, a physiologic interrogation may be more useful than an anatomic one.

Methods: Using a pressure/thermistor tipped guidewire to measure coronary pressure and estimate flow using a thermodilution technique, Fractional Flow Reserve (FFR), Coronary Flow Reserve (CFR), and Index of Microcirculatory Resistance (IMR) were measured in 64 patients 1 year after transplantation. The primary endpoint was occurrence of congestive heart failure requiring hospitalization, cardiac re-transplant, or death. A secondary endpoint included change in left ventricular function based on echocardiography. Our goal was to determine if FFR, IMR, and CFR measured 1 year after transplantation predict the primary or secondary endpoints.

Results: Nine of the 64 patients reached the primary endpoint during an average of three years follow-up. The mean FFR, CFR and IMR in patients who reached the primary endpoint were not significantly different compared to those that did not. Values were FFR .83 and .86 (p-value 0.26), CFR 3.1 and 3.5 (p-value 0.53), and IMR 30.7 and 20.8 (p-value 0.27). We found no significant correlation between left ventricular ejection fraction (LVEF) and FFR, CFR or IMR, however those patients with an abnormal LVEF had a significantly lower CFR compared to those with a normal LVEF, with a CFR of 3.4 and 2.7, respectively (p-value 0.04).

Conclusion: Our findings are promising but we will need to continue to follow our present cohort and recruit more patients for future study. We expect that the ability to distinguish physiologic dysfunction in the epicardial vessels and microcirculation may help identify modifiable factors that lead to CAV.

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ENDOGENOUS THROMBOSPONDINS 1 AND 2 ARE NECESSARY FOR SYNAPTIC PLASTICITY AND SPONTANEOUS FUNCTIONAL RECOVERY AFTER STROKE

Stanley Hoang*, Jason Liauw, Michael Choi, Matt Choi, Matt Percy, Ben Wildman-Tobriner, Cagla Eroglu, Ben Barres, Tonya Bliss, Raphael Guzman, Gary Steinberg
Department of Neurosurgery, Department of Neurobiology

Thrombospondins 1 and 2 (TSP-1/2) are secreted multimeric extracellular glycoproteins that have angiostatic as well as synaptogenic properties. While the robust expression of TSP-1/2 after stroke has been postulated to drive the resolution of post-ischemic angiogenesis, little is known about the role TSP-1/2 may play in synaptogenesis and subsequent functional recovery after stroke. Recently, it was shown that TSP-1/2 mediate the formation of synapses in the developing murine brain. As a result, we investigate whether TSP-1/2 are necessary for synaptic and motor recovery after stroke.

An ischemia model was generated in 8 to 12 weeks old wild-type and TSP-1/2 knockout (KO) mice by unilateral occlusion of the distal middle cerebral artery. Spontaneous recovery of motor functions was assessed through rotarod, tongue protrusion, corner turning, and limb asymmetry behavioral paradigms for four weeks after stroke. At 4 weeks post-stroke, synaptic quantification with antibodies to pre- and post- synaptic proteins was compared in the peri-infarct area. Biotinylated dextran-amine (BDA) was injected into the contralesional homotopic cortex to quantify axonal sprouting from the contralesional homotopic cortex to the ipsilesional penumbra and striatum at 5 weeks post-ischemia.

TSP-1/2 KO mice exhibited significant deficits in their ability to recover motor function following stroke in the tongue protrusion test at week 4 ($p < 0.05$). Moreover, TSP-1/2 KO have reduced synaptic density and synaptogenesis in the peri-infarct area ($p < 0.05$). BDA tracing also showed reduced axonal sprouting in the peri-infarct area from the contralesional cortex in TSP-1/2 KO mice ($p < 0.05$). No significant differences in infarct size or blood vessel density were found between the 2 groups. These results demonstrate that functional recovery after stroke requires TSP-1/2 expression, which may play a necessary role in neuroplasticity, synaptogenesis and axonal sprouting. This understanding of the mechanisms underlying post-stroke neuroplasticity may give rise to new therapies aimed at augmenting recovery after stroke.

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MOLECULAR RESPONSE TO TARGETED INACTIVATION OF BCR-ABL IN CHRONIC MYELOID LEUKEMIA

Melissa Horoschak*¹, Alice Fan¹, Amy Shirer¹, Jan van Riggelen¹, Jason Gotlib ²,
Dean W. Felsher¹

1. Division of Oncology, Departments of Medicine and Pathology, Stanford University School of Medicine, 269 Campus Drive, CCSR 1120, Stanford, CA 94305, United States,

2. Stanford Cancer Center, Stanford University School of Medicine, 875 Blake Wilbur Drive, Room 2327B, Stanford, CA 94305-5821, USA.

Chronic myeloid leukemia (CML) is known to respond well to targeted inactivation of the Bcr-Abl fusion protein by treatment with Gleevec, Dasatinib, and the experimental compound AMN-107. While these drugs have been successful in the treatment of CML, to date there has been no insight into the molecular mechanism of the response. Using CML cell lines *in vitro* and *in vivo*, as well as primary patient samples, we have developed a panel of assays that will be eventually be tested for its ability to predict patient response to treatment.

Cellular senescence can be observed in many cell types for various reasons, eg. during normal cell division and telomere shortening or in response to unscheduled oncogene activation. Using beta-galactosidase assays, as well as cell cycle protein analysis, it has been observed in our lab that upon oncogene inactivation, senescence plays a role in tumor regression in various cancer models. Recently, we observed that human CML cell lines exhibit senescence *in vitro* after treatment with Gleevec. Together, these data support the hypothesis that senescence may be a conserved mechanism of response by cancer cells to various therapeutic interventions.

By combining the results of beta-galactosidase assays, Western gels, real-time PCR, and cell-cycle analysis we have established a profile of what our cells “look like” when they are responding to treatment. In addition to using CML cell lines *in vitro*, we will also investigate the cell lines *in vivo* (in SCID mice), samples from a mouse model of CML, and primary patient samples. Preliminary results suggest that these molecular profiles may correlate with response to treatment. Future studies will incorporate the data from all experimental assays in the various cell types.

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MULTI-MODALITY MOLECULAR IMAGING OF GLIOBLASTOMA GROWTH INHIBITION USING VASCULAR-TARGETING FUSION TOXIN VEGF₁₂₁/RGEL

Andrew R. Hsu*,¹ Weibo Cai,¹ Anand Veeravagu,² Khalid A. Mohamedali,³ Kai Chen,¹ Hannes Vogel,⁴ Lewis C. Hou,² Victor Tse,² Michael G. Rosenblum,³ and Xiaoyuan Chen¹

¹ The Molecular Imaging Program at Stanford (MIPS), Department of Radiology and Bio-X Program, Stanford University School of Medicine, Stanford, CA 94305

² Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305

³ Immunopharmacology and Targeted Therapy Laboratory, Department of Experimental

Therapeutics, M. D. Anderson Cancer Center, Houston, TX 77030

⁴ Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

* A.R.H. and W.C. contributed equally to this work

Abstract: VEGF-A and VEGF receptors, Flt-1/FLT-1 (VEGFR-1) and Flk-1/KDR (VEGFR-2), are key regulators of tumor angiogenesis and tumor growth. The purpose of this study was to determine the anti-angiogenic and anti-tumor efficacy of vascular-targeting fusion toxin VEGF₁₂₁/rGel in an orthotopic glioblastoma mouse model using non-invasive in vivo bioluminescence imaging (BLI), magnetic resonance imaging (MRI), and positron emission tomography (PET). **Methods:** Tumor-bearing mice were randomized into two groups and balanced according to BLI and MRI signals. PET imaging using ⁶⁴Cu-DOTA-VEGF₁₂₁/rGel was performed before VEGF₁₂₁/rGel treatment. ¹⁸F-fluorothymidine (¹⁸F-FLT) scans were performed before and after treatment to evaluate VEGF₁₂₁/rGel therapeutic efficacy. In vivo results were confirmed with ex vivo histology and immunohistochemistry.

