EPIGENETIC DYSREGULATION IN HUMAN BRAIN CANCER: PTEN PROMOTER METHYLATION AND MICRORNA UPREGULATION IN GLIOBLASTOMA

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As the primary negative regulator of oncogenic AKT pathway signaling, PTEN functions as one of the most important tumor suppressors in glioma biology. Contrary to numerous findings that imply PTEN expression should be retained in a significant portion of glioblastomas multiforme (GBMs) (~60%), our own immunohistochemical data indicates that this number is considerably lower (10-20%), implying additional, nongenomic mechanisms for PTEN silencing. In the present study, we investigated the role of PTEN promoter methylation and microRNA PTEN silencing and associated GBM oncogenesis.

We studied a collection of 80 GBM tumor samples from patients undergoing craniotomy at a major academic cancer center. Methylation-specific PCR of PTEN promoter was performed by DNA bisulfite conversion of methylated cytosines to uracils, followed by PCR using primers complementary either to the original PTEN promoter sequence (where mC->mC) or to the mutated promoter sequence (where C->T). microRNA profiling was done using RT-PCR with primers for miR-19a, 19b, 21, 26a, 106b, and 214 (previously identified in low-grade gliomas). Quantitative PCR was performed relative to endogenous RNU6B and normal brain. Our immunocytochemistry and DNA mutation data indicated that the loss of PTEN protein can happen even in absence of gene mutation or deletion. Both promoter methylation and upregulation of microRNAs was found to be associated with silencing of PTEN expression in glioblastoma.

Our results suggest that promoter methylation and microRNA overexpression may be two distinct mechanisms responsible for epigenetic silencing of PTEN in brain tumors. Understanding these epigenetic mechanisms can lead to better prognostic tools and new drug targets for GBM.

DIRECT EVIDENCE OF POSTNATAL CARDIOMYOCYTE GENERATION IN MURINE MODELS OF AGING AND CARDIAC INJURY

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The dogma of the adult mammalian heart as a post-mitotic organ has recently come under question. Radiolabeled isotope studies have demonstrated that the human heart exhibits a low rate of renewal of cardiomyocytes throughout one's lifespan. Furthermore, a recent study has elegantly shown that a neonatal mouse can regenerate its ventricle if the chamber is resected within the first week of birth. However, a number of questions remain unanswered about the nature of the cell type that gives rise to cardiomyocytes postnatally. The field has yet to clonally address whether cardiomyocytes divide symmetrically upon birth, or if a resident progenitor differentiates into cardiomyocytes. Rather than rely on proxies for cell division (e.g. BrdU incorporation studies), we use genetic mouse models in which cell division results in asymmetric, indelible labeling of the daughter cells ("Mosaic analysis of double markers" (MADM)) and a stochastic multi-color Cre reporter in the Rosa26 locus akin to the "Brainbow" mouse to investigate postnatal cell division clonally in order to identify which cell type(s) generate cardiomyocytes.

Our studies have demonstrated limited symmetric division of cardiomyocytes during normal aging up to six weeks, with a significantly high rate of cardiomyocyte division during the first postnatal week. While we provide evidence for cell division in the peri-infarct region of a myocardial infarction model, the extent of cell division and proliferation appears to be limited 24 hours after the infarct. We are doing further studies to elucidate whether cardiomyocytes are generated in the one week following infarction.

BIODESIGN: REDESIGNING THE TREATMENT OF HEART FAILURE

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Introduction:
Volume overload is a common consequence of heart failure and accounts for more than 900,000 admissions each year. Excess fluid accumulates in the interstitium and can lead to pulmonary edema, causing shortness of breath and potentially respiratory arrest. Despite widely used therapeutics, 90% of patients re-admit with the same symptoms within 6 months. Our group is studying the effects of lymphatic access and drainage as a way to influence interstitial fluid balance. Here we present a novel method of reconstructing vascular and lymphatic anatomy to develop a benchtop model of the lymphatico-venous confluence.

Methods:
Manual segmentation (Analyze 10.0, AnalyzeDirect, Inc.) was performed on high resolution axial computed tomography (CT) of the neck and chest anonymized from patient data at Stanford Hospital. The confluence of the internal jugular (IJ), subclavian, and innominate veins was isolated, followed by identification and segmentation of smaller branching veins and the thoracic duct terminus. Three dimensional reconstruction (Solidworks 18.0, Dassault Systèmes SolidWorks Corp.) was used to produce sterolithographic models of the venous confluence and thoracic duct. Next, compliant resin models were cast from a novel elastic polymer (SynDaver Labs, Inc.) and integrated into a benchtop perfusion model (Masterflex L/S, Cole Parmer, Inc.).

Results:
Compliant models of the lymphatico-venous junction were successfully constructed from actual patient data through stepwise segmentation and reconstruction. Dimensional accuracy was preserved throughout the reconstruction process (subclavian vein diameter = 12.9 mm, our model ≈ 13.5 mm; IJ vein diameter = 17.4 mm, our model ≈ 13.1 mm, innominate vein diameter = 17.0 mm, our model ≈ 19.9; model diameters calculated by taking the average of the major and minor diameters). Using a variable speed perfusion system, physiological venous flow rates can be recreated in a benchtop model.

Conclusions:
Here we demonstrate a highly accurate method for reconstructing the lymphatico-venous junction in a benchtop model. As a valuable tool in our on-going research, this model will be used to study flow and pressure patterns around the thoracic duct outlet and the performance of novel catheter-based interventions to control duct output.

INDUCED ABORTION AND THE MEXICO CITY POLICY IN AFRICA

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The Mexico City Policy is an intermittent American foreign policy prohibiting non-governmental organizations receiving federal support from performing or promoting abortion. The effect of the policy on abortion rates is unknown.

We use survey data from 20 African countries between 1994 and 2008 to identify women who had induced abortions. Using data on US support for family planning, we assume that women living in countries with greater support while the policy was inactive were more exposed to its effects after its reinstatement. Using a difference-in-differences approach, we estimate the odds ratio of having an induced abortion for women living in highly exposed countries when the policy was active. Our study population includes 261,116 women (1.38 million women-years) between 1994-2008. The mean abortion rate was 10.4 per 10,000 woman-years from 1994 to 2000 and 14.5 from 2001 to 2008 ($p=0.01$ for difference). The increase was pronounced among women living in highly exposed countries and attenuated in countries with less policy exposure. The odds ratio of having an induced abortion for a woman living in a highly exposed country between 2001 and 2008 was 2.73 (unadjusted, 95% CI 1.92-3.82; adjusted odds ratio 2.55, 95% CI 1.76-3.71).

These patterns suggest that abortion in Africa increased under the Mexico City Policy. This may be due to declining support for some family planning providers coupled with substitution to abortion among African women. Regardless of one's view about abortion, our findings have substantial implications for future public policies governing abortion-related services.

PREGNANCY OUTCOMES FOLLOWING GASTRIC BYPASS SURGERY

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Background:
Nationwide, 80% of gastric bypass patients are women, many in their childbearing years. This study assesses the risks and health outcomes of pregnancies following surgery. We hypothesized that pregnancy outcomes would improve for both mother and child following gastric bypass. At a single institution, women between 18 and 45 years of age undergoing gastric bypass surgery from 2004 and 2009 were surveyed. Of the completed surveys, there were 27 pregnancies before gastric bypass surgery and 15 pregnancies following gastric bypass surgery.

Results:
For preoperative pregnancies, there were 19 live births, 4 miscarriages and 4 abortions. The mean age of mothers was 26.9 years and the mean a BMI was 33.0 kg/m^2. For postoperative pregnancies, there were 8 live births, 6 miscarriages and 1 abortion. The mean age was 33.7 years and the mean BMI was 35.3 kg/m^2. Of the 15 women who became pregnant following gastric bypass, those who became pregnant within the first 2 years had fewer live births than miscarriages compared to women who delivered after 2 years postop [1 (12.5%) vs. 7 (87.5%), $p = 0.036$)]. In addition, when comparing outcomes before and after gastric bypass surgery, women who became pregnant before surgery had a greater weight gain from the start of pregnancy to the time of delivery than women who became pregnant after gastric bypass (30.3 lbs vs. 4.31 lbs, $p = 0.0179$).

Conclusion:
This is a preliminary study looking at pregnancy outcomes before and after gastric bypass surgery. Data suggests that women are likely to gain more postpartum weight from pregnancies before compared to pregnancies after gastric bypass. Future large-scale studies need to be conducted to understand the specific biological changes as well as the long-term weight changes in the late postpartum period following weight loss surgery.

Cost-Effectiveness Analysis Of Bariatric Surgery Versus Medical Treatment For Patients With Bmis Between 30 And 35

Gaurav Banka

Background: Despite Its Effectiveness In Resolving Hypertension, Diabetes, Hyperlipidemia, And Obesity, Bariatric Surgery Is Currently Restricted To Those With A Body Mass Index (Bmi) >40 Kg/M2 Or A Bmi >35 Kg/M2 With Obesity Related Co-Morbidities. There Is A Large Population Of Patients With Bmis Between 30 And 35 And Obesity Related Co-Morbidities. Offering Bariatric Surgery To These Patients May Cause Substantial Health Care Improvements And Large Cost-Savings Benefits.

Specific Aims: To Assess The Cost-Effectiveness Of Bariatric Surgery Versus Medical Treatment For Patients With Bmis Between 30 And 35, Hypertension, Diabetes, And Hyperlipidemia

Methods: We Will Create A Cohort Of 46 Year Old Patients Diagnosed With Diabetes Within The Last Year, Hypertension, Hyperlipidemia, And A Bmi Of 32.5 Kg/M2. Quality Adjusted Life Years (Qalys) In This Study Will Be Estimated Using The United Kingdom Prospective Diabetes Study (Ukpds) Computer Simulation Model. Data From Bariatric Surgery And Medical Treatment Studies Will Be Extracted And Inputted Into This Model. The Input Variables For The Model Include Age, Sex, Weight, Height, And Yearly Biochemical Values Including Lipids, Hba1c, And Sbp And The Output Will Be A Yearly Health Utility. These Yearly Utilities And Costs Will Be Discounted At A Rate Of 3% And Used To Calculate The Qalys And Then Calculate The Incremental Cost-Effectiveness Ratio. A Sensitivity Analysis Will Be Performed.

Temporal Association between Introduction of Human Milk Fortifier and Onset of Necrotizing Enterocolitis in Premature Infants.

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Lucile Packard Children's Hospital at Stanford, March 2011

Introduction One of the major challenges for neonatologists is to provide premature infants with adequate nutrition for optimal growth and development, while minimizing the incidence of feeding intolerance and necrotizing enterocolitis (NEC). Breast milk is widely believed to be the best nutrition for all neonates. However, it is often fortified in order to increase calories, protein and mineral content of human milk. Many concerns have been raised regarding safety and efficacy of cow's milk-based human milk fortifiers (HMF), in particular in relation to the risk of NEC. No study to date has been able to demonstrate a direct causal relationship between HMF and NEC; however, a recent multicenter study has shown a dramatic reduction in NEC incidence when HMF was avoided altogether. Based on the experience in our NICU we suspected a very concerning temporal association between introduction of HMF and development of NEC symptoms in premature infants.

Objective To determine the incidence of HMF use among infants with NEC (prior to developing the disease); as well as to investigate a suspected temporal association between introduction of HMF and onset of NEC.

Study design We identified 268 infants who received a diagnosis of NEC at our NICU between 1999 and 2009. We excluded patients who were transferred to our institution with a pre-existing diagnosis of NEC, had significant congenital heart disease or other major anomalies. 139 patients born between 22 and 35 weeks gestation were included in the study and their feeding histories were reviewed in detail. Patients were divided into three groups, those that received no enteral feeds prior to NEC diagnosis (NPO, n = 26), those that were fed, but did not receive HMF (HMF (-); n = 82) and those that received HMF prior to NEC onset (HMF (+), n=31)

Results Patients in HMF(+), HMF(-) and NPO groups were similar in respect to gestational age, birth weights and NEC mortality rates. Enteral feeds were initiated at similar ages in HMF (-) and HMF (+) patients. 31 (22%) of the 139 patients received HMF prior to diagnosis of NEC and 15 (48%) of them developed symptoms of NEC within the first 3 days after receiving HMF (P<0.001 applying the Poisson distribution). Interestingly, only 6% of HMF(+) patients, compared to 38% of NPO patients and 21% of the HMF (-) patients developed perforated NEC, suggesting a different quality and possibly different pathogenesis of the disease between the three groups.
Additionally, distribution of postnatal ages at the time of NEC diagnosis predictably clustered around 10 days of age among patients in HMF(-) and NPO groups, while infants who received HMF did not follow the same pattern, instead timing of NEC in these patients appeared to be correlating with HMF exposure.