Results: Logarithmic transformation of peak BLI signal intensity showed a strong correlation with MRI tumor volume ($r = 0.89$, $n = 14$). PET imaging using ⁶⁴Cu-DOTA-VEGF₁₂₁/rGel pre-treatment showed a tumor accumulation of 11.8 ± 2.3 %ID/g at 18 h post-injection (p.i.) and the receptor specificity of the tumor activity accumulation was confirmed by successful blocking of the uptake in the presence of excess amount of VEGF₁₂₁. PET imaging using ¹⁸F-FLT showed significant decreases in tumor proliferation in VEGF₁₂₁/rGel-treated mice compared with controls. Histologic analysis showed specific tumor neovasculature damage following four doses of VEGF₁₂₁/rGel treatment, accompanied by a significant decrease in peak BLI tumor signal intensity.

Conclusions: The results of this study suggest that future clinical multi-modality imaging and therapy using VEGF₁₂₁/rGel may provide an effective means to prospectively identify patients who will benefit from VEGF₁₂₁/rGel therapy and then stratify, personalize, and monitor treatment to obtain optimal survival outcomes.

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STEERABLE SHEATH FOR ENDOSCOPIC AND TRANSLUMENAL SURGERY

Natalia Isaza, BS; Tom Low, MS; Pablo Garcia, MS; Sanjeev Dutta, MD
Department of Surgery, Stanford University School of Medicine & SRI International

Objective: 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.

Methods and Results: 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.

Conclusion: 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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NO_x AND ADMA CHANGES WITH FOCAL ISCHEMIA, AMELIORATION WITH THE CHAPERONIN GROEL

Lijun Xu^{1*}, Bingyin Wang^{2*}, **Kirandeep Kaur**¹, Melanie F. Kho¹, John P. Cooke², Rona G. Giffard¹, Department of Anesthesia¹ and Cardiovascular Center², Stanford University School of Medicine, Stanford, CA, USA

Both nitric oxide and asymmetrical dimethylarginine (ADMA) play a critical role in the regulation of cerebral blood flow, though their neuroprotective and cytotoxic effects are still under investigation.

In this study we evaluated whether NO and ADMA levels change in plasma, ischemic brain tissue and cerebral spinal fluid (CSF) in response to focal ischemia. Male S.D rats (280-320G) were subjected to 2 hrs MCAO using a suture. Venous blood samples were collected at baseline, after 2 hrs MCAO, and at 24 hrs reperfusion. CSF and tissue samples were taken at 24 hrs reperfusion. Plasma total nitrate/nitrite concentration (NO_x) and ADMA were measured by ELISA kit.

We found NO_x levels in plasma, ischemic brain tissue, and cerebrospinal fluid (CSF) increased significantly 24h after 2h transient middle cerebral artery occlusion (MCAO) in rats. ADMA levels were unchanged in plasma, but decreased significantly in CSF 24h following MCAO. The CSF ADMA/NO_x ratio decreased markedly following ischemia. Rats protected by expression of the chaperonin GroEL or its folding deficient mutant D87K had lower plasma NO_x levels at 24h reperfusion.

ADMA, NO, and their ratio in CSF correlates with extent of injury in the protected rats. A peripherally detectable biomarker that rises quickly and correlates with injury is still being sought in stroke. The possibility of using serum and CSF ADMA/ NO levels to markers of ischemic stroke is worth further study.

Funded by the Stanford Medical Scholars Research Program.

GENOMIC ANALYSES IDENTIFY ABNORMALITIES IN LIPID METABOLISM IN DERMATOMYOSITIS PATIENTS

Bory Kea, Robert Pesich, Lorinda Chung, Patrick Brown, and David Fiorentino

Institutions: School of Medicine, Stanford University; Dept. of Biochemistry, School of Medicine, Stanford University; Division of Immunology and Rheumatology, Department of Medicine, Stanford University; Dept. of Dermatology, School of Medicine, Stanford University

Dermatomyositis (DM) is a complex autoimmune disease in which the characteristic skin inflammation is the only constant, defining feature. Despite this, the mechanism of cutaneous disease in DM has not been extensively investigated and remains poorly understood. We sought to identify gene expression patterns that could lead to novel hypotheses regarding pathogenesis of skin disease in DM. We used printed oligonucleotide DNA microarrays that represent nearly all human genes (44,544 70mer sets) to analyze the skin biopsies from 9 healthy controls and 12 adult patients with DM. We performed SAM (Statistical Analysis of Microarray) analysis to identify genes that discriminate between active skin disease and control subjects. With a false discovery rate of 5%, we found 207 and 355 genes whose expression was strongly increased or decreased, respectively, in DM relative to controls. Functional annotation clustering of these genes identified two major biological processes, as defined by Gene Ontology—that of lipid metabolism and host-pathogen interaction. These processes included a major signature for lipid metabolism (43 genes, $p=2E-13$), steroid biosynthesis (10 genes, $p=6E-10$), and carboxylic acid metabolism (39 genes, $p=8E-14$), and to a lesser extent, biotic stimulus (54 genes, $p=3.7E-8$), response to pest, pathogen, or parasite (31 genes, $p=2E-7$), and defense response (49 genes, $p=9E-7$). Our data are consistent with previous findings from muscle biopsies in DM indicating activation of type I interferon responsive genes, and we extend these findings to include the intriguing possibility that lipid metabolism contributes to the pathogenesis of skin disease in dermatomyositis.

Funded by the Stanford Medical Scholars Research Program & Genentech

THE EFFECTS OF DIGITAL DERMOSCOPY ON SKIN SELF-EXAMINATION IN PATIENTS AT INCREASED RISK FOR MELANOMA

Layton, Christle. Pollit, Ricardo. Ngyuen, Josephine, MD. Susan Swetter, MD.
Stanford Department of Dermatology.

In recent decades, the rate of melanoma has been on the rise, becoming one of the most common preventable cancers.¹ Melanoma prevention and education are aimed at decreasing its morbidity and mortality, especially for high-risk populations such as patients with atypical moles. This project's main objective is to evaluate the effect that *digital dermoscopy* imaging has on increasing *skin self-examination (SSE)* in patients who are at increased risk for melanoma based on abnormal mole phenotype (atypical mole syndrome and familial atypical mole-melanoma syndrome). *SSE* is the purposeful inspection of one's skin for new or changing moles. *SSE* is estimated to reduce melanoma mortality by 63%.² *Dermoscopy* is a non-invasive technique that magnifies skin features and pigmented skin lesions that are not visible to the unaided eye.

A study is being conducted at the Stanford Pigmented Lesion and Cutaneous Melanoma Clinic (PLCMC) using digital dermoscopy to identify melanoma and melanoma precursors. In addition, a patient survey is being conducted to examine whether digital dermoscopy improves the quality and frequency of *SSE*. Using the melanoma ABCDE (asymmetry, border irregularity, color variation, diameter, and evolution) criteria, patients are shown normal and abnormal mole features. Patients are surveyed before and after dermoscopy to evaluate if this intervention helps to educate high-risk patients about *SSE* and their ability to detect suspicious lesions for early melanoma detection.

We hypothesize that an intervention focused on digitally imaging pigmented skin lesions will increase patients' self skin-examination and awareness of melanoma warning signs. This type of intervention may help to reduce melanoma morbidity and mortality in this high-risk population and lay the groundwork for using digital dermoscopy to help educate patients regarding normal and abnormal skin features.

Funded by the Stanford Medical Scholars Research Program.

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1. Howe HL, Wingo PA, Thun MJ, et al. Annual report to the nation on the status of cancer (1973 through 1998), featuring cancers with recent increasing trends. *J Natl Cancer Inst* 2001;93:824-842
2. Berwick M, Begg CB, Fine JA, Roush GC and Barnhill RL. Screening for cutaneous melanoma by skin self-examination. *J Natl Cancer Inst*. 1996;88:17-23.

FACTORS ASSOCIATED WITH POOR FOLLOW-UP AMONG GLAUCOMA PATIENTS IN SOUTH INDIA

Bradford W. Lee, Kuldev Singh (Stanford Medical School, Ophthalmology), Parthasarathi Sathyan (Aravind Eye Hospital, Coimbatore, India), Alan L. Robin (Johns Hopkins University, Baltimore, MD).

Purpose: To determine the factors associated with poor attendance of follow-up glaucoma examinations (FGEs) among glaucoma patients in South India. **Methods:** This prospective case-control study enrolled 300 established patients with primary glaucoma who did or did not attend FGEs as advised in the past year at Aravind Eye Hospital. Responses regarding various factors hypothesized to be associated with poor attendance of FGEs were collected by oral questionnaire. Unadjusted and adjusted odds ratios were then calculated using step-wise multiple logistic regression.

Results: The factors most associated with poor attendance of FGEs included: lower perceived importance of attending FGEs [Adj. OR—10.80, 4.40-26.50], non-use of glaucoma medications [Adj. OR—2.10, 1.10-4.00], and means-tested waiving of clinic fees for low-income patients (“free patients”) [Adj. OR—3.10, 0.91-10.50]. Notable factors *not* significantly associated with FGE attendance included: severity of disease, convenience of transportation to clinic, and FGE-related expenses (direct and indirect).

Conclusions: Despite the provision of free clinical services for low-income patients, having one’s clinic fees waived is still independently associated with poor attendance of FGEs. Lower perceived importance of attending FGEs and non-use of glaucoma medications are also associated with poor attendance of FGEs. Meanwhile, many factors traditionally believed to explain poor attendance of FGEs, such as inconvenient transportation to clinic and less severe disease, were found to have little to no effect in this study. These findings suggests that efforts to improve patient attendance of FGEs should focus on changing patients’ perceptions about the importance of attending regular FGEs, since even marginal differences in patients’ perceived importance of follow-up (“somewhat important” vs. “very important”) were associated with significant differences in FGE attendance. Furthermore, administering short questionnaires that elicit factors associated with poor follow-up may be a valuable means of identifying patients at greater risk for poor follow-up. These patients can then be counseled, educated, and treated appropriately in order to minimize disease progression and unnecessary glaucoma-induced vision loss.

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WHY WE SHOULD SCREEN ALL FOREIGN-BORN ASIAN AMERICAN ADULTS FOR HEPATITIS B: A CROSS-SECTIONAL STUDY OF 3,163 ASIANS IN CALIFORNIA

Steven Y. Lin, Ellen T. Chang, and Samuel K. So
Department of Surgery

Asian Americans are at disproportionately high risk for liver disease due to their high prevalence of chronic hepatitis B virus (HBV) infection – a disease that, if undetected, is associated with a 25% chance of death from cirrhosis or liver cancer. Our objective was to study the prevalence of chronic HBV infection and hepatitis B vaccination among Asian adults. From 2001 to 2006, we provided free HBV serological screening to Asians in the San Francisco Bay Area. Participants completed a survey assessing hepatitis B vaccination status. The study was conducted in Asian communities in San Francisco, San Jose, Cupertino, Millbrae, Milpitas, Sunnyvale, and a screening clinic at Stanford Hospital. Among a volunteer sample of 3,163 Asian adults (age range: 18 to 101 years, median: 52.9 years), over 93% were foreign-born. The main outcome measures were seroprevalence of hepatitis B surface antigen (HBsAg) and surface antibody (HBsAb).

Of 3,163 Asian adults screened, 8.9% were chronically infected with HBV. Alarming, 2 in 3 (65.4%) of those chronically infected were unaware that they were infected. Participants born in East Asia, Southeast Asia or the Pacific Islands (10.7% HBsAg-positive) were approximately 20 times more likely to be chronically infected than participants born in the U.S. (0.7% HBsAg-positive) (relative risk=19.4, 95% confidence interval: 2.6, 141.8). Of those who were not infected, 44.8% lacked protective antibodies against HBV and were susceptible to future infection. Only 12.0% of participants reported having been vaccinated against HBV. Of these individuals, 20.3% lacked protective antibodies and 5.2% were found to be chronically infected with HBV.

Given the serious medical implications of this study, a strong public health response is needed. In support of the newly released Centers for Disease Control and Prevention national recommendations, we call for all foreign-born Asian adults to be screened for HBV – regardless of their vaccination status.

Funded by the Asian Liver Center at Stanford University

THE ROLE OF CSN5 IN UV-MEDIATED DNA DAMAGE

Helen Liu, David J. Wong, Howard Y. Chang
Stanford University School of Medicine

The nucleotide excision repair (NER) pathway is essential for prevention of DNA damage and skin cancers induced by ultraviolet radiation. CSN5 is the catalytic subunit of the COP9 signalosome and a key regulator of the ubiquitin ligase activities of DDB2 and CSA complexes that mediate NER. The CSN5 gene resides on the long arm of human chromosome 8, which is frequently amplified in human squamous cell carcinomas (SCC). However, the role of CSN5 in ultraviolet-induced carcinogenesis remains unclear.

Here we show that CSN5 protein is overexpressed in 60% of human SCCs. Enforced expression of CSN5 in epithelial cells, but not a catalytically inactive mutant, is sufficient to confer protection against apoptosis induced by UV radiation. To better define the roles of CSN5 in stratified epidermis *in vivo*, we generated *K5-CSN5* and *K5-CSN5(D151N)* transgenic mice that express CSN5 or the catalytically inactive CSN5(D151N) in murine epidermis.

The role of CSN5 on UV-induced apoptosis, DNA repair, and mutagenesis in our transgenic mouse models will be presented. These studies will also better define CSN5 as a potential therapeutic target for human epidermal neoplasia and light-regulated dermatoses.

Funded by the Stanford Medical Scholars Research Program

EPENDYMOMA INCIDENCE IS A FUNCTION OF GENDER AND AGE BUT NOT TIME: A SEER STUDY

Courtney S McGuire, Kristen L Cobb¹ and Paul G Fisher²

¹Health Research and Policy - Epidemiology

²Neurology and Pediatrics

Previous small studies disagree about how clinical risk factors influence ependymoma incidence, perhaps due to limited sample size. We aimed to show the relationship of incidence to gender, race, and tumor location by rigorous analysis of a cancer registry, and to determine incidence trends over the past three decades. Data were obtained from Surveillance Epidemiology End Results (SEER-9) from 1973 to 2003. Histologic site codes (ICD-O-3: 9391-9394) were used to define ependymomas. Differences in age-adjusted incidence rates were compared by confidence intervals in SEER*Stat 6.2. A multiplicative Poisson regression and joinpoint regression were performed to determine annual percentage change and look for sharp changes in incidence, respectively.

1402 subjects (798 males, 604 females; 1213 whites, 112 blacks) were identified. Incidence (/100,000) was significantly higher in males than females (males 0.227 +/- standard error [SE] 0.029, females 0.166 +/- SE 0.030). For children (18 years and younger), age at diagnosis differed significantly by tumor location, with mean ages of incidence for infratentorial 5.04 +/- SE 0.41 years, supratentorial 7.77 +/- SE 0.60 years, and spinal 12.16 +/- SE 0.80 years. Between 1973 and 2003, there were no significant changes in incidence over time and no sharp changes at any one year.

Males have higher incidence of ependymoma compared to females. A biologic explanation for this finding remains elusive. Ependymoma occurs at distinctly different locations at different ages, consistent with hypotheses postulating distinct populations of radial glia stem cells within the central nervous system. Contrary to prior research, ependymoma incidence does not appear to have increased over the past three decades.

Funded by the Stanford Medical Scholars Research Program

CURCUMIN EXPOSURE INDUCES G1 ARREST IN *SACCHAROMYCES CEREVISIAE* BY IRON STARVATION

Steven C. Minear, Allyson O'Donnell, Guri Giaever, Corey Nislow, Tim A. Stearns, Martha Cyert. Dept. of Biological Sciences.

Curcumin, a naturally occurring substance, shows great potential as a therapeutic compound. Clinical trials are underway using curcumin as an anti-cancer therapeutic, and curcumin treatment is proposed for many other diseases, including Alzheimers and atherosclerosis. Studies on curcumin activity report pleiotropic downstream effects, however curcumin's mechanism of action *in vivo* remains unclear. We show that curcumin impairs iron uptake in cells. This role for curcumin in modulating iron uptake helps explain previously described curcumin-related phenomenon.