Conclusions There appears to be a temporal association between supplementation with HMF and onset of NEC. While this finding does not establish a direct cause-and-effect relationship between HMF and NEC, we urge clinicians to be hyper vigilant in their monitoring of feeding intolerance and other NEC symptoms in the first few days after adding HMF to an infant's feeds.

OneBreath: A Low Cost Ventilator for Pandemic Preparation and the Developing World
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Catastrophic disasters, particularly an influenza pandemic, force allocation decisions for mechanical ventilation due to the high cost of these devices. Pandemic modeling suggests 750,000 additional ventilators may be required to meet demand in the US. Globally, even in the absence of pandemic many countries face an extreme shortage of ventilators. In India, there are only 35,000 intensive care ventilators available for a population of 1.1 billion compared to 205,000 ventilators for 300 million in the United States. Through observation and interviews in India we identified clinical and design features needed for a low-cost ventilator. We compared the novel device with current ventilators using in-vivo and simulated ARDS models.

A low-cost ventilator was constructed around a unique microprocessor and solenoid assembly to support adults and children in accordance with the ARDSnet protocol. ARDS was induced in swine with oleic acid injection (0.3mg/kg) and defined as $PaO2/FiO2 <200$. Ventilation targets were PaO2 of 60-100mmHg and tidal volumes of 10-15cc/kg. In simulation studies (Ingmar 4000 Servo Lung), ARDS was defined by 30ml/cmH2O compliance and 10cmH2O/l/s airway resistance. An Oceanic/Magellan and Drager Evita were used for comparison, respectively.

In swine studies, there was no significant difference in performance between ventilators. Both maintained tidal volumes of 10-15cc/kg by delivering inspiratory pressures up to 50cmH2O with PEEP of 15cmH2O. Above 35cmH2O, the error in displayed pressure was 10%+/-2 on the Oceanic/Magellan and <1% on the experimental device. In simulation studies, there was no significant difference in performance or display accuracy (<1%) between ventilators. Trigger accuracy and response times (+/- 0.5cmH2O and <0.3sec) were similar in A/C modes. We describe a novel low-cost mechanical ventilator capable of supporting ARDS patients with comparable performance in-vivo and in simulation to existing devices. At approximately one-tenth the cost of current ventilators, this device represents an alternative solution for mechanical ventilation in pandemics and the developing world.

MATURATION AND CLUSTERING OF MYELOID DENDRITIC CELLS: A NOVEL PROGNOSTIC INDICATOR IN TUMOR-DRAINING LYMPH NODES OF BREAST CANCER PATIENTS

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Tumor-draining lymph nodes (TDLNs) are often the first site of metastasis for breast cancer, as well as a critical location for the interaction of tumor cells and the immune system. Understanding the alterations of immune populations within the TDLN is therefore crucial to understanding the first step in tumor escape from immune control and eventual systemic spread. Pathological analyses of TDLNs, however, have remained largely qualitative. Our laboratory thus developed a quantitative image analysis approach that incorporates 1) multi-color tissue staining, 2) high-resolution, automated whole-section imaging, 3) custom image analysis software utilizing user-driven machine-learning to identify cell types and locations, and 4) spatial statistical analysis to obtain accurate numerical and spatial data of each immune cell subtype in TDLN samples. This novel approach provides objective assessment of immune alterations in TDLNs, and the data generated is of great prognostic and mechanistic value. We are also working to increase the throughput of tissue analysis to gather numerical data in a shorter amount of time, which will result in a realistic model for a quick, reliable and highly informative prognostic tool.

Our current studies in myeloid dendritic cell maturation and grouping patterns further reinforce the importance of quantifying numerical and spatial immune cell data in TDLNs. We have found, utilizing a mathematical algorithm for defining clusters, that TDLNs from patients whose breast cancer relapsed during the observation period had a greater number of clusters, but fewer mDCs within each cluster, than TDLNs from patients who remained disease-free during the observation period. The literature suggests that increased DC clustering correlates with the maturation of these cells, while our samples with relapsed patients have a greater proportion of immature mDCs than patients who had a disease-free survival at the time of the study. This novel finding suggests that mDC clustering may be an important step in promoting an effective anti-tumor immune response and that it may be related to the maturation state of mDCs.

Thus far our work has demonstrated that both numerical and spatial distribution of immune cells in breast cancer TDLNs provides valuable prognostic and mechanistic information. In the near future, we expect further insights by extending our quantitative tissue analysis approach to immune cells within the primary tumor mass and microenvironment.

DOES EARLY RESPONSE TO CHEMOTHERAPY BY FDG-PET PREDICT OUTCOMES AFTER DEFINITIVE CHEMORADIATION IN PATIENTS WITH NON-SMALL CELL LUNG CANCER?

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In 2010, there were 222,520 new cases of cancer of the lung and bronchus in the United States, with non-small cell lung cancers (NSCLC) making up approximately 85-90% of all lung cancers (Jemal 2010). Positron-emission tomography (PET) with the tracer 18F-fluorodoxyglucose (FDG) is often used as a non-invasive imaging technique in diagnosis as well as for staging and restaging purposes.

Our group recently conducted one of the first studies to look at PET response during radiotherapy. We found mid-treatment PET/CT scans to be prognostic for overall progression and distant progression but not local progression occurring in the field of treatment. The majority of patients received concurrent chemotherapy (80%). Thus, FDG activity measured may have been more related to chemotherapy response than radiation response. A handful of studies to date also suggest that change in PET during and after chemotherapy may be predictive of progression.

Our current study retrospectively evaluates the prognostic value of PET/CT scans before and after induction chemotherapy for NSCLC to evaluate whether there is a correlation with patient outcomes. Thus far, we have identified 36 patients fitting criteria for the study and through followup scans have scored whether these patients progressed after treatment or remain without evidence of progression. Following this, quantitative measurement of PET will be through the program MimVista (including maximum standard uptake value, change in SUV between scans, and metabolic tumor volume). We will then analyze whether FDG avidity following induction chemotherapy correlates with survival/mortality or progression of disease. The overall goal is to identify a tool to predict prognosis at a time point where a change in therapy is still possible.

A COMPARATIVE STUDY MAINSTREAM MEDIA REPRESENTATION OF EXPERIMENTAL TREATMENTS OF EXPERIMENTAL STEM CELL PROCEDURES AND LAETRILE

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Today, one controversy surrounding experimental medical procedures stems from the marketing of these treatments to vulnerable patient populations with few or no therapeutic options. In many cases, there is sparse data about the potential harm that these procedures may bring. The phenomenon of patients traveling to foreign countries or unsanctioned clinics to undergo unproven procedures is not new and was seen in past controversies such as Laetrile, an anti-cancer drug of the 1960s and 1970s, and presently with experimental stem cell procedures (ESCPs), which are offered as cures for diseases like Alzheimers, Multiple Sclerosis, and diabetes.

This project will compare stakeholder perceptions of laetrile and stem cell procedures, through popular press portrayals of patients, physicians, government representatives, and advocacy groups during two periods in American history. What can we learn from the Laetrile controversy that will better inform public policy on ESCPs and future unproven medical technologies?

Methods include content analysis of articles found in a systematic review of the popular press from 1970-1980 (Laetrile) and 2000-present (ESCPs). Coding schemes will focus on how individuals are portrayed, for example the desperation of last-option patients, and whether the unproven treatments are framed in a positive, neutral, or negative light.

Preliminary analysis shows different reasons for support between the two technologies. For Laetrile, the most prevalent reason focused on treatment choice, usually articulated by patient advocates or patients. For ESCPs, the prevalent reason was based on perceived efficacy, a view shared by both patients and physicians. In both cases, clinician-scientists and government representatives were more likely to be skeptical of the procedures.

This project will attempt to explain the differences between the reasoning of the supporters of either procedure, the differences in the regulatory environments of the two periods, and whether current policy is leading vulnerable patients to pursue these unproven treatments.

NEUROGENETIC INFLUENCE ON SOCIAL COGNITION

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Williams syndrome (WS) is a neurogenetic disorder resulted from a deletion in chromosome 7. Fragile X syndrome (FXS) is a disorder resulted from a single gene mutation on the X chromosome. Previous experiments suggest that the prefrontal cortex may be morphologically abnormal in both disorders. The prefrontal cortex has been implicated to play an important role in social cognition and behavior. Interestingly, although both show abnormality in this area, WS and FXS are characterized by very opposite behaviors: people with WS are usually highly sociable while those with FXS tend to display social inhibition. In this study, using high-resolution MRI data and advanced image processing software, the prefrontal cortex of 10 WS subjects and 10 FXS subjects were compared anatomically, and its association with social cognition and behavior was explored. Functional MRI and cognitive-behavioral assessment data obtained from 10 normal subjects were used as controls to compare with the findings. In-house software, Brain Image Java was used to perform volumetric MRI analysis of the brain images.

Results showed that there were statistically significant differences in the gray matter of the middle prefrontal gyrus among the WS, FXS, and control groups. The gray matter of the middle prefrontal gyrus in FXS subjects was found to be larger in volume than that of the control group ($p=0.015$) and WMS subjects ($p=0.005$).

Behavioral neurogenetic studies on WS and FXS can ultimately lead to better understanding of the specific roles particular genes play in the development and functioning of the brain. In addition, insights into how the brain functions differently in people coping with such disorders and how they behave differently, can aid in developing more specific treatments for such patients.

FUNCTIONAL ASSESSMENT OF THE ACUTE LOCAL AND DISTAL TRANSPLANTATION OF HUMAN NEURAL STEM CELLS FOLLOWING SPINAL CORD INJURY

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The human central nervous system is not readily able to regenerate and reconnect axons following insult, which leads to debilitating functional impairment in patients with spinal cord injury (SCI). Primary injury and secondary oxidative damage, apoptosis, demyelination, and glial scarring are barriers to axonal recovery, and there are limited treatment options for preserving neuronal function following SCI. Neuronal stem cells have been shown to aid in functional recovery after SCI in rat models when transplanted at the site of injury. We hypothesize that acute transplantation of human fetal neural stem cells (hNSCs) to sites local and distal to the site of injury will lead to a measurable functional improvement in a rat model of spinal cord contusion injury.

METHODS: Adult female Long-Evans hooded rats were divided into 4 groups: 2 experimental, 2 control. Each subject underwent posterior spinal exposure and laminectomy at T10, with standardized cord contusion using the Multicenter Animal Spinal Cord Injury Study (MASCIS) impactor. Experimental subjects received a subdural injection of hNSCs at the injury site (local) or an intrathecal injection of hNSCs through a separate lumbar laminectomy (distal). Control subjects received media injection in these regions. Functional assessment was measured weekly for 6 weeks using the Basso, Beattie, and Bresnahan (BBB) locomotor rating score. These BBB scores were analyzed using ANCOVA and mixed effect regression.

RESULTS: 24 subjects were analyzed, 6 in each group. At the end of 6 weeks, both local and distal experimental groups improved their functional BBB scores over their control counterparts (Local injection: 10.67 vs 2.58 control; Distal injection: 9.17 vs. 3.4 control), and these observations were statistically significant. There was no statistical difference between local and distal experimental subjects.

CONCLUSION: Acute local and distal transplantation of hNSCs into the spinal cord of rats improved function in an established model of SCI. Distal injection of stem cells may allow for an easier approach for clinical treatment of SCI, in that patients could potentially receive an acute lumbar puncture delivery of therapeutic hNSCs.