Using genomic screens, we identified mutations of non-essential genes in *Saccharomyces cerevisiae* that result in hypersensitivity to curcumin. Our analysis shows that curcumin influences the high-affinity copper and iron import pathways. Iron supplementation of media rescued all hypersensitive strains, while copper supplementation rescued only a subset of strains. Rescue of all mutants by iron, including those with defects in copper regulation, suggests that the primary function of curcumin is to starve cells of iron. This is not surprising since high-affinity iron import is copper-dependent. Curcumin exposure depletes cellular iron and elicits a transcriptional response consistent with iron starvation. Moreover, curcumin induces a G1 arrest, an effect alleviated by iron supplementation. We expanded our studies to include human tissue culture cells and found striking similarities between yeast and mammalian cellular responses to curcumin. Like yeast, human cell growth is inhibited when curcumin is present, and iron addition rescues this effect.

Our novel findings link the previously reported iron chelation by curcumin to curcumin-induced cell cycle arrest, and begins to define the mechanism by which curcumin produces its wide variety of effects. The curcumin-induced iron starvation is conserved in yeast and human cells. Findings from our genetic screens in yeast can be used as a framework for further investigations in humans. Further exploration of curcumin's iron-transport modulation will enable a more targeted therapeutic application of this compound.

Funded by the Stanford Medical Scholars Research Program

IN VIVO DELIVERY OF WNT PROTEINS BY LIPOSOMAL PACKAGING

Nathan T. Morrell¹, Jae-Beom Kim¹, Philipp Leucht¹, Derk ten Berge², Roel Nusse², and Jill A. Helms¹

1: Department of Surgery, Division of Plastic and Reconstructive Surgery,

2: Department of Developmental Biology

The Wnt family of lipoproteins exercises strong cell-biological effects of therapeutic value, but, until now, no method for *in vivo* delivery existed. We exploited the lipophilicity of Wnts by packaging purified Wnt3a protein into lipid vesicles and assessed its activity using both *in vitro* and *in vivo* assays.

When purified Wnt3a was added to liposomes, 55% of input activity was detected when assessed via an *in vitro* reporter assay. Because Wnt proteins contain post-translational lipid modifications, we assumed that, rather than being encapsulated, Wnt3a protein would be tethered to the liposomal surface where it could stimulate target cells. A series of experiments involving enzymatic cleavage and Western blot analyses revealed that 55% of the input protein was localized to the exo-liposomal surface where it could stimulate target cells; 15% of the input protein was localized to the endo-liposomal surface where it was unavailable to induce the Wnt signaling cascade. This packaging scheme allows us to conclude that liposomal packaging does not adversely affect Wnt3a activity.

Finally, *in vivo* studies using reporter mice demonstrated that Wnt3a liposomes elicit a biological response at a concentration where purified protein does not. The delivery of Wnt3a liposomes to skeletal injury sites induced reporter activity and led to a dramatic enhancement of tissue repair and regeneration. Taken together, liposomal packaging may turn purified Wnt protein into a powerful therapeutic reagent. Because the endogenous transport of Wnt proteins may occur through association with lipid vesicles, Wnt3a liposomes may represent a biomimetic strategy for the delivery of Wnts for the treatment of diseases, aid in regeneration, or enhancement of stem cell self-renewal and proliferation. Future experiments will aim to optimize this system for therapeutic benefit.

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A UBIQUITOUS DOUBLE-FLUORESCENT CRE REPORTER MOUSE

Mandar D. Muzumdar, Kazunari Miyamichi, Ling Li, Bosiljka Tasic, and Liqun Luo
Howard Hughes Medical Institute and Department of Biological Sciences

The Cre/loxP system has been used extensively for conditional mutagenesis in mice. In this system, Cre recombinase regulated by a tissue-specific and/or temporally-regulated promoter can excise essential loxP-flanked (“floxed”) genes via *intra*-chromosomal recombination to generate conditional knockouts. Reporters of Cre recombinase activity are important to define the spatial and temporal extent of Cre-mediated recombination. Here we describe *mT/mG*, a double-fluorescent Cre reporter mouse that expresses membrane-targeted tandem dimer Tomato (*mT*) prior to Cre-mediated excision and membrane-targeted green fluorescent protein (*mG*) following excision.

Homozygous *mT/mG* mice are viable and fertile, demonstrating minimal toxicity of the fluorescent markers. We show that reporter expression is ubiquitous, allowing visualization of fluorescent markers in live and fixed samples of all tissues examined. We further establish that mG expression is Cre-dependent and complementary to mT at single cell resolution. *mT/mG* is the first demonstration of tandem-dimer Tomato expression *in vivo* in mice. Our results suggest that tdTomato is a sufficiently bright and photostable red fluorescent protein for complementary use with existing green fluorescent protein lines. Both single-copy membrane-targeted markers outline cell morphology, highlight membrane structures, and permit visualization of fine cellular processes *in vivo*.

mT/mG represents the first ubiquitously-expressed double-fluorescent Cre reporter mouse. We conclude that *mT/mG* will serve not only as a ubiquitous Cre reporter but also as a tool for lineage tracing, mosaic analysis, transplantation studies, and analysis of cell morphology *in vivo*.

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PPAR γ CONTROLS ALTERNATIVE MACROPHAGE ACTIVATION TO AMELIORATE OBESITY-INDUCED INSULIN RESISTANCE

Justin I. Odegaard^{1,2}, Roberto R. Ricardo-Gonzalez^{1,2}, Matthew H. Goforth¹, Christine R. Morel¹, Vidya Subramanian⁴, Lata Mukundan¹, Alex Red Eagle^{1,3}, Divya Vats¹, Frank Brombacher⁵, Anthony W. Ferrante⁴, Ajay Chawla^{1,2}

1 Division of Endocrinology, Metabolism and Gerontology, Department of Medicine,

2 Graduate Program in Immunology,

3 Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305-5103

4 Department of Medicine, Naomi Berrie Diabetes Center, Columbia University College of Physicians and Surgeons, New York, NY

5 Institute of Infectious Disease and Molecular Medicine, Division of Immunology, Health Science Faculty, University of Cape Town, Werhner Beit South, Cape Town, South Africa

Obesity and insulin resistance, cardinal features of metabolic syndrome, are closely associated with a state of low-grade inflammation. In adipose tissue chronic overnutrition leads to macrophage infiltration, resulting in local inflammation that potentiates insulin resistance. Because macrophages also actively participate in the resolution of inflammation, we postulated that macrophage activation programs that terminate inflammation might ameliorate obesity-induced insulin resistance. In particular, the interleukin-4 (IL-4) driven program of alternative macrophage activation has been shown to dampen inflammation and enhance repair in tissues; however, its role in obesity and insulin resistance remains unknown.

Using mice with macrophage-specific deletion of peroxisome proliferator activated receptor- γ (PPAR γ), we show here that PPAR γ is required for maturation of alternatively activated macrophages. Disruption of PPAR γ in myeloid cells impairs alternative macrophage activation, thereby predisposing these animals to development of diet-induced obesity, insulin resistance, and glucose intolerance. Furthermore, gene expression profiling revealed that downregulation of oxidative phosphorylation gene expression in skeletal muscle and liver leads to decreased insulin sensitivity in these tissues.

Together, our findings demonstrate that resident alternatively activated macrophages have a beneficial role in regulating nutrient homeostasis and suggest that macrophage polarization towards the alternative state might be a useful strategy for treating obesity and type 2 diabetes.

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THE ROLE OF BONE MARROW-DERIVED CELLS IN THE PROCESS OF HYPERTROPHIC SCAR FORMATION

Shola B. Olorunnipa, Shahram Aarabi, and Geoffery C. Gurtner

Hypertrophic scars occur following cutaneous wounding and result in severe functional and aesthetic defects. The pathophysiology of this process remains unknown and, as a result, treatment remains a challenge. Recent studies by our laboratory have demonstrated that mechanical stress applied to a healing wound is sufficient to produce hypertrophic scars in mice. These scars are histopathologically identical to human hypertrophic scars and show equivalently dramatic increases in volume and cellular density. The development of this novel murine model opens the door to further investigation of the underlying pathophysiology of hypertrophic scarring. Our study utilizes this model to investigate the contribution of cells derived from the bone-marrow to the formation of hypertrophic scars.

Bone marrow from Green fluorescent protein (GFP) labeled mice was transplanted into irradiated C57BL/6 mice. Hypertrophic scars were generated on the dorsa of transplant recipient mice using a mechanical strain device. Normal scars were also generated on each mouse to serve as internal controls. The resulting scars were harvested and analyzed via fluorescent microscopy enabling us to visualize the contribution of bone-marrow derived cells to both hypertrophic scars and control wounds.

Preliminary results based on histologic analysis suggest that the 25-fold increase in cellular density seen in murine hypertrophic scars can be attributed in part to greater recruitment of bone-marrow cells to the site of injury. Ongoing studies are utilizing cell markers and flow cytometry to determine the cellular phenotypes of these GFP-positive marrow-derived cells and to provide a quantitative measurement of their contribution to hypertrophic scars when compared to internal controls.

Funded by the Stanford Medical Scholars Research Program

OVERCOMING DELAYS IN CHILDBIRTH DUE TO HEMORRHAGE: A QUALITATIVE STUDY OF THE NON-PNEUMATIC ANTI-SHOCK GARMENT (NASG) IN NIGERIA

Adeoti Oshinowo¹, Hadiza Galadanci, MD², Mohammed Awwal, MD³, Oladosu Ojengbede, MD⁴, Lyndsay McDonough, MPH⁵, Elizabeth Butrick, MPH, MSW⁶, Suellen Miller, PhD, CNM⁷. (1) School of Medicine, Stanford University. (2) Obstetrics and Gynecology, Aminu Kano Teaching Hospital, Kano, Kano, Nigeria. (3) Obstetrics and Gynecology, Murtallah Mohamed Speciality Hospital, Kano, Kano, Nigeria. (4) Obstetrics and Gynecology, University College Hospital, College of Medicine, Ibadan, Nigeria. (5) School of Public Health, Tulane University. (6) Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco. (7) OB/GYN and Reproductive Sciences, University of California, San Francisco.

Background: Obstetric hemorrhage (OH) is the leading cause of maternal mortality due to delays in obtaining Emergency Obstetric Care. Women die during transports to facilities or while awaiting appropriate care in facilities. One strategy for decreasing MMR from OH is a first aid device, the Non-pneumatic Anti-Shock Garment (NASG), a low technology compression suit. The NASG is being pilot tested in Kano, Nigeria.

Objectives: To understand provider, patient and family perceptions of the NASG in order to enhance its acceptability and decrease delays in application.

Methods: 10 focus groups of 134 health care providers (doctors, nurse-midwives, nurses, and staff) and 6 in-depth, individual interviews of patients who survived severe OH and shock and/or their family members, were conducted and analyzed using grounded theory.

Results and conclusions: Providers agreed that the NASG was easy to use, improved management of OH, but was difficult to fold correctly, and was not always the first thing providers would think of to apply in frantic emergency situations. Patients generally accept the NASG, but those who received less information about it before it was applied (they were unconscious at the time of application) who wake up in it are confused and uncomfortable with it; some try to remove it. There are clear policy implications from this study. As a new device, more training in pre-service education may help incorporate the NASG into providers' emergency response algorithm. Ante-natal and community education activities may help women and family members learn about the NASG as a life-saving device, not something to fear.

Learning objectives:

1. Recognize obstacles to implementing a new technology to reduce maternal mortality associated with obstetric hemorrhage in Nigeria.
2. List training methods that will enhance providers incorporating a new technology into their emergency response algorithm.
3. List community outreach activities that can be conducted to improve families understanding of a new device to keep women alive during transport to emergency obstetrical care.

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ENCODING SKELETAL MORPHOLOGY IN THE GENOME

Luiz C. Pantalena Filho, Catherine Guenther, Christine Ham, [David Kingsley](#)
Department of Developmental Biology, Stanford University

There is great variation in the skeletal system of vertebrates. This variation has a strong genetic component. The skeletal system is also modular; in other words, it is possible to change one skeletal element and its shape, while allowing other elements to remain unaltered. The following experiments present a role for the gene *Bone Morphogenetic Protein 5 (Bmp5)* in this process. Isolation of *Bmp5* cis-regulatory elements show that skeletal elements, such as ribs, can be further subdivided into compartments. They also allow the manipulation of skeletal morphology.

By altering levels of BMP signaling in rib compartments during development, I have been able to alter both rib cross sectional shape and vertebrae-to-sternum trajectory in transgenic animals. In addition, I was able to detect impaired bone deposition, in a compartment-specific manner, in regulatory *Bmp5* mutants lacking specific cis-regulatory elements. Finally, I also describe experiments implicating the use of cis-regulatory elements in fracture response.

Therefore, these experiments show that *Bmp5* cis-regulatory elements affect skeletal morphology. I have also added to the concept of compartmentalization of skeletal elements, which add to the modularity of the skeleton. Finally, I have shown that processes using *Bmp5* to shape bone during development are re-capitulated during fracture healing. These experiments describe a method to encode skeletal morphology in the genome.

Funded by the Stanford Medical Scholars Research Program

EFFECT OF PULSE DURATION ON THE SIZE AND CHARACTER OF THE LESION IN RETINAL PHOTOCOAGULATION

Yannis M. Paulus, ATul Jain, Michael W. Wiltberger, Dan E. Andersen, Phil Huie, Mark S. Blumenkranz, Daniel Palanker
Department of Ophthalmology, Stanford University School of Medicine

The recently-developed semi-automated Patterned Retinal Photocoagulation (PRP) system transmits multiple spots in a prearranged pattern, thus decreasing procedure time and increasing reproducibility from conventional PRP. To utilize this new technology in clinical practice, an assessment of laser parameters must be performed. The effects of laser beam size, power, and pulse duration from 1 to 100ms on the characteristics of ophthalmoscopically visible retinal coagulation lesions were systematically evaluated. A 532nm Nd:YAG laser was used to irradiate 36 retinas in Dutch-Belt rabbits with retinal beam sizes of 66, 132, and 330 μm . Lesions were clinically graded 1 minute after lesion placement, lesion size was measured by digital imaging, and lesion depth was assessed histologically at different time points.

Retinal lesion size increased linearly with laser powers from 50 to 250 mW and logarithmically with pulse durations from 10 to 100 ms. Ophthalmoscopically visible retinal lesions obtained with 10 and 20 ms pulses were 3 and 2 times smaller than those produced by 100 ms exposures, respectively. The width of the therapeutic window, defined by the ratio of the threshold power for producing a rupture to that of a mild coagulation, decreased with decreasing pulse durations. For 330 μm retinal beam sizes, the therapeutic window declined from 5.4 to 3.7 as pulse duration decreased from 100 to 20 ms. At pulse durations of 1 ms, the therapeutic window decreased to unity, at which point rupture and a mild lesion were equally likely to occur.

This data shows that at shorter pulse durations, the diameter of retinal lesions is smaller and less dependent on variations in laser power than at longer durations. The width of the therapeutic window, a measure of relative safety, increases with the beam size. Clinically, pulse durations of 10 to 20ms represent an optimal compromise between the favorable impact of speed, higher spatial localization, and reduced collateral damage on one hand, and sufficient width of the therapeutic window (>3), on the other. This may translate into less damage to nerve fiber layer, decreased choroidal swelling, and reduced pain due to decreased penetration of heat into the choroid, but this will need to be confirmed in clinical trials.

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THE EFFECTS OF DIGITAL DERMOSCOPY ON SKIN SELF-EXAMINATION IN PATIENTS AT INCREASED RISK FOR MELANOMA

Pollit, Ricardo. Layton, Christle. Ngyuen, Josephine, MD. Susan Swetter, MD.
Stanford Department of Dermatology.

In recent decades, the rate of melanoma has been on the rise, becoming one of the most common preventable cancers.¹ Melanoma prevention and education are aimed at decreasing its morbidity and mortality, especially for high-risk populations such as patients with atypical moles. This project's main objective is to evaluate the effect that *digital dermoscopy* imaging has on increasing *skin self-examination (SSE)* in patients who are at increased risk for melanoma based on abnormal mole phenotype (atypical mole syndrome and familial atypical mole-melanoma syndrome). *SSE* is the purposeful inspection of one's skin for new or changing moles. *SSE* is estimated to reduce melanoma mortality by 63%.² *Dermoscopy* is a non-invasive technique that magnifies skin features and pigmented skin lesions that are not visible to the unaided eye.