PRESCRIPTION OPIOID ANALGESICS RAPIDLY CHANGE THE HUMAN BRAIN

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Chronic opioid exposure is known to produce neuroplastic changes in animals; however, it is not known if opioids used over short periods of time and at analgesic dosages can similarly change brain structure in humans. In this longitudinal, magnetic resonance imaging (MRI) study, 10 individuals with chronic low-back pain were administered oral morphine daily for one month. High-resolution anatomical images of the brain were acquired immediately before and after the morphine administration period. Regional changes in gray matter volume were assessed on the whole brain using tensor-based morphometry (TBM), and those significant regional changes were then independently tested for correlation with morphine dosage. Thirteen regions evidenced significant volumetric change, and degree of change in several of the regions was correlated with morphine dosage. Dosage-correlated volumetric decrease was observed primarily in the right amygdala. Dosage correlated volumetric increase was seen in the right hypothalamus, left inferior frontal gyrus, left superior temporal gyrus, right ventral posterior cingulate, and right caudal pons. A follow-up scan conducted 4.7 months after cessation of opioids found all morphine-induced changes to be persistent.
In a separate study, nine individuals consuming blinded placebo capsules for six weeks evidenced no significant morphologic changes over time. The results add to a growing body of literature showing that opioid exposure causes neuroplastic changes in reward- and affect-processing circuitry. Morphologic changes occur rapidly in humans during new exposure to prescription opioid analgesics.

Arrangement – Use three paragraphs. In general, the paragraph content should be:
First paragraph: general statement of the research topic, including two-to-three sentence background, objective, and approach (the methods can be in the second paragraph also)
Second paragraph: research findings to date
Third paragraph: conclusion, implications, further studies
Graphics – Do not use charts, diagrams or tables unless essential.
Greek letters – Use symbols (α) to designate or spell out (alpha).
References – In general try to avoid citing references in your abstract.
Abbreviations/acronyms – It is necessary to define all initially except those commonly used such as DNA, cAMP.
Length – Stay under 300 words and/or one page (using 12pt Aria font).
Funding – Acknowledge funding source in separate final sentence in *italics* (e.g., *Funding provided by the Stanford Medical Scholars Fellowship Program*).

USING THE SEMANTIC WEB FOR RADIOLOGY DECISION SUPPORT OF FOCAL LIVER DISEASE

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Due to the rapidly increasing volume of radiology in the diagnosis and treatment of disease, investigating automated systems of decision support for biomedical images becomes imperative. A promising approach known as the Semantic Web allows for a scalable knowledge-base of logical relationships between image phenotypes and clinical diseases. This technique has the added potential of reducing variation in readings by standardizing to a canonical radiology database.

In this project, we are building a decision support application that harnesses the biomedical informatics resources and are evaluating the system for the challenging case of focal liver disease diagnosis. This work entails three phases to 1) construct a Semantic Web knowledge resource to encode radiological understanding; 2) create an image-based computer reasoning application that interfaces with the Web knowledge resource to assist radiologists in formulating diagnoses; and 3) evaluate the accuracy of the system. In crafting the radiological ontology, a translation of widely used biomedical knowledge sources into the standards of the Protégé semantic web environment makes information on various focal liver diseases accessible to intelligent Web agents. As the underlying pathologies can often mimic each other in aspects of radiological imaging appearance, our decision support GUI application generates a differential diagnosis based on observed imaging features.

By benchmarking against a set of curated test images with known diagnoses, the utility and accuracy of our tool is being evaluated. A semantic architecture to represent radiological findings connected to clinical etiologies serves as an enabling technology for more efficient and accurate diagnosis.

EVALUATION OF AN INTERACTIVE WEB-BASED INFORMATION VISUALIZATION SYSTEM FOR DATA-MINING RELATIONSHIPS WITHIN A BIOMEDICAL RESEARCH NETWORK USING MEDLINE PUBLICATION TITLES AND ABSTRACTS

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CAPSig is software, developed by the Stanford Center for Clinical Informatics, that uses the Vector Space Model to generate similarity scores using attribute vectors. CAPSig has been used to generate publication similarity scores based on shared MeSH terms for Stanford faculty within the School of Medicine's Community Academic Profiles (CAP) system. As an extension of the CAPSig program, we aimed to use titles and abstracts from MEDLINE publications alone or in conjunction with MeSH terms to generate a ranked list of similar faculty. Our goal was to data-mine publicly available data to extract information regarding the possibilities for interdisciplinary research and collaboration between our medical school faculty. We hypothesize that use of the primary source data may lessen the loss of information we suspect occurs when using hand-coded MeSH terms alone.

Our CAP database included 1330 faculty to be compared. For each faculty, the titles and abstracts for all publications were extracted along with a full list of MeSH terms for each publication. Using natural language processing (NLP) techniques, common terms ("stop words" with little information content) were removed and individual words were parsed out (a process known as tokenization). These tokens were then processed using a computational method known as 'stemming' which extracts only the root of a particular term. Running a vector of these terms through CAPSIG, the ranked lists for all faculty were generated for human review. Further, the relevance scores generated for each faculty member were exported to a database to be normalized and combined into an integrated MeSH+Stems score. The scores were integrated using both a CombSUM+ZMUV and a CombMNZ+Sum approach.

All faculty were sent four ranked ordered lists of comparable factory and asked to score the accuracy of each list from a scale of 1-5 with 5 being the most accurate. With a 38% response rate, MeSH terms alone had a mean of 3.46, median of 4 and std-dev of 1.06. Stemmed terms alone had a mean of 3.24, median of 3 and std-dev of 0.99. Combined MNZ+Sum had a mean of 3.28, median of 3 and std-dev of 0.98. Combined SUM+ZMUV score had a mean of 3.27, median 3, std-dev of 1.05. These findings suggest that the use of MeSH terms alone, without extracted key terms in titles and abstracts is the best method for determining the similarity between faculty members in a research network.

Further studies include assessment of the impact of age, gender, faculty status (instructor, assistant, associate, full) and the number of publications. Further it is unclear to what extent those responding are representative of the entirety of CAP faculty.

DEVELOPMENT AND REGENERATION OF THE CHICKEN INNER EAR

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Progressive hearing loss affects over 30 million people in the United States. It is caused by the irreversible loss of sensory hair cells, which are mechanoreceptors located in specialized sensory epithelia of the inner ear. Unlike humans, birds have the ability to regenerate hair cells and restore hearing, though the factors that allow for regeneration in birds are unknown. Given that regeneration is often seen as recapitulating development, the goals of this study are two-fold: (1) to determine the role of Sox2 and other candidate transcription factors in the developing chicken inner ear and (2) to develop an assay for studying regenerating chicken sensory epithelia in the adult using time lapse imaging. Our lab's ultimate goal is to restore hair cell function using inner ear stem cells.

Given that a comprehensive set of genes expressed in otic progenitors has not been described, we conducted a Serial Analysis of Gene Expression (SAGE) of mRNA present in the chick otocyst at E 3. Our analysis identified 303 transcriptional regulators that are previously unknown in the context of the inner ear. We performed in situ hybridization screening of this library and identified several candidates for specification of the prosensory region, most notably in the Sox family of genes.

Of this gene family, Sox2 has been shown to be involved in prosensory specification in mice. While Sox2 knockout mice fail to develop any prosensory region, Sox2 overexpression later in development seems to suppress hair cell formation, indicating that the timing of Sox2 expression may contribute to multiple roles in prosensory region development. We have developed a method for Sox2 overexpression and knockdown using RNAi by injecting an avian-specific retrovirus into embryonic chicken otocysts at different time points in development, and we are studying the outcome on prosensory region markers, hair cell and supporting cell development, and Notch signaling markers.

In addition to these embryonic studies, we are concurrently developing an assay using live cell time-lapse imaging to study regenerating chicken sensory epithelia from adult chickens, after inducing hair cell loss with an ototoxic aminoglycoside antibiotic. This assay will allow us to identify the supporting cell populations that serve as somatic stem cells in the adult chicken.

OUTCOMES AND COST EFFECTIVENESS OF SIMULATION-BASED MEDICAL EDUCATION: A CASE STUDY OF CENTRAL VENOUS CANNULATION

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Central venous cannulation (CVC) is an extremely common procedure performed on acutely-ill patients requiring invasive monitoring or delivery of life-saving medications and fluids. Although the safe practice of CVC has become a national priority, rates of complications remain fairly high, resulting in deaths, longer hospital stays, and higher healthcare costs. Simulation training may increase CVC success and reduce complications. We therefore sought to analyze outcomes from simulation-based versus traditional bedside-based CVC training by performing a literature review and meta-analysis.

Searching MEDLINE/PubMed databases returned a core set of 13 articles focused on CVC simulation, including four randomized controlled trials, seven prospective cohort designs, one retrospective analysis, and one cost-effectiveness analysis. Many cohort studies suggest that CVC simulation increases procedural knowledge, skills, success rates, and confidence, up to 12-18 months post training, while reducing complications, notably catheter-related bloodstream infections. Randomized trials, however, have been less supportive, arguing at best for a short-term catalyzed skill acquisition but no long term improvement compared to traditional instruction. One cost effectiveness study highlighted the potential value in reducing complications, but a thorough analysis remains to be done. Current research bears a number of significant flaws including the inability to isolate the value of simulation versus gross additional education time.

This research will provide the basis for an upcoming cost effectiveness analysis, using outcome variables of the meta-analysis and literature-supported cost estimates to determine overall value and efficiency. We also plan to place this research in context by analyzing two specific simulation-based education programs, Stanford's Department of Anesthesia and Stanford's Department of Emergency Medicine. In total, this study will provide further clarification on the role of simulation-based education amid potential adoption of new simulation-based training requirements.

EXPRESSION QUANTITATIVE TRAIT LOCI MAPPING OF IBUPROFEN EFFECTS IN INFLAMED HUMAN SKIN

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The non-steroidal anti-inflammatory drugs (NSAID) have a well-studied anti-nociceptive mechanism of action. However, inabilities to individualize treatment and quantify subjective pain remain. Little is known about how genes modulate anti-inflammatory events. This study relates genomic measures to behavioral and objective measures to identify biomolecular markers associated with acute inflammation and pain. A placebo-controlled, double blinded, crossover protocol was implemented to assess responses to 800 mg ibuprofen. Participants were aged 18-45, 11 males and 4 females. UV light sunburns were used to induce inflammation, and skin biopsies were subjected to gene expression profiling. Heat and mechanical nociception levels were measured with a thermostimulator and weighted von Frey hairs, respectively. Inflammation was assessed objectively by blood flow change using laser Doppler imaging. Ibuprofen anti-inflammatory effects were assessed using two-sample t-test for paired samples. Gene expression fold change p-values and eQTL linear regression coefficients were adjusted using permutation based multiple hypothesis correction.

We confirmed the anti-inflammatory effects of ibuprofen by measuring a statistically significant decrease in inflammation ($p=0.0007$), increase in heat required for nociception ($p=0.002$), and increase in mechanical weight required for nociception ($p=0.002$). Nociceptive responses were statistically correlated to inflammation (heat pain, $r^2=0.46$, $p=0.00004$; mechanical pain, $r^2=0.39$, $p=0.0002$). Differential gene expression level analysis generated a list of up and down-regulated genes induced and repressed upon drug treatment in inflamed tissue. We performed an eQTL analysis to reveal gene expression changes, and we identified PODXL expression as the most significant marker associated with inflammation level (laser Doppler imaging).

This study demonstrates the potential in correlating gene expression measurements to subjective and objective pain and inflammatory measurements. Future work will include a careful examination of our gene lists to identify novel biomarkers for both inflammation and pain. Additionally, our analysis suggests PODXL expression levels are associated with blood flow changes in skin during inflammation. This finding is encouraging considering the suggested role that PODXL has in influencing cell migration and diapedesis as well as neurite growth during development. Furthermore, biological validation and genetic polymorphism investigations will be undertaken.