A study is being conducted at the Stanford Pigmented Lesion and Cutaneous Melanoma Clinic (PLCMC) using digital dermoscopy to identify melanoma and melanoma precursors. In addition, a patient survey is being conducted to examine whether digital dermoscopy improves the quality and frequency of *SSE*. Using the melanoma ABCDE (asymmetry, border irregularity, color variation, diameter, and evolution) criteria, patients are shown normal and abnormal mole features. Patients are surveyed before and after dermoscopy to evaluate if this intervention helps to educate high-risk patients about *SSE* and their ability to detect suspicious lesions for early melanoma detection.

We hypothesize that an intervention focused on digitally imaging pigmented skin lesions will increase patients' self skin-examination and awareness of melanoma warning signs. This type of intervention may help to reduce melanoma morbidity and mortality in this high-risk population and lay the groundwork for using digital dermoscopy to help educate patients regarding normal and abnormal skin features.

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1. Howe HL, Wingo PA, Thun MJ, et al. Annual report to the nation on the status of cancer (1973 through 1998), featuring cancers with recent increasing trends. *J Natl Cancer Inst* 2001;93:824-842
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LATERAL DISPLACEMENT IS AN INDICATOR OF STENT-GRAFT MIGRATION IN ENDOVASCULAR ANEURYSM REPAIR

Benjamin Y. Rafii*, Oscar J. Abilez, and Christopher K. Zarins
Department of Surgery, Division of Vascular Surgery

Background and Purpose: Positional stability of stent-grafts (endografts) is important in the long-term durability of endovascular aortic aneurysm repair (EVAR). Longitudinal migration of endografts may lead to loss of fixation and development of endoleaks, potentially exposing the patient to continued risk of aneurysm rupture. Previous studies have described inadequate proximal (aortic neck) fixation and distal (iliac) fixation as predictors of subsequent longitudinal migration. However, the importance of lateral stability of the endograft within the aneurysm sac is unknown. The present study examines whether longitudinal migration of the endograft corresponds with lateral displacement, defined as significant movement of the mid-portion of the endograft within the transverse plane.

Methods: A retrospective review of computed tomography scans taken immediately and one year postoperatively from 43 patients undergoing endovascular aneurysm repair at Stanford Hospital between 1998 and 2005 was conducted. The study population included 19 patients with ≥ 5 mm longitudinal migration at one year (migrators) and 24 patients with < 5 mm longitudinal migration at one year (non-migrators). A novel measurement approach was employed to quantify absolute and percent lateral displacement of the endograft, using the vertebral body as an anatomical reference point. Multivariate data analysis was performed using JMP 6 statistical software.

Results and conclusions: The mean longitudinal displacement in the migrator group was 8.1 ± 3.7 mm, with a range of 5.0 to 19.0 mm. The mean longitudinal displacement in the non-migrator group was 1.7 ± 1.6 mm, with a range of 0.0 to 4.0 mm. The mean absolute lateral displacement among migrators was 5.3 ± 5.8 mm, with a range of 0.0 to 21.0 mm. The mean absolute lateral displacement among non-migrators was 3.4 ± 3.5 mm, with a range of 0.0 to 13.0 mm. There was a significant ($p=0.008$) correlation between longitudinal migration and absolute lateral displacement of the endograft relative to the vertebral body. When measured separately, there was a significant ($p=0.0045$) correlation between longitudinal migration and absolute lateral displacement among migrators; in contrast, there was no significant correlation between longitudinal migration and absolute lateral displacement among non-migrators ($p=0.2$). Taken together, these results suggest a relationship between longitudinal migration of the endograft and lateral displacement of the endograft in the transverse plane. This raises the possibility that positional instability in the transverse plane may contribute to longitudinal migration of the endograft over time. Future interventions or preventative measures may be directed toward lateral stabilization of the endograft within the aneurysm sac.

CHARACTERIZATION OF PARENTAL PSYCHOPATHOLOGY IN ANOREXIA NERVOSA

Sheila Ravi*, Sarah Forsberg and James Lock,MD,Ph.D
Department of Psychiatry

Although there are many studies that have characterized patients suffering from eating disorders, few have focused on characteristics of their parents. The current study aimed to both assess the levels of psychopathology displayed by the parents of adolescents suffering from anorexia nervosa (AN), and examine the relationship between specific adolescent eating disorder characteristics and parental psychopathology.

75 female adolescent subjects suffering from AN were administered the Eating Disorder Examination (EDE) and their parents were given the SCL-90-R questionnaire. Analysis showed that significant numbers of both fathers and mothers of anorexic adolescents suffer from sub-clinical and clinical levels of obsessive compulsive behaviors, hostility, depression, and anxiety as measured by the subscales of the SCL-90-R. Further analysis showed a significant relationship between hostility and depression scores among mothers and fathers, respectively, and the duration of their child's illness. There were no significant relationships between other aspects of eating disorder symptom severity and parental psychopathology.

While these results cannot delineate the direction of the relationship between parental psychopathology and adolescent eating disorders, it suggests that parental psychopathology may play a limited role in either the development or maintenance of AN.

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OPTIMIZATION OF FLEXOR TENDON TISSUE ENGINEERING: THE ROLE OF MECHANICAL FORCES

Jonathan Riboh¹ BS; **Alphonsus Chong**¹ MD; **Hung Pham**¹ BS; **Michael Longaker**¹ MD, MBA; **Chris Jacobs**² PhD; **James Chang**¹ MD, FACS

1: Division of Plastic and Reconstructive Surgery, Department of Surgery

2: Department of Bioengineering

Flexor tendon injuries are both frequent and devastating. Despite recent progress in operative and rehabilitative care, contractions, fibrous adhesions, and long-term disability are common. These challenges are magnified in severe trauma, where the amount of tendon lost exceeds the supply of autologous grafts. Tissue engineering promises to help address these issues. In this study we applied Functional Tissue Engineering (FTE) techniques to the regeneration of flexor tendons. FTE focuses on combining biological and mechanical stimuli to recreate environments in the laboratory that mimic those encountered in situ. The goals of this study were four-fold: to identify the best cell line for flexor tendon engineering, and to study the effects of mechanical forces on cell proliferation, collagen production and morphology.

Four candidate cell lines were tested: epitenon tenocytes (E), tendon sheath fibroblasts (S), bone marrow-derived stem cells (bMSC), and adipoderived stem cells (ASC). These cells were first tested for their ability to adhere to extracellular matrix (fibronectin), an essential component for scaffold seeding. S and ASC adhered significantly better than the others. Cells were then subjected to 3 different strain regimens: Continuous Cyclic Strain (CCS: 8% elongation, 1 Hz, 100% duty cycle), Intermittent Cyclic Strain 1:2 (ICS 1:2: 4% elongation, 0.1 Hz, 33% duty cycle) and ICS 1:5 (4% elongation, 0.1 Hz, 17% duty cycle). CCS caused a decrease in cell proliferation in all four cell lines, and induced moderate levels of apoptosis. However, collagen I production doubled in E, S and ASC and increased 10x in bMSC. S and ASC had the fastest growth rates. Based on these experiments, only S and ASC were retained for further study. ICS (1:2 and 1:5) caused a 20% increase in cell proliferation in ASC, and caused a 35% increase in collagen I production in S. All forms of cyclic strain caused parallel alignment of cells, with parallel organization of their cytoskeleton, as well as nuclear and cellular elongation. In order to better understand the response to mechanical strain, microarray analysis of the ASC transcriptome before and after cyclic strain was performed.

In this study we identified ASC as the ideal candidate cell line for flexor tendon engineering, which is encouraging given the ease of isolation of these adult stem cells. Furthermore, we established the dose-dependent effects of cyclic strain on cell proliferation and collagen production. We are currently using our functional genomics data to understand the influence of cyclic strain on the differentiation of ASC into various mesenchymal tissues.