Investigation of a Tumor-Specific Laminin Domain Involved in Normal and Cancerous Tissue

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Laminin-332 is a glycoprotein in the extracellular matrix that is needed in promoting tissue integrity. Previous research has shown that two of its three processed domains play a crucial role in squamous cell carcinoma (SCC) tumorigenesis. The third processed domain, domain IIIa of the $\alpha3$ chain is a domain whose function, both in normal tissue and carcinomas, is not yet known. This project investigates domain IIIa of laminin-332 $\alpha3$ chain, which is expressed in normal wound healing and prominently in most human SCC cells.

In order to produce domain IIIa antibody, we have used cDNA constructs coding for the following human laminin sequence: laminin $\alpha3$ domain IIIa. This construct has been produced by PCR using full length human cDNAs coding for human laminin $\alpha3$ chain as template. In order to prepare the antibody, laminin $\alpha3$ domain IIIa cDNA is being cloned in a bacteria vector. The antibody will soon be sent to a commercial laboratory for polyclonal antibody production.

In order to produce keratinocytes expressing laminin-332 trimers with $\alpha3$ chain domain IIIa deletions (ΔIIIa cells), we will be doing the following. We recently produced a mutant cDNA construct coding for laminin $\alpha3$ with a deletion of the laminin $\alpha3$ domain IIIa. We are in the process of cloning this into our LZRS retroviral vector which will be expressed in human epidermolysis bullosa cells with null mutations for the LAMA3 gene, which codes for the laminin $\alpha3$ chain.

Using the antibody and the domain IIIa deleted keratinocytes, we hypothesize that primary human keratinocytes lacking this domain may show a breakdown in epithelial- mesenchymal cohesion, impaired attachment complex formation, and altered migration and invasion. Furthermore, we hypothesize that grafting these cells into immunodeficient mice may lead both clinically and histologically to the development of blistering, erosion, abnormal granulation tissue, and only rudimentary hemidesmosomes. After SCC transformation, the domain IIIa deficient cells may show reduced tumorigenic capacity, increased apoptosis, and an impaired ability to proliferate and invade. Lastly, we hypothesize that human primrary transformed SCC cells as well as human SCC cell lines treated with domain IIIa antibody could also show a similar reduction in tumor proliferation and invasion. If our hypotheses are correct, this knowledge could be lead to the characterization of domain IIIa as a therapeutic cancer target and knowledge of IIIa's role in normal tissue homeostasis could lead to improvement of processes such as wound healing.

POSSIBLE DYSFUCTION OF FIBROBLAST POSITIONAL IDENTITY IN SCLERODERMA PATIENTS

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Scleroderma is a disease characterized by progressive fibrosis of the skin and internal organs and has mortality of approximately 5-8 times that of the general population. There is some evidence that perturbations in the proximal distal axis via the WNT and or HOX signaling systems may play a role in the fibrosis seen in Scleroderma. Scleroderma often starts at specific anatomic sites that are sites of expression of WNT5A and excessive activation of WNT signaling can cause aggressive fibromatosis and fibroblast proliferation in response to wounding. In order to search for perturbations in HOX gene expression or objective is to compare the RNA expression profile of scleroderma patient skin to that of normal skin on HOX tiling arrays.

We procured normal and diffuse scleroderma forearm skin RNA and/or tissue samples from two sources Dr. D Fiortentino and Dr. ML Whitfield. RNA was isolated from tissue biopsies, reverse transcribed into CDNA and then amplified using in vitro transcription. The RNA concentration, quality was checked via nanodrop and then it was run out on a gel to check for degradation.

In the future the scleroderma and normal skin RNA samples will be reverse transcribed into DNA, labeled with dye and hybridized to HOX tiling arrays in order to look for differences in HOX RNA expression. In addition it would also be beneficial to analyze the RNA expression profiles using human genome microarrays which would allow one to analyze whether there are connections between certain HOX perturbations and microarray gene signatures.

WEIGHT-BEARING AXIAL RADIOGRAPHY IN EVALUATION OF PATIENTS WITH PATELLOFEMORAL PAIN

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Patellofemoral pain is characterized by anterior knee pain with activities such as running, ascending/descending stairs, or squatting and is one of the most common disorders of the knee amongst young athletes. Lateral maltracking of the patella is a commonly cited cause of pain, resulting in increased joint stress, which may eventually result in osteoarthritis of the patellofemoral joint. Therefore, accurate diagnosis of pain mechanism with appropriate treatment is important to prevent continued loss of joint function. Standard clinical assessment includes non-weight-bearing axial radiography for assessment of the patellofemoral joint, but does not reflect the physiologic conditions in which patellofemoral pain occurs and may therefore misdiagnose patients with lateral patellar maltracking. We propose upright, weight-bearing radiography as a more appropriate diagnostic imaging method to differentiate patients with patellar maltracking from those without maltracking.

We are in the process of recruiting a cohort of 20 subjects with patellofemoral pain. Each patient will have a clinical exam and two radiographs taken of their knees: an axial X-ray of knees flexed at 30º in a supine position and an axial X-ray in a standing position with the same angle of knee flexion. From these images, we can calculate bisect offset index and patellar tilt, well-established measurements of patellar alignment, and compare these for weight-bearing vs. non-weight-bearing conditions. Preliminary results will be reported.

Our major objective is to validate weight-bearing axial radiography as a method for tracking patellar alignment. We expect to find that the patella moves closer into the trochlear groove with weight-bearing, thereby decreasing bisect offset and patellar tilt. Given that a subset of patients with patellofemoral pain have patellar maltracking, we may find that not all subjects respond the same to weight-bearing, which would allow for a classification system that could be used in future studies to determine what treatments are most appropriate for different patient subsets, for instance.

Biomechanical Evaluation of a Novel Reverse Coracoacromial Ligament Reconstruction for Acromioclavicular Joint Separation

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Purpose

The purpose of this study was to compare the initial stability of coracoclavicular (CC) tendon graft acromioclavicular joint reconstructions with and without augmentation by either (1) a novel "reverse" coracoacromial (CA) transfer from the coracoid to the distal clavicle or (2) an intramedullary acromioclavicular tendon graft. The hypothesis was that our new technique of reverse CA ligament transfer would reinforce the acromioclavicular joint capsule and stabilize the joint in the anterior-posterior direction compared with CC reconstruction alone. Six matched pairs of cadaveric shoulders (n=12) underwent 10mm distal clavicle resection and CC tendon graft reconstruction. Specimens were cyclically loaded with 20 cycles of 25N (compression and tension at 3.3mm/s) along the anterior-posterior (AP) axis of the acromioclavicular joint while displacement data was collected. Without moving the setup, each shoulder was manually loaded with 10N superior-inferior (SI) compression and 70N tension for a minimum of 3 cycles, until recorded end displacement ranges equilibrated. Each right shoulder was then randomized to receive augmentation by either reverse CA transfer or intramedullary tendon graft, with the left contralateral side receiving the other treatment option. Post augmentation AP and SI displacement was then reassessed with the same loading protocol.

Results

A series of paired t-tests were performed to evaluate each augmentation techniques' ability to improve acromioclavicular joint restraint. Reverse CA transfer augmentation improved AP stability by significantly decreasing translation compared with CC reconstruction alone, with 3.7mm average reduction in displacement (1.3mm SEM; 95%CI: 0.458- 7.014, p=0.03). Intramedullary acromioclavicular tendon graft augmentation also reduced AP motion in response to loading by an average of 3.4mm (1.1mmSEM; 95% CI: 0.521- 6.345, p=0.03). SI stability did not differ between first and second treatment groups in a statistically significant manner, although a trend towards increased stability was seen. Using a clinically significant displacement value of 5mm in all directions, a test of equivalence of means supports that there is no difference between reverse CA ligament transfer versus intramedullary tendon graft in AP or SI stabilizing effect (alpha=0.05, confidence intervals for AP difference -1.46 to 0.85, SI difference -3.04 to 3.63).

Conclusions

Acromioclavicular joint reconstruction with CC tendon graft augmented with either intramedullary acromioclavicular tendon graft or reverse CA ligament transfer demonstrates improved anterior-posterior restraint and provided similar superior-inferior stability compared with CC reconstruction alone. Our data suggests that anterior-posterior stability of a CC tendon graft acromioclavicular reconstruction can be enhanced with minimal increase in soft tissue

Identification of serum protein biomarkers for small cell lung cancer

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Lung cancer is the most common cause of cancer mortality in the world. Small cell lung cancer (SCLC) is characterized by rapid progression, extensive metastasis, and very high mortality (~95%). The fact that SCLC prognosis improves when it is detected early underscores the urgent need for a non-invasive method such as serum protein quantification for timely diagnosis. Many researchers have tried to identify serum protein biomarkers for various diseases using a proteomics approach, but challenges such as low sensitivity of mass spectrometry, variable protein concentrations in serum, small sample sizes, and clinical confounding factors still exist. We hypothesized that integrative meta-analysis of a number of SCLC gene expression data sets would increase the sample size as well as address the issue of confounding factors, which in turn will allow us to identify serum protein biomarkers for SCLC.

We performed meta-analysis on 9 SCLC gene expression data sets consisting of total 749 samples. We characterized the genes discovered from meta-analysis to identify candidate serum protein biomarkers and utilized ELISA to validate our *in silico* findings in human SCLC patient serum samples *in vivo*. Meta-analysis of gene expression data revealed 100 genes (false discovery rate < 0.1) that are over-expressed in SCLC. These included not only ASCL1, a well-known neuroendocrine marker, but also possible drug targets such as HDAC1 and RXRG. We demonstrated that this meta-analysis approach is more successful at identifying over-expressed genes in SCLC compared to widely used Significant Analysis of Microarrays method. We selected a subset of genes from our initial genes based on their presence in serum, expression in SCLC mouse model, and potential involvement in SCLC pathogenesis. We are currently conducting ELISA of human SCLC patient serum samples to validate our predicted serum protein biomarkers.

Candidate SCLC serum protein biomarkers were identified using meta-analysis of gene expression profiles. The methods that we described in this study will enable us to discover serum protein biomarkers for other diseases and potentially apply them clinically for their early detection and better diagnosis in the future.

USING SLAMS TO IDENTIFY UPSTREAM REGULATORS OF HOTAIR EXPRESSION

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Less than 2% of genomic transcripts are translated into proteins. Recent evidence suggests that greater than 1000 conserved, large intergenic non-coding RNAs (lncRNAs) exist throughout the genome and may perform a significant role in transcriptional regulation and cellular function. These 300nt – 10KB lncRNAs are transcribed above background level and are polyadenylated but are not capable of being translated into functional proteins and therefore must carry out their cellular functions in RNA form. Examples of lncRNAs whose cellular functions have been well characterized include Xist, which epigenetically silences one X chromosome in female XX cells in order to normalize the copy number of X chromosomes in males and females and Nron, which functions in the regulation of nuclear import.

Utilizing tiling array technology, Rinn et. al. discovered 231 lncRNAs embedded within the intergenic regions of the four human hox loci. One of these lncRNAs, HOTAIR, is transcribed from the HOXC cluster and regulates HOXD gene cluster expression. HOTAIR is over-expressed in breast cancer patient samples and correlates with increased metastasis, poor prognosis and decreased overall survival. HOTAIR has been shown to promote invasion and migration in vitro and metastatis in vivo. In order to study the mechanism by which HOTAIR leads to this aggressive form of breast cancer, we used a bioinformatic approach, Stepwise Linkage Analysis of Microarray Signatures (SLAMS), to identify upstream drivers of HOTAIR expression. This approach allows us to correlate genome-wide copy number variation and gene expression in hundreds of primary breast cancer samples simultaneously to uncover chromosomal regions that are consistently amplified or deleted when HOTAIR is overexpressed. Using this method, we identified multiple candidate regulators, including the breast cancer metastasis promoting gene, MTDH. We believe that this work will better define the molecular regulation of lncRNA expression and also contribute to our understanding of cancer pathogenesis.