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POST-TRANSLATIONAL MODIFICATION OF TUMOR SUPPRESSORS AND THE METHYLATION OF RETINOBLASTOMA

Louis Saddic, Or Gozani, and Julien Sage
Sage Lab, CCSR South, Stanford University

The retinoblastoma (RB) tumor suppressor controls cell cycle progression at the G1/S transition of the cell cycle. Deletion of RB is found in a wide range of human cancers, including pediatric retinoblastomas and osteosarcomas, and carcinomas of the breast, bladder, and prostate. Proper regulation of RB activity during normal cell cycle is critical to prevent the development of these tumors. Most of this regulation takes place through phosphorylation by cyclin-dependent kinases, but emerging evidence suggests that RB activity may be controlled by other kinases as well as by other forms of post-translational modification such as acetylation.

Arginine and lysine methylation of histones has been initially implicated in the regulation of chromatin structure. Since then, the discovery that non-histone protein can also undergo methylation has extended the importance of this post-translational modification in cells. For instance, methylation of the p53 protein controls its stability and its tumor suppressor activity.

Thus far, there is no evidence that RB activity is controlled by methylation events. However, based on the abundance of lysines on the RB protein and the presence of RB in high molecular weight complexes containing methyltransferases, I hypothesize that RB is methylated in mammalian cells and that this methylation affects RB's ability to act as a regulator of the cell cycle. Preliminary results have identified a putative lysine residue of RB that is methylated by the lysine methyltransferase Set9. The importance of this modification and the identification of additional methylated residues by other methyltransferases are currently being investigated.

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ASSESSING PREOPERATIVE AND POSTOPERATIVE VISUAL ACUITY IN PATIENTS RECEIVING FREE CATARACT SURGERY BY OPHTHALMOLOGISTS AT FOUR EYE CLINICS IN GHANA AND INDIA

Jennifer B Staple and Dr. Peter Egbert. Department of Ophthalmology.

The objective of this four-site interventional study is to assess visual outcome after community-based screening and hospital-based cataract extraction programs. The outcomes measures include retention at follow-up, surgical complications, individual visual acuity results, and program level outcomes (efficiency rates). A total of 991 patients receiving free surgery at eye clinics in Ghana and India were included in the study. The surgical techniques vary for the free surgery patients at each eye clinic due to cost-effectiveness, training, and equipment available. One eye clinic in India provided 618 Phacoemulsification cataract surgeries, another clinic in India provided 49 Small Incision Cataract Surgeries (SICS), one eye clinic in Ghana provided 105 SICS, and another provided 219 ECCE+IOL. The results assessed the postoperative acuity improvement as well as the percentage of operated eyes achieving postoperative functional acuity of equal or better than 20/40.

This study analyzed patient outcomes, principally pre- and post- operative vision in the operated eye. Of the 618 patients receiving Phacoemulsification in the eye clinic in India, 50% (311) had a preoperative visual acuity of "Count Fingers" (CF) or worse, which is defined as a best acuity of viewing fingers held directly in front of the eyes. Of the patients receiving surgery, 87% (537) had a final postoperative visual acuity of 20/40 or better in the operated eye. At the eye clinic in India providing SICS, 84% (41) patients had visual acuity of CF or worse, and 88% (44) had a final postoperative acuity of 20/40 or better. At the clinic in Ghana providing SICS, 79% (83) started with visual acuity of CF or worse. Postoperatively, 37% (39) of the patients had a visual acuity of 20/40 or better, and 58% (61) patients had visual acuity of 20/50 or better. At the Ghanaian eye clinic providing ECCE+IOL surgery, 100% (219) had a preoperative visual acuity of CF or worse. Postoperatively, 5% (11) had visual acuity of 20/40 or better, and 14% (31) of the patients had visual acuity of 20/50 or better. Age, pre-operative acuity, and any significant complications were also documented and analyzed in comparison to the outcomes.

This prospective study is important to assess the quality of outcomes of community-based surgery programs in two countries with differing levels of equipment, environmental conditions, and training opportunities. The goal is to contribute data to the international ophthalmology community about outcomes of cataract surgeries in medically underserved regions of the world.

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THE MIGHTY MOUSE: UBIQUITOUS EXPRESSION OF TRI-FUSION IMAGING MULTIMODALITY (BIOLUMINESCENCE, FLUORESCENCE, PET) REPORTER GENE IN TRANSGENIC MOUSE

Ricky T. Tong, Pritha Ray, and Sanjiv S. Gambhir
Department of Radiology and Bioengineering

Reporter genes are extremely useful in following the gene expression and cellular behavior in development and disease studies in mice. While bioluminescence and fluorescence reporter genes provide valuable information, they are not tomographic, quantitative nor do they have great tissue depth penetration. Similarly, PET reporter genes are especially useful for whole body imaging, but they lack the sensitivity that is provided by bioluminescence or fluorescence imaging system at superficial depths. This present study aims to create a transgenic mouse that will ubiquitously express a multimodality imaging reporter construct and can be imaged by the three most common imaging techniques (bioluminescence, fluorescence, PET) used in small animal imaging research. This transgenic mouse model will be crucial in stem cell, cancer, and tissue engineering research as this universal donor mouse can serve as the source of any cells or tissues for transplant experiments.

The tri-fusion reporter vector harbors a bioluminescence reporter gene (a mutated thermo-stable firefly luciferase), a fluorescence reporter gene (a monomeric red fluorescence protein) and a positron emission tomography (PET) reporter gene (truncated herpes simplex virus type 1 sr39 thymidine kinase). We first test and confirm the activity levels of the tri-fusion protein in multiple cell lines. To create the transgenic mouse, we insert the plasmid into fertilized eggs and implant them in female mice. Since the tri-fusion reporter gene is driven by the chicken β -actin promoter, all cells of the transgenic mouse, as expected, produce strong bioluminescence, fluorescence, and PET signals when the proper substrate (PET/bioluminescence) or light (fluorescence) is provided.

In conclusion, we have demonstrated that our transgenic mouse provides strong bioluminescence, fluorescence, and PET signals in all cells. The first and current application of this mouse is to examine the contribution of circulating stem cells in tumor development. By performing a parabiosis surgery, we are “stitching” a transgenic mouse with a wild type mouse such that the two mice share blood circulation. A tumor is implanted in the flank of the wild type mouse and the circulating cells from the transgenic mouse are monitored using one of the three imaging modalities. Similarly, solid organ transplant experiments (using the transgenic mouse as the donor mouse) will also be performed in the near future.

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BLOOD AND LYMPHOID IMMUNE RECONSTITUTION FOLLOWING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION IN MICE

Gabriel J. Tsao, Jessica A. Allen, Kathryn Logronio, Judith A. Shizuru.
Division of Blood and Marrow Transplantation.

Introduction: Allogeneic hematopoietic cell transplantation (HCT) is an established way to cure many hematologic malignancies and bone marrow (BM) failure states. Unfortunately, there remain considerable risks involved with the procedure, in particular, the potential for graft-versus-host disease (GVHD) and the post-transplantation immunocompromised state. While pharmacotherapy is used to prevent and control GVHD, a more effective approach is through the transplantation of purified hematopoietic stem cells (HSC) which are devoid of T cells. However, decreased engraftment rates, reduced blood counts and increased infectious complications have been observed following HSC transplants, suggesting that the risks of using such grafts outweigh the potential benefits. While it is generally believed that HSC grafts result in impaired immune function post-HCT, there are surprisingly few reports interrogating immune recovery in recipients of T cell reduced compared to unmanipulated BM grafts.

Methods: We compared immune reconstitution in mice transplanted with unmanipulated BM and purified HSC grafts, including MHC-matched, MHC-mismatched, and haploidentical transplant pairs. Immune reconstitution was evaluated quantitatively with complete blood and lymph node (LN) cell counts and by phenotyping and immunohistochemical analysis of LN size and architecture. Qualitative function was assessed by lymphocyte proliferation assays to MHC-restricted peptides.