40 DAYS AND 40 NIGHTS: SEQ-ING THE TRANSCRIPTOME OF 22Q13 DELETION SYNDROME IN NEURONS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Phelan-McDermid syndrome, or 22q13 deletion syndrome, is a neurodevelopmental disorder characterized by autistic features, neonatal hypotonia, and global developmental delay. In order to study the cellular and molecular consequences of this deletion *in vitro* we reprogrammed human fibroblasts from patients with Phelan-McDermid syndrome and from unaffected control individuals into induced pluripotent stem cells (iPSC). This was accomplished by infecting fibroblasts with retroviruses expressing Sox2, Oct3/4, Klf4, and c-Myc. We subsequently differentiated iPSC into human neurons in culture and will apply whole transcriptome shotgun sequencing or RNA-seq to examine the transcriptional signatures and cellular phenotypes association with Phelan-McDermid syndrome. In addition we will use single-cell PCR arrays to study alterations in the subpopulations of neurons produced from IPSC derived from patients.

We next differentiated the iPSC lines into neural progenitor cells and neurons using conditions that favor the generation of cortical neurons. The iPSCs were suspended to generate embryoid bodies and plated to produce neural rosettes. The rosettes were mechanically isolated and expanded as neurospheres and then plated for differentiation into neurons. Preliminary single cell PCR analysis of neurons confirmed expression of cortical markers Satb2, Ctip2, Foxp1, Etv1, and Reelin. Moreover, RT-PCR experiments confirmed reduced expression of Shank3, a gene deleted in 22q13 deletion syndrome, in iPSC, neural progenitors, and neurons derived from patients with Phelan-McDermid syndrome relative to controls. At day 40 of neural differentiation, we harvested RNA and single cells and are now performing RNA-seq and single cell PCR arrays.

This approach represents an unbiased means of identifying genes and signaling cascades that are misregulated in Phelan-McDermid syndrome. Cellular endophenotypes identified via this approach will set the stage for the development of cell based pharmaceutical screens to discover treatments.

PRECLINICAL EVALUATION OF A GAMMA-SECRETASE INHIBITOR TO TARGET HEAD AND NECK CANCER STEM CELLS

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Head and neck squamous cell carcinoma (HNSCC) is an epithelial malignancy of the mucosa lining the structures of the upper aerodigestive tract. Our laboratory has been investigating CD271 (a.k.a. nerve growth factor receptor, NGFR, and p75NTR), a receptor on HNSCC that may mark a subpopulation of "cancer stem cells" having tumor-initiating capacity. This project aimed to inhibit HNSCC cancer stem cells by targeting CD271. Upon binding ligand this receptor normally undergoes proteolytic processing within the cell membrane by an enzyme called gamma-secretase. The intracellular domain that is released upon cleavage by gamma-secretase translocates to the nucleus and regulates transcription. Specifically, we hypothesized that using DAPT, a gamma-secretase *inhibitor*, would decrease tumor growth.

Using a standard XTT assay, we assessed the effects of DAPT on cell proliferation in 4 cell lines *in vitro*. Notably, in all cell lines gamma-secreatse inhibition significantly decreased cell proliferation in a dose-dependent manner. Additionally, a HNSCC cell line was xenografted into the subcutaneous compartment overlying the flanks of immunocompromised mice. Cohorts were treated with and without DAPT, and tumor growth was measured by whole animal imaging of luciferase. To our surprise, no difference was found between tumor growth or metastases between the treated and untreated groups *in vivo*. Finally, the mechanism of DAPT's action was investigated. Flow cytometry and qPCR were performed on cell lines treated for 5 days with DAPT. Interestingly, no difference in CD271 expression was detected after treatment.

In total, this study shows that DAPT may have detrimental effects on tumor growth. However, using an *in vivo* tumor model of immunocompromised mice, no tumor growth inhibition was found. The potential mechanism of *in vitro* growth inhibition is still unclear too. Potentially, CD271 may regulate cellular processes through mechanisms besides proteolytic processing by gamma-secretase. Other methods of inhibition of CD271 could be tested.

Characterization of Wnt-Responsive Cells in the Mouse Cochlea

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Hearing loss is one of the most common sensory disorders, affecting both the pediatric and adult populations. In the United States alone 30 million people suffer from this disability. The pathology underlying sensory hearing loss is the irreversible loss of the inner ear sensory hair cells. These cells are highly specialized and function to transform sound pressure waves to electrical neural signals. Since mammals are unable to regenerate lost sensory hair cells, there is much interest in investigating the potential of endogenous progenitor cells in the cochlear and vestibular systems of mice.

Past experiments have demonstrated that Wnt-responsive Axin2-positive cells show signs of proliferation after Wnt activation. These findings draw similarities between the inner ear to other systems including skin, mammary glands, central nervous system, eyes and prostate where Wnt signals also play a critical role in modulating their respective endogenous stem/progenitor cells. However, we are interested in understanding the mechanism by which we can modulate proliferation among progenitor cell population in the cochlea by controlling the Wnt pathway, and have therefore designed the following experiments interrogating Wnt agonists and antagonists.

Using organotypic cultures of cochlea from three-day-old wildtype mouse, we assessed the effects of Wnt agonists (Wnt3a and R-spondin1) and antagonists (IWP-2, and XAV-939) on proliferation as measured by EdU uptake. Cochleae were cultured for 60 hours in varying drug-growth media conditions. Wnt3a-treated cultures showed increased proliferation when compared to vehicle only controls, while treatment with IWP-2 or XAV-939 caused decreased proliferation, suggesting that proliferation of the Axin2-expressing progenitor cell population is dependent on the canonical Wnt pathway. R-spondin1 showed variable proliferation when compared to vehicle controls, which differed from previous data. Additional experimentation is planned to elicit the role of R-spondin1 in modulating cochlear cell proliferation. During experimentation, there were notable differences in proliferation between cochlear apex, middle and base. The anatagonists showed the greatest variation in proliferation at 0.1μM in the base and middle when compared to DMSO vehicle controls. Further experiments have been planned to assess drug dose-response relationships, and the fate of our proliferating cell population.

COMPARISON OF RECIST 1.1, WHO AND COG RESPONSE CRITERIA IN PATIENTS WITH EWING SARCOMA FAMILY OF TUMORS

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Purpose
The optimal method for assessing treatment response in patients with primary bone tumors is not clear. We sought to compare radiographic response of Ewing sarcoma family of tumors (ESFT) to determine whether response classification differs between the RECIST 1.1 (Response Evaluation Criteria in Solid Tumors), WHO (World Health Organization) and COG (Children's Oncology Group) response criteria.

Materials and Methods
We retrospectively analyzed MR imaging scans of fifty-six patients with EFST, who were treated at Stanford and UCSF Medical Centers. Tumor size was assessed on T2-weighted sequences and postcontrast T1-weighted sequences before, during and after therapy. Tumor measurements were obtained according to the RECIST 1.1 (longest single diameter), WHO (byproduct of the longest perpendicular diameters) and COG criteria [tumor volume (V) calculated using the maximum diameters from sagittal (S), coronal (C) and axial (A) planes: $V = S \times C \times A \times F$ (where $F = \pi/6$ for ellipsoid tumors; $F = \pi/4$ for cylindrical tumors)]. All three guidelines share the same four response categories: progressive disease (PD) (>20% increase in RECIST/COG; > 25% in WHO), stable disease (SD) (neither PR nor PD), partial response (PR) (< 30% decrease in RECIST/COG; <50% decrease in WHO) and complete response (CR) (100% decrease). Numerical values were assigned to each response category: 1 = PD, 2 = SD, 3 = PR and 4 = CR. Concordance between the three response classification systems was assessed using Cohen's kappa (κ) coefficient and percentage of disagreement per response category.

Results
The κ statistic for concordance in RECIST/WHO, RECIST/COG and WHO/COG were 0.443, 0.215 and 0.349 respectively. Disagreement rates for RECIST/WHO, RECIST/COG and WHO/COG were 30.36, 48.21, and 32.14% respectively. Twenty-seven patients coded as SD by RECIST were reclassified as PR by COG. Similarly, eighteen patients originally coded as SD by WHO were re-categorized as PR by COG.

Conclusions
This study demonstrates a fair level of agreement, by the κ statistic, between the RECIST 1.1, WHO and COG response criteria in ESFT.

Clinical Relevance
Given the degree of discordance between WHO, RECIST and COG response criteria in ESFT, evaluation of the prognostic impact in each classification system may guide selection of the optimal system for future use in this disease.

TRANSGENDER CONTENT IN MEDICAL CURRICULA: DEANS' AND MEDICAL STUDENTS' PERSPECTIVES

Lesbian, Gay, Bisexual, & Transgender Medical Education Research Group. Department of Medicine

Transgender patients suffer from major health disparities compared with non-transgender individuals. While many factors impact transgender persons' health status, health care providers' perceived or real lack of understanding and competency in handling transgender patients' health concerns may play a part in these disparities. Two questionnaires (of Deans of medical education [n=150], and of medical students [n=8,551]) were combined with insights from focus group interviews [n=37, across 5 schools] to assess transgender-related content of medical curricula.

The surveys show that transgender-related topics often lag behind the LGB topics assessed, in terms of coverage in medical schools across the United States and Canada. According to Deans of medical education, the least-covered content areas included transitioning (30.9% of schools) and sex reassignment surgery (covered in 36.5% of schools). Students feel that these topics receive too little coverage in the medical curriculum. For example, approximately 50% of medical students surveyed believe that sex reassignment surgery and transitioning receive too little coverage at their school, while less than 5% claim in-depth coverage of these topics. Student focus groups demonstrated that some medical students feel much less prepared and comfortable handling the health needs of transgender patients as opposed to LGB patients. For some, LGB patients were like any other patient, while transgender patients were different.

Medical education has yet to reach consensus on transgender topics in the curriculum. If care for transgender patients is to improve through the education of health professionals, understanding the current situation in medical schools across the United States is one necessary step.

CORRELATION OF SERUM LEVELS OF THE ENVIRONMENTAL TOXIN BISPHENOL-A (BPA) WITH REPRODUCTIVE OUTCOMES IN HUMANS

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Bisphenol-A (BPA) is an endocrine disrupting chemical found ubiquitously in household items such as the lining of metal food and drink cans, plastic baby bottles, hard plastic water bottles, medical devices, and dental sealants, with detectable levels of BPA found in 93% of people six years and older in the United States. There is growing concern of a potential relationship between BPA and negative effects on human health, including research that low-dose *in vivo* BPA exposure increases meiotic aneuploidy in oocytes and causes recurrent miscarriage and decreased fertility in mice. Limited research investigating BPA and miscarriage in humans suggests that exposure to BPA may be associated with recurrent human miscarriage. Although BPA use in baby products has been banned in Canada and the European Union, its widespread use continues in the United States.

This case-control study aims to assess whether maternal serum BPA levels during conception and early pregnancy are associated with subsequent pregnancy outcomes. We hypothesized that elevated maternal serum BPA levels are correlated with aneuploid miscarriage. This study analyzed BPA levels in frozen serum samples collected during early pregnancy from 117 subjects with known subsequent pregnancy outcomes (47 aneuploid miscarriages, 24 euploid miscarriages, and 46 live births). There was no significant difference in demographic characteristics between study groups.

Data analysis of serum BPA level results is pending completion of sample analysis. Planned statistical analysis includes ANOVA of mean BPA levels by study group and odds ratios for miscarriage associated with BPA levels according to fetal karyotype. Further analysis will determine if in fact there is a correlation between serum BPA level and miscarriage, and whether BPA is more closely associated with aneuploid miscarriage. If an association between BPA and miscarriage in humans is demonstrated, this research may significantly impact the future regulation of the use of BPA in food packaging and consumer products.

MOLECULAR INVERSION PROBES (MIP) IDENTIFY NOVEL GENOMIC SIGNATURES IN PEDIATRIC LOW GRADE GLIOMAS

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Childhood brain tumors (CBTs) are the leading cause of cancer death in children under the age of 18 years old. Pediatric gliomas account for 76% of CBTs diagnosed annually in the United States. Despite the high prevalence, morbidity, and mortality from chemotherapy and radiation therapy, very little is known in regard to the genomics of tumor occurrence and progression. Accordingly, we used Molecular Inversion Probes (MIP) in a pilot study to identify novel copy number alterations (CNAs) in an effort to better classify and risk-stratify low grade pediatric gliomas (LGGs).

DNA was extracted from 22 fresh-frozen pediatric gliomas (pilocytic astrocytoma (PA), n=14, WHO grade I; ependymoma, n=2, grade II; subependymal giant cell astrocytoma, n=3, grade I) and non-neoplastic brain tissue control samples (n=9).   The MIP assay was run using 37ng of genomic DNA/sample on a customized Affymetrix MIP 330K platform with probes spread throughout the genome focused predominately on known cancer genes. We observed the full spectrum of genomic CNAs scattered throughout the tumor genome with implications of heterogeneity demonstrated by non-integer copy number seen only in CBT samples but not in controls. Similar to high grade gliomas (HGGs), LGGs appear to possess multiple clones within the same sample. CNAs were composed of single copy duplications, high-level focal amplifications, heterozygous and homozygous deletions. PA-specific CNAs on chromosome 7 included *BRAF* and an *EGFR* deletion including all tumor types (LGG cases n=13/19 vs. controls n=2/9, p=0.0418). Cytobands 17q21.32-33 also contained two significant deleted regions. 24 genes were affected in these two regions, and the most commonly deleted gene found exclusively in tumor samples was *CACNA1G* (LGG cases n=13/19 vs. controls n=0/9, p=0.0008).

In summary, our pilot study revealed known and novel regions of CNAs in pediatric LGGs, including *CACNA1G* in 17q21.33 previously implicated in HGGs. We also found that LGGs contain a significant amount of tumor heterogeneity within specific regions, implying multiple clones within a single specimen. Identifying recurring CNAs in pediatric gliomas will provide a better understanding of the molecular genetics of pediatric brain tumors and when combined with clinical data, may help to predict which LGGs will progress despite treatment.

ESTABLISHING A HEPATOCELLULAR CARCINOMA SERUM BIOMARKER PANEL VIA A MULTIPLEXED PROTEIN LIGATION ASSAY

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Hepatocellular carcinoma prognosis has been consistently poor among the GI cancers. One reason is because of the limited time period to treat these patients once the cancer is discovered.

Recently, the Koong Lab as well as others has developed a research tool known as the protein ligation assay (PLA), which can be used to create a biomarker panel for diagnosis. PLA consists of paired antibody probes tagged with unique DNA barcodes that will ligate upon protein recognition once placed in a patient serum sample. PLA signal is quantitated via real time qPCR of the ligated DNA. In the age of personalized medicine, PLA serves as a tool to uncover serum proteins associated with various cancers and may lead to better therapy selection and prognosis. The procedure is relatively noninvasive, requires little sample, and may have high predictive value for diagnosis. Additionally, PLA was used because it utilizes both antibody specificity and PCR accuracy to assess protein concentration.

In this ongoing study, we used multiplex PLA to develop a biomarker panel for hepatocellular carcinoma diagnosis. PLA probes were made, tested with pure protein and control serum both singly and in multiplex. A panel of 12 probes will be used for this study. We will use serum collected from 25 hepatocellular carcinoma patients and 25 age-matched controls with HBV infection and analyzed their blood based on biomarkers associated with hepatocellular carcinoma. Preliminary testing on all the probes made indicate that they work and we will be testing these probes on samples shortly. This work will hopefully lead to improved diagnosis and therapy assessment for hepatocellular cancer patients. We are also working on a 56-probe panel for pancreas cancer patients to examine prognosis.

EVALUATION OF AWHONN WEANING PROTOCOLS IN TRANSITIONING INFANTS FROM INCUBATORS TO BASSINETTES AT LPCH.

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General clinical practice requires that all premature infants be transitioned from incubators to bassinettes prior to discharge from the nursery. This is to ensure that each infant has developed an independent capacity to self-regulate body temperature without adversely affecting energy expenditure and losing weight. Clinical and nursing practices have evolved over time to gradually "wean" an infant from an incubator, culminating in a protocol developed by Barbara Medoff-Cooper in 1994 that was subsequently adopted by AWHONN. Based on consistent trends, temperature stability, weight gain, and cardio-respiratory stability, Medoff-Cooper's weaning protocol is now the standard at many nurseries including LPCH. Based upon anecdotal evidence, however, there are reports of wide variations in practices that ultimately affect transition from incubators and subsequent discharge from the nursery. This goal of this study, therefore, was to evaluate the AWHONN weaning protocols at LPCH.

A retrospective chart review (Stanford IRB#19788) examined 121 premature infants (20 infants less than 29 weeks gestational age, 96 between 29 - 34 weeks, and 5 greater than 34 weeks) in the Packard Intermediate Care Nursery over a two-year span (July 1, 2008 - June 30, 2010). Results showed that birth weights were 941 ± 220, 1759 ± 480, and 2414 ± 264 grams, respectively for <29 weeks, 29 - 34 weeks, and >34 weeks gestational age.   At transition, weight and post-menstrual age were 1758 ± 245 grams at 34.8 ± 2.3 weeks, 2052 ± 304 grams at 34.5 ± 1.2 weeks, and 2344 ± 287 grams at 36.2 ± 0.4 weeks, again respectively. Transition incubator temperatures were 26.1 ± 0.8, 26.3 ± 0.6, and 26.7 ± 0.8 Celsius, p = 0.34). Ages at discharge were 38.1 ± 3.5, 36.6 ± 1.5, and 37.6 ± 0.2 weeks, respectively.

The results showed variations in weaning practices at LPCH. Even though the AWHONN guidelines stipulate a baby can be transitioned after five continuous days of weight gain and stable temperature profile, the large variances seem to indicate wide interpretation and adherence. However, the data does indicate that there is a developmental component to the weaning process (e.g. increasing prematurity led to more time spent in incubators but earlier age at transition from incubators). In addition, even though literature has supported safe transition at weights down to 1600 grams, a majority of infants at LPCH are transitioned around 2000 grams. Therefore, further study may accertain how LPCH nurseries can more optimally wean infants from incubators.

DETERMINING THE ROLE OF SMOOTH MUSCLE CELLS IN AORTIC ANEURYSM OF A MARFAN SYNDROME MOUSE MODEL

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Marfan syndrome (MFS) is an autosomal dominant inherited disorder with clinical manifestations in the skeletal, ocular and cardiovascular system. MFS is caused by a genetic mutation in the fibrillin-1 gene that results in extracellular matrix disarray and ultimately in connective tissue dysfunction. The most prominent features of MFS include long bone growth, ocular lens dislocation and aortic aneurysms. The aortic aneurysms are the most life threatening phenotype of this disorder due to aortic dissections that can lead to death. Even though the mutation of fibrillin-1 that leads to TGFB up-regulation resulting in many cytological abnormalities has been determined, the exact process of the pathogenesis of aneurysm development has yet to be finely understood. This study focuses on studying the mechanism of aneurysmal formation in a fibrillin-1 haploinsufficiency mouse model of MFS and developing a cell line of MFS smooth muscle cells to test that mechanism.

Firstly, we determined the baseline parameters of aneurysm formation by calculating the root diameter of heterozygous MFS mice compared to wild type mice utilizing MRI and Echo imaging modalities. Our data show that the diameter of the aortic root in MFS mice at weeks 2, 4, 6, and 8 are significantly larger than their wild type littermate counterparts by both Echo and MRI data analysis. After this was established, aortic samples of the ascending, descending and abdominal aorta were collected and analyzed for apoptosis by western blot, cell death ELISA, and microRNA expression. We established that several markers of the apoptotic pathway and microRNA expression were significantly up-regulated in the MFS samples compared to wild type. Finally and central to this project, numerous different methods to grow vascular smooth muscle cells from the MFS mice by the most effective and efficient techniques were established and refined. Currently the cell line is being propagated and eventually the apoptotic and microRNA markers will be targeted in-vitro to confirm their role in the pathogenesis of aneurysmal formation. From determining the role of microRNA expression influence on apoptosis as the pathogenesis of aneurysm formation in Marfan syndrome, targets of this pathway could be potentially developed for future treatment of this disease.

CULTURAL ADAPTATION OF A SURVEY TO ASSESS MEDICAL PROVIDER'S KNOWLEDGE OF AND ATTITUDES TOWARDS HIV/AIDS IN ALBANIA

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Sustaining low HIV/AIDS prevalence in South Eastern Europe depends on assessment of local epidemics and implementation of prevention and treatment strategies. Previous studies in Albania have shown that patient-reported medical provider's lack of knowledge of HIV/AIDS is major barrier to attaining HIV medical services. This study set out to culturally adapt an instrument to assess medical provider's knowledge of and attitudes towards HIV/AIDS in Albania. Cultural adaptation was completed through development of a survey from previously validated instruments, translation of the survey into Albanian, blinded back translation, expert committee review of the draft instrument, focus group pre-testing with physicians and nurses from the greater Tirana-community and University Hospital Center of Tirana, and test-retest reliability testing.

Blinded back translation of the instrument supported the initial translation with slight changes to the idiomatic and conceptual equivalences. Focus group pre-testing generally endorsed the instrument, yet some experiential and idiomatic changes were made. Based on unweighted kappa and/or prevalence adjusted bias adjusted kappa (PABAK), 20 of the 43 questions tested were deemed statistically significant at kappa and/or PABAK ≥ 0.5, while 12 others did not cross zero on the 95% confidence interval for kappa, indicating their probable significance. These 32 questions were retained for the final instrument.

A final instrument to assess medical provider's knowledge of and attitudes toward HIV/AIDS was developed for an Albanian population. This will be useful in order to gain a more comprehensive understanding of the HIV/AIDS educational background of Albanian medical professionals and the level of discrimination against patients with HIV/AIDS within the medical field. The current paucity of data attributed to these areas limits future healthcare reform and innovative policy recommendations in regards to HIV/AIDS in Albania; a niche that can potentially be filled by this instrument. The survey can also likely be expanded nationally within Albania or to outside regions within the Balkans like Kosovo or Former Yugoslavian Republic of Macedonia.

Investigating the Sufficiency of CCL2 In Promoting Diapedesis And Specific Migration Of Neural Progenitor Cells Transplanted Intra-Arterially After Stroke

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Stroke continues to exert a large burden on millions throughout the world. In spite of this, few treatment options are available to limit the extent of injury. The use of neural progenitor cells (NPCs) presents an exciting new therapeutic strategy to do just that through a variety of mechanisms. However, a fundamental challenge with this approach which must be overcome is the design of an effective delivery method.

Prior work has shown that intra-arterial delivery of NPCs is likely the best candidate, since NPCs possess receptors to detect molecular signals released by ischemic tissue and can diapedese through blood vessel walls to reach damaged areas. Though our group has demonstrated that C-C chemokine ligand 2, CCL2, and its receptor, CCR2, play a pivotal role in directing this migration, it remains unclear whether this signaling is sufficient to allow for NPC diapedesis, or if the blood-brain barrier (BBB) compromise which accompanies stroke is also required.

Here we attempt to answer that question through the intraparenchymal injection of CCL2 in healthy, non-stroked mice. If NPCs injected into the common carotid artery are able to diapedese and migrate specifically to the CCL2 source, this would indicate that CCL2-CCR2 signaling is indeed sufficient to promote this process. Preliminary results suggest that this is indeed the case, with bioluminescence indicating that specific migration of NPCs is indeed possible in the presence of exogenously administered CCL2, even in the absence of stroke. This could shift therapeutic strategies to capitalize on the CCL2-CCR2 interaction, resulting in increased NPC migration to ischemic tissue and improving the likelihood for a positive therapeutic outcome.

CHARACTERIZING THE NO SHOW PATIENT AT LUCILE PACKARD CHILDREN'S HOSPITAL

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For outpatient care, most healthcare systems require patients to schedule appointments in advance. Patients who fail to keep their appointments, without rescheduling or canceling, are considered "No-Show" (NS) patients. This lost opportunity for provider / patient interaction has potentially detrimental effects on the patient, the health care system, and on society. The objective of our study was to determine positive and/or negative predictors for NS patients at Lucile Packard Children's Hospital's outpatient subspecialty clinics. A database of potential predictor variables, as suggested in previously published articles, for NS patients was generated from clinical encounters between the fiscal years of 2005 to 2010 at the 15 subspecialty outpatient clinics (5 surgical and 10 medical) at Lucile Packard Children's Hospital. Using STATA Data Analysis and Statistical Software (College Station, TX), we analyzed the 301,299 outpatient encounters of which 17,024 were no shows. Univariate and multivariate regression analyses were performed to determine the variables that were associated with clinic NS events.

The positive predictors for NS events are government-subsidized insurance, younger age of the patient, medical subspecialty appointments, follow up visits, appointments during summer months, afternoon appointment times, increased traveling distances to the clinic, and requiring a language interpreter. Government-subsidized insurance types (MediCal and CCS), compared to private insurance or self-pay, had increased probability of NS (OR 2.19, 95% CI 2.10 – 2.28, $p<0.0005$ for MediCal; OR 1.56, 95% CI 1.50 – 1.62, $p<0.0005$ for CCS). Compared to patients 21 to 30 years of age, patients <12 years (OR 2.08, 95% CI 1.77 – 2.45, $p<0.0005$) and adolescents 13 to 20 year of age (OR 1.93, 95% CI 1.63 – 2.26, $p<0.0005$) had increased probability of NS. Overall, medical subspecialties (Allergy-Immunology, Cardiology, Dermatology, Gastroenterology, Infectious Diseases, Neurology, Oncology, Pulmonology, Nephrology, Rheumatology) had higher NS rates than the surgical subspecialties (ENT, Neurosurgery, General Surgery, Urology, Orthopedic Surgery) (OR 1.69, 95% CI 1.63 – 1.75, $p<0.0005$).

The most significant predictors of increased clinic NS are government subsidized insurance, younger age of patients, and seeing a non-surgical subspecialist. Future studies should focus on how interventions can reduce the NS rate.

IDENTIFYING INHIBITORS OF NITROGLYCERIN-INDUCED INACTIVATION OF ALDH2

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Nitroglycerin is a potent drug that has been used to effectively treat cardiovascular diseases for over a century. Nitroglycerin is converted into 1,2-glyceryl dinitrate and nitrite by aldehyde dehydrogenase 2 (ALDH2), and ultimately into the vasodilator nitric oxide (NO). Nitroglycerin has wide clinical use because of its strong effect, quick action and limited side-effects. However, the effects of nitroglycerin are reduced after as little as 24 hours of sustained nitroglycerin exposure. This "nitroglycerin tolerance" has been linked to the inactivation of ALDH2 by the products of nitroglycerin bioactivation. Nitroglycerin tolerance represents an unresolved issue in clinical settings, and poses a challenge for long-term patient management.

We previously identified several small molecule activators of ALDH2 activity. To determine whether these compounds also prevented the inactivation of ALDH2 by nitroglycerin, I incubated ALDH2 enzyme with both the compounds and nitroglycerin, and then tested the dehydrogenase enzymatic activity of ALDH2 using an established assay. In order to expand our search, we also synthesized a library of compounds that were chemically modified forms of our original small molecule activators of ALDH2. In total, approximately 300 compounds were tested. Although treatment with nitroglycerin reduced ALDH2 activity by 90%, nine of the tested compounds were able to overcome this reduction and fully restore ALDH2 activity.

Our results suggest that it may be possible to design and synthesize a small molecule that, when given in conjunction with nitroglycerin, will be able to prevent nitroglycerin-induced inactivation of ALDH2. Further studies will assess the effect of these small molecules on other aldehyde dehydrogenase enzymes and will confirm that the compounds do not inhibit the production of nitroglycerin-derived NO. Ultimately, the identification of a small molecule inhibitor of nitroglycerin-induced inactivation of ALDH2 could lead to a novel treatment approach for patients with cardiovascular disease.

MEDICAID FUNDING AND ABORTION COMPLICATION RATES: INSIGHTS FROM LONGITUDINAL DATA ON COMPLICATION RATES IN AMERICA*

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Although states are prohibited from banning abortion, many have states imposed regulations on the procedure, such as restricting Medicaid funding for abortion and imposing mandatory delays prior to undergoing the procedure. Multiple studies have investigated how these rules affect abortion rates, birth rates, and the timing of abortion in gestation. Studies have shown that legal restrictions are associated with higher gestational age at the time of abortion. Clinical studies demonstrate that complications rates of abortion are higher in the second and third trimesters. No studies have investigated the impact of legal restrictions on rates of abortion complications.

We studied the association between the restriction of Medicaid funding on abortion complication rates in 31 states from 1997 to 2008, using data on hospital discharge diagnoses from the Healthcare Cost and Utilization Project. There were 175,291,190 patient discharges during the study period, including 17,766 with abortion complications. The percentage of post-first trimester abortions across all states studied was 10.1% (95%CI 9.45-10.7) and in states without Medicaid funding was 8.82 (95%CI 7.98-9.67). The average abortion complication rate across all states studied was 1.89 (95%CI 1.77-2.01) and in states without Medicaid funding was 1.74 (95%CI 1.57-1.90). The abortion complication rate was significantly lower in states without Medicaid funding($p < 0.003$). Several demographic and socioeconomic characteristics have been shown to potentially affect abortion complication rates. A regression of the effect of funding restriction on the abortion rate controlling for these characteristics found a significant effect (β = -.23, 95%CI -.409- -0.0415, $p < 0.016$).

Over the states and time periods studied, we demonstrated that the absence of Medicaid funding was associated with lower abortion complication rates; however our study is limited as there was a paucity of data that longitudinally spanned the imposition of removal of Medicaid funding in a given state.

*CO-PRESENTED WITH JOSHUA ROLNICK

miR-142-5p As A Novel Biomarker And Mediator of Experimental Autoimmune Encephalomyelitis

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Multiple Sclerosis (MS) is a chronic demyelinating autoimmune condition thought to require the dysregulation of T helper cell responses to the protein components of myelin. Interleukin-17 (IL-17) producing T helper cells (Th$_{17}$ cells) and interferon-gamma (IFNg) producing T helper 1 (Th$_1$ cells) have established roles in the pathophysiology of MS and its mouse model, experimental autoimmune encephalomyelitis. The role of micro-RNA (miRNA) as a biomarker and contributor to T cell phenotype in EAE was investigated.

6-8 week old female SJL/J mice were subcutaneously immunized with a mixture of complete freund's adjuvant (CFA) and proteolipid protein (PLP$^{139-151}$). At days 0, 9, 12, 19, 27, and 35 post immunization spinal cords were isolated from three mice and RNA purified. Agilent miRNA arrays were analyzed using GeneSpringGX 11. Fold change was obtained by dividing the signal intensity from samples with a CFA treated control. Treatments and electroporations were done using locked nucleic acid sequences.

The most highly upregulated miRNA at the peak of EAE on day 12 was miR-142-5p (160-fold). This miRNA was not present in the CSF of healthy animals. Principal component and hierarchical cluster analyses suggest temporal and clinical severity of EAE is observable in global miRNA expression. In vivo knockdown of miR-142-5p by two i.v. treatments of 9mg/kg with anti-miR-142-5p on days 0 and 12 was unable to suppress EAE. However, in vitro electroporation of a miR-142-5p mimic in mouse splenocytes significantly suppressed IFNg under Th$_1$ conditions (alphaCD3, IL-12) but not IL-17 under Th$_{17}$ conditions (alphaCD3, IL-6, TGF-beta). Additional experiments on sorted cells suggested the effect was not via naïve T cells (CD4+CD62L+).

miR-142-5p is a useful biomarker in EAE spinal cords. The role of miR-142-5p in the inflammatory microenvironment may operate by non T-cell mediated regulation of Th$_1$ not Th$_{17}$ responses.

A FEASIBILITY STUDY TO EVALUATE KNOWLEDGE AMONG BREAST CANCER PATIENTS

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Despite the growing emphasis on informed decision-making in patient care, little is known about what breast cancer patients know about their disease and treatment or how best to measure this knowledge. The primary aim of this study was to assess breast cancer patients' knowledge of their cancer diagnosis, clinical and pathologic features, and treatment. A questionnaire was developed to measure patients' knowledge about their breast cancer, perceptions of knowledge, and interest in receiving a personalized breast cancer medical summary. Participants completed an interviewer-administered electronic survey in person or by telephone. Medical records were abstracted to verify participant responses.

Among 38 eligible patients who were approached for the study, 35 (92%) participated and 33 (94%) completed the questionnaire. The median time from diagnosis was 3.2 years (0.2 - 9.2 years). Two-thirds of participants completed the survey in person, one-third by telephone. Survey administration required 11-25 minutes (median, 16.5 minutes) and medical record abstraction required 15-45 minutes (median, 27 minutes) per participant. Most participants thought they were knowledgeable about their breast cancer; still, 70% wanted to know more. Actual knowledge accuracy varied across domains of knowledge. Most participants knew their breast cancer stage (88%); however, fewer knew their type of breast cancer (70%), tumor size (58%), or histologic grade (33%). All correctly reported whether they had surgery, radiation, and chemotherapy, but only 77% knew the names of all chemotherapy drugs, 41% knew the type of radiation, and none knew the dose of chemotherapy or radiation. Nearly all participants (32 of 33) were interested in receiving a breast cancer medical summary.

An interviewer-administered electronic survey is a feasible method to assess breast cancer patients' knowledge of their diagnosis and treatment. Preliminary data suggests that patients undergoing treatment have gaps in their knowledge, particularly related to tumor pathology and details of radiation and chemotherapy. There was a universal desire to receive a breast cancer medical summary. Larger studies are warranted to further characterize breast cancer patients' knowledge gaps, predictors of poor knowledge accuracy, and uses of a comprehensive medical summary.

A Novel Approach To Training: Using High Fidelity Video For Simulation Based Training of Health Care Providers Caring For Pediatric Patients Requiring Mechanical Circulatory Support With the Berlin Excor Device – A Work In Progress

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Ventricular assist devices (VADs) are mechanical devices used to support circulation as a bridge to heart transplantation in critically ill patients. Due to the severity of illness in this patient population, the use of these devices requires rapid decision-making as well as technical competence in their controls. Simulation with extracorporeal membrane oxygenation (ECMO) circuits has been employed with success in improving technical and clinical skills, yet to our knowledge, no equivalent training exists for VADs, which are increasingly the preferred method of support in critically ill children. The aim of this ongoing project is to develop and evaluate a curriculum for a simulation based training environment for health care professionals managing pediatric patients supported with VADs, and assess it in conjunction with the didactic based curriculum currently in place.

With parental consent, we are acquiring photographic and video images of Berlin Excor biventricular assist devices supporting patients at LPCH. We are preparing a learning module based on commonly encountered clinical scenarios as well as a pre and post tests to be used in conjunction with the already existing training for bedside nurses in both the cardiovascular intensive care unit and the inpatient floor. At the time of the next training session on May 19, 2011 we will randomly assign half of the nurses undergoing training to complete the learning module. All nurses undergoing training will complete the pre and post tests as well as a survey about their perception of their preparedness to care for patients on VADs.

In pediatric critical care simulator settings, simulation based training programs are enthusiastically received by trainees and improve trainee clinical performance and technical skills as well as trainee confidence. It stands to reason that this approach will enhance learners' experience by providing more opportunities for active learning and by allowing participants to practice behavioral and technical skills as well as cognitive skills in a high-fidelity, safe environment in order to build competence and confidence in caring for these critically ill patients.

Cingulum Bundle in First-Episode Psychosis

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Background & Objectives: Recent research into the underlying pathophysiology of schizophrenia have suggested that abnormal white matter connectivity may be the principal abnormality underlying the pathogenesis and symptomatology of this debilitating disease (Friston and Frith, 1995). As the major white matter tract connecting the cortex and limbic system, the cingulum bundle (CB) acts as the gateway of information passing between these two complex parts of the brain, incorporating functions of memory, visual processing, reasoning and emotion, and abnormalities in this pathway have been found in patients with chronic schizophrenia. The relationship remains poorly understood, and most data has been collected in chronic schizophrenic patients, where medications and disease development may independently affect white matter integrity (Kubicki, 2007). This study aims to analyze differences in CB connectivity in first-episode psychosis compared with controls and look at correlations between symptomatology, white matter integrity, and outcomes 3 months after diagnosis.

Methods: All individuals in defined catchment areas in South London seen by a medical professional for first episode psychosis were included in the study population. Individuals with organic causes for psychosis were excluded. Controls were selected from the same geographical areas as cases. Using diffusion tensor imaging (DTI) tractography, a method for analyzing MRI data utilizing the diffusion properties of water, CB white matter tracts were dissected in line with virtual *in vivo* dissections described by Catani et al (2002). Fractional anisotropy (FA), axial diffusivity, radial diffusivity, mean diffusivity and trace diffusivity were calculated for these tracts. Repeated-measures ANOVA was used to compare cases and control, with case status as between-subject factor and side (right, left) as within-subject factor. Spearman's correlation coefficient was used to investigate the relationship between DTI data and psychopathological measures in the patient group. Data on outcomes is currently being collected.

Results: To date, baseline data on 39 controls and 35 cases was analyzed to compare differences in DTI metrics as well as correlations between symptomatology and markers of cingulum connectivity. There was no significant difference between cases and controls on key demographic factors. No statistically significant difference was found between FA in patients and controls, although there was a trend towards decreased FA in cases. Axial and trace diffusivity in the right hemisphere were found to be statistically different between the two groups. Of note, these metrics were significantly inversely correlated with positive symptoms scores, utilizing the well-standardized and validated PANSS (Positive and Negative Syndrome Scale) rating criteria.

Conclusion: These results suggest that radial and trace diffusivity contribute to changes in white matter integrity that can be associated with abnormalities characteristic of schizophrenia. This suggests that abnormal myelination is involved in the pathophysiology of schizophrenia. Moreover, these changes correlate with positive symptoms in schizophrenia. Further analysis of longitudinal data will help determine if imaging data using DTI can be used to guide treatment and prognostic evaluations in first-episode psychosis patients.

TB RESPONSES BEFORE AND AFTER TREATMENT OF H. PYLORI

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Tuberculosis (TB) and *H. pylori* are the most common bacterial infections in the world. Past studies have shown lower rates of *H. pylori* infection in people who have active TB versus those who have latent TB suggesting that *H. pylori* may interact with mechanisms to maintain TB in the latent state. Also, persons with latent TB infection were shown to generate a stronger IFN-γ response against TB-specific antigens if they were *H. pylori* co-infected. The relationship between TB and *H. pylori* may be related to the fact that both infections are associated with the Th1 cellular immune response. One aspect of TB and *H. pylori* co-infection that has not been studied is how immune responses to TB are affected by treatment of *H. pylori*. Therefore, this study sought to examine whether IFN-γ levels against TB antigens decrease after treatment of *H. pylori*.

The study was conducted in The Gambia, West Africa. Sixty-three participants were recruited after they had endoscopy and tested positive for *H. pylori* by rapid urease test. IFN-γ response to TB-specific antigens was measured before and two and six months after *H. pylori* treatment was initiated. Preliminary data has shown 30% positivity for latent TB at enrollment by using the Quantiferon IFN-γ assay. At the two months follow-up visit more patients have increased IFN-γ as compared to enrollment. The results of this study may have implications for TB vaccine development.

BARRIERS TO EYE CARE SERVICE UTILIZATION AND POTENTIAL STRATEGIES TO IMPROVE FOLLOW-UP IN PATIENTS WITH GLAUCOMA AND AGE-RELATED MACULAR DEGENERATION*

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Abstract:

Noncompliance with follow-up appointments has been demonstrated in numerous studies of Glaucoma and Age-related Macular Degeneration (AMD), and can lead to irreversible blindness.[1,2,3,4] To better understand barriers to follow-up, we conducted a cross-sectional survey of 119 Glaucoma patients attending Stanford Eye Center and 86 AMD patients at California VitreoRetinal Center between June and November 2009. Eligibility criteria included (1) Glaucoma or AMD (2) follow-up visits for the prior 12 months (3) age >18 years. Using a modified form of the Aravind questionnaire,[3] patients were asked questions concerning demographics, travel, insurance, education, and knowledge of glaucoma or AMD. Patients rated their visual function using the Visual Functioning Questionnaire-25.[5] Finally, patients were surveyed on strategies to improve compliance with follow-up.

The protocol was approved by Stanford University's IRB. Data analysis was completed using SAS Enterprise. Patients were determined to have poor follow-up if they no-showed to any appointment or cancelled at least 3 appointments in the past year. Prevalence percentages were calculated in the primary analysis, and unadjusted OR for poor follow-up were investigated using a univariate logistic regression model.

Forty-eight percent of Glaucoma and fifty-six percent of AMD patients had poor follow-up. They were more likely to be non-whites and lack private insurance. In AMD, poor follow-up patients were more likely to need an escort to the clinic, and were uncertain if AMD requires treatment for life. Compared to good follow-up patients, a greater percentage of glaucoma poor follow-up patients had a high school degree or less, had missed their eyedrops in the past week, were unsure if vision loss from glaucoma was permanent, and felt like they would benefit from knowing other people with glaucoma or an eye condition. While this study was limited in sample size and in demographics, future larger studies are needed especially to better analyze minority populations.

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BARRIERS TO EYE CARE SERVICE UTILIZATION AND POTENTIAL STRATEGIES TO IMPROVE FOLLOW-UP IN PATIENTS WITH DIABETIC RETINOPATHY*

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Abstract:

According to the American Diabetes Association, diabetic retinopathy (DR) has become the leading cause of blindness in America.[1,2] While numerous studies of diabetics have demonstrated poor compliance with annual eye screenings,[3,4,5,6] recent data shows that patients continue to have inconsistent follow-up when diagnosed with DR.[5,7] To better understand barriers to follow-up, we conducted a cross-sectional survey of 33 DR patients attending appointments at California VitreoRetinal Center between June and November 2009. Eligibility criteria included (1) proliferative or nonproliferative DR (2) follow-up visits for the prior 12 months (3) age >18 years. Using a modified form of the Aravind questionnaire,[8] patients were asked questions concerning demographics, travel, insurance, education, and knowledge of DR. Patients rated their visual function using the Visual Functioning Questionnaire-25.[9] Finally, patients were surveyed on strategies to improve compliance with follow-up.

The protocol was approved by Stanford University's IRB. Data analysis was completed using SAS Enterprise. Patients were determined to have poor follow-up if they no-showed to any appointment or cancelled at least 3 appointments in the past year. Prevalence percentages were calculated in the primary analysis, and unadjusted OR for poor follow-up were investigated using a univariate logistic regression model.

Sixty-nine percent of DR patients had poor follow-up. They were more likely to be non-whites, older than 80 years, have a high school degree or less, and have nonproliferative DR. A larger percentage thought their eye disease was less severe. Yet 35% more patients reported need for an escort to the clinic in the poor follow-up group. Thirty-five percent of poor follow-up patients believed they would benefit from knowing other people with DR or an eye condition, compared with 0% of good follow-up patients. While this study was limited in sample size and in demographics, future larger studies are needed especially to better analyze minority populations.

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SALIVARY CORTISOL INCREASES AFTER BARIATRIC SURGERY IN WOMEN

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Background and Methods: Cortisol increases have been associated with psychological and physiological stress; however cortisol dynamics after weight loss (bariatric) surgery have not been defined. Obese participants not using exogenous glucocorticoids were eligible to participate. Female participants (N = 24) provided salivary cortisol samples at bedtime, upon awakening the following morning, and 30 minutes after awakening before and at 6 or 12 months after bariatric surgery. The Medical Outcomes Study Short Form-12 version 2 questionnaire regarding health-related quality of life was also completed.

Results: Preoperatively, mean body mass index was 45.1 ± 8.1 kg/m^2. Mean late night (1.8 ± 1.1 nmol/L), awakening (10.7 ± 7.4 nmol/L), and after-awakening (11.5 ± 7.9 nmol/L) salivary cortisol values were within normal ranges. The cortisol awakening response (mean $21.1\%\pm79.7$, median 13.7%) was at the low end of normal. Preoperatively, participants had lower mental and physical health-related quality of life scores than US adult norms ($P<0.001$). Salivary cortisol was not correlated with measures of health-related quality of life. Mean BMI decreased over time ($P<0.001$) and participants experienced improved physical and mental health-related quality of life ($P\leq0.011$). Postoperative late night salivary cortisol was not different from preoperative values. Awakening and after-awakening cortisol levels were higher than preoperative values (15.3 ± 7.7 nmol/L, $P=0.013$; 17.5 ± 10.2 nmol/L, $P=0.005$; respectively), but the cortisol awakening response was not changed (mean $26.7\pm66.2\%$; median 7.8%).

Conclusions: Morning salivary cortisol increased at long-term follow-up after bariatric surgery. Although self-evaluated mental and physical health improved after surgery, the cortisol awakening response is at the low end of normal, which may indicate continued physiological stress.

MANDATORY DELAYS AND ABORTION COMPLICATION RATES: INSIGHTS FROM LONGITUDINAL DATA ON COMPLICATION RATES IN AMERICA*

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Although states are prohibited from banning abortion, many have states imposed regulations on the procedure, such as restricting Medicaid funding for abortion and imposing mandatory delays prior to undergoing the procedure. Multiple studies have investigated how these rules affect abortion rates, birth rates, and the timing of abortion in gestation. Studies have shown that legal restrictions are associated with higher gestational age at the time of abortion. Clinical studies demonstrate that complications rates of abortion are higher in the second and third trimesters. No studies have investigated the impact of legal restrictions on rates of abortion complications.

We studied the association between the presence of a mandatory 24-hour delay on abortion complication rates in 31 states from 1997 to 2008, using data on hospital discharge diagnoses from the Healthcare Cost and Utilization Project. There were 175,291,190 patient discharges during the study period, including 17,766 with abortion complications. The percentage of post-first trimester abortions across all states studied was 10.1% (95%CI 9.45-10.7) and in states with mandatory delays was 8.54% (95%CI 7.52-9.54). The average abortion complication rate across all states studied was 1.89 (95%CI 1.77-2.01) and in states with mandatory delays was 1.86 (95%CI 1.65-2.09). There was no significant difference in complication rate between states with and without delays. Several demographic and socioeconomic characteristics have been shown to potentially affect abortion complication rates. A regression of the effect of delays on the abortion rate controlling for these characteristics found no significant effect (β = -.033, 95%CI -.227- .150).

Over the states and time periods studied, we did not demonstrate an effect of mandatory delays on abortion complication rates; however our study is limited as there was a paucity of data that longitudinally spanned a the imposition or removal of a mandatory delay law in a given state.

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MULTIMODALITY THERAPY FOR ESOPHAGEAL CANCER – THE BENEFIT OF CHEMORADIATION

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The purpose of this study was to evaluate and compare the survival of patients with esophageal cancer according to treatment modality. The records of 392 patients with esophageal carcinoma treated at Stanford University from 1994-2010 were reviewed. Patients with T1N0 disease, concurrent malignancies, prior radiation to the thorax, or radiation treatment at an outside institution were excluded. Information was collected from medical records and direct patient follow-up. Four separate treatment modalities were analyzed: surgery alone (S), chemoradiation alone (CRT), preoperative chemoradiation followed by surgery (CRT-S), and surgery followed by postoperative chemoradiation (S-CRT). There were 34 (24%) patients in the S group, 59 (41%) in the CRT group, 28 (20%) in the CRT-S group, and 21 (15%) in the S-CRT group. Thirty-three (22%) patients were diagnosed with squamous cell carcinoma, 109 (76%) adenocarcinoma, and 3 (2%) an unknown histology.

The median follow-up among the entire group was 21.3 months (range: 1.4-168.1), and among living patients was 29.8 months (range: 2.7-168.1). The median age at diagnosis was 66 years (range: 36-87). Two-year overall survival (OS) was 50% (median=24.4 months) for the S group, 50% (median=24.3) for the CRT group, 60.5% (median=34.6) for the CRT-S group, and 83% (median=56.7) for the S-CRT group. Patients in the combined CRT-S and S-CRT group had significantly improved OS