Results: Peripheral blood reconstitution showed markedly increased white counts across all lineages in the BM as compared to the HSC transplants. However, lymphoid reconstitution as measured by LN cell counts, size and architecture was significantly improved in the purified HSC transplant groups, especially in the MHC-mismatched setting. Qualitative measures of immune function revealed that LN proliferative responses were significantly increased in the HSC compared to BM transplant groups. Using MHC-restricted peptides, we determined that T cell restriction was dictated by donor rather than host elements. No significant differences in T-regulatory cell populations were found in the blood or LNs in the transplanted recipient groups.

Conclusions: Our results show that although HSC grafts initially reconstitute slower than BM grafts as measured by parameters in the peripheral blood, their lymphoid reconstitution is superior, both quantitatively and qualitatively. Furthermore, when donor and host are mismatched at the MHC, the peripheral T cells are restricted to the donor MHC type. These studies challenge two tenets in clinical HCT and in basic immunology: First, it is generally thought that T cell depletion and graft manipulation leads to impaired immune reconstitution. Our studies suggest that even subclinical GVHD can significantly impair immune function and that transplantation of highly purified grafts may lead to superior long-term immune function post-allogeneic HCT. Second, these studies directly show that hematopoietic lineage cells can determine the MHC restriction of peripheral T cells, traditionally understood to be mediated exclusively by thymic epithelium cells.

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THE ROLE OF THE COMPLEMENT CASCADE IN GLAUCOMA

Luis E Vazquez¹, Beth Stevens¹, Navid Nouri¹, Gareth R Howell², Simon WM John², Ben A Barres¹

¹Department of Neurobiology, Stanford University School of Medicine; Stanford, California 94305

²Howard Hughes Medical Institute, The Jackson Laboratory; Bar Harbor, Maine 04609

Glaucoma is the leading neurodegenerative cause of blindness worldwide. Blindness results from death of the retinal ganglion cells (RGCs) of the retina. Although important risk factors that render RGCs susceptible to death have been identified, the cause of their death remains unknown. We hypothesize that RGCs degenerate because they lose their connections (synapses) with the rest of the cells in the retina. This has indeed been demonstrated in other neurodegenerative diseases, such as Alzheimer's disease. We took advantage of the *DBA/2J* mouse model of Glaucoma to test this hypothesis, and to get at the molecular underpinnings of Glaucoma.

Here, we demonstrate that there is synapse loss in the retina of glaucomatous mice. Moreover, within the retina, the area most severely affected is the inner plexiform layer (IPL), which hosts the RGC synaptic terminals. More importantly, synapse loss seems to precede RGC death, supporting the idea that synapse loss may lead to RGC degeneration. In addition, we found that C1q, a complement protein known to be upregulated in Glaucoma, accumulates in the IPL around the time of synaptic elimination. Similar to the synaptic stain (PSD-95), the C1q stain is also punctate, suggesting that C1q protein may be decorating synapses and targeting them for destruction. This C1q accumulation was not observed in pre-Glaucoma or in control mice.

These findings, together with extensive data obtained by Beth Stevens in the Barres lab, suggests that pathological C1q upregulation by RGCs results in accumulation of C1q protein around synapses, targeting them for destruction. This synapse elimination may underlie the RGC death observed in glaucomatous mice. We believe that our findings shed light on the pathophysiology of Glaucoma in humans, and may open new avenues for treatment of this disease.

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HOW DO CYP1B1 MUTATIONS CAUSE GLAUCOMA?

Jack T. Wang¹, Jeffrey L. Goldberg², and Ben A. Barres¹.

¹Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305-5125, and ²Bascom Palmer Eye Institute, University of Miami, Miami, Florida 33136

Glaucomas are a group of ophthalmic diseases marked by excavation of optic disc, progressive death of retinal ganglion cells (RGCs), and eventually visual field loss. It is thought that obstruction of aqueous humor drainage secondary to structural deformities in the trabecular meshwork raises intraocular pressure (IOP) in the anterior chamber, causing direct physical damage and consequently optic nerve degeneration and RGC death. However, high IOP is neither necessary nor sufficient for the onset and progression of glaucoma. Furthermore, in glaucoma the RGCs specifically degenerate while other retinal cell layers remain relatively intact. It is unclear what makes RGCs more susceptible to degeneration than other retinal cell types in glaucoma, though the highly dynamic transcriptome of developing RGCs suggests that a genetic, cell-autonomous event may influence RGCs' viability and susceptibility to external insult. Furthermore, the early onset and developmental abnormalities seen in congenital glaucoma indicate that such genetic events may occur during development.

To address the possible developmental and molecular changes that may predispose RGCs to cell death in glaucoma, I examined differential gene expressions by RGCs at various stages of development. I found high RGC expression and developmental regulation of *CYP1B1*, a gene linked to the disease loci for congenital glaucoma and encodes an enzyme that catalyzes the rate limiting step of all-trans retinoic acid (RA) synthesis. The gene is enriched in the cytoplasm of embryonic RGCs and there was a dramatic downregulation of CYP1b1 of almost 21.7 folds by developing RGCs. I further demonstrated that CYP1b1 significantly promotes RGC survival but not neurite outgrowth in both postnatal and embryonic ages upon CYP1b1 overexpression, and that siRNA knockdown of CYP1b1 transcript abolished the survival effect. Co-culturing RGCs transfected with CYP1b1 in RA-containing medium was found to further enhance the survival effect of CYP1b1 compared to controls, although RA by itself was not sufficient to increase RGC viability. This indicates that CYP1b1 is sufficient and necessary to promote RGC survival during development putatively through a downstream RA-mediated process.

The study is first to identify CYP1b1 in the neural retina, as its expression was previously unidentified in RGCs possibly due to the gene's developmental downregulation. The results further suggest that CYP1b1 may normally be neuroprotective in the developing neural retina, and its mutations can lead to glaucoma by decreasing RGC survival through decreased RA production or RA-dependent mechanisms. This indicates that rather than an external problem of aqueous drainage, some cases of glaucoma may arise from an intrinsic, cell-autonomous cause and that CYP1b1 mutations lead to early-onset glaucoma by failing to support survival *within* RGCs.

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A NOVEL ADJUVANT'S IMMUNOGENECITY IS NOT MEDIATED BY PLASMACYTOID DENDRITIC CELLS

Thierry Giffon, **Lena Winestone**, David Lewis

Department of Pediatrics, Division of Immunology and Transplantation Biology

Each year, influenza A causes extreme morbidity and mortality, particularly in infants, the elderly, and the immunocompromised. The recent spread of pathogenic avian influenza A increases the likelihood of a human influenza pandemic. Current vaccines protect through the induction of neutralizing antibodies against hemagglutinin and neuraminidase surface proteins. This requires that the vaccine be closely matched in subtype to the virus that will be circulating six months after the start of production. However, a human pandemic would likely emerge and spread significantly more rapidly. It has been shown that the cytolytic T lymphocyte (CTL) response is essential for viral clearance from the respiratory tract. Furthermore, CTL are generally specific against internal proteins and have been shown in mice to be less dependent on viral subtype, and thus are more likely to protect against a previously unseen subtype. A unique adjuvant based on a complex of lipid carrier and non-coding DNA (CLDC) has been shown to stimulate a robust CTL immune response when administered with protein antigens.

We hypothesized that the robust CTL response seen with CLDC is mediated by plasmacytoid dendritic cells (pDC) acting as antigen presenting cells. Thus, we examined the role that pDCs play in vivo in the protection provided by vaccination with CLDC. Two mice were injected with anti-plasmacytoid dendritic cell antigen 1 (PDCA-1) in order to ablate their pDC immune response. After the depletion was confirmed, at Day 1, both these 2 mice and 2 controls were administered CLDC with influenza vaccine. Twenty-eight days later both mice were sacrificed and their immune responses were quantified. ELISA was used to track IFN-gamma secretion in response to live virus and purified antigen at 4 time points. ELISA was also used to quantify IgG2c and IgG1 antibody responses at 2 and 4 week time points. In addition, hemagglutination-inhibition (HAI) antibody titers were quantified at these time points. In all cases, there was no significant difference between the immune response mounted by the controls and that mounted by the pDC-depleted mice.

These experiments suggest that CLDC's immunogenicity is mediated by a mechanism independent of pDCs. Future studies should attempt to identify the mechanism that mediates the increased immunogenicity that CLDC provides.

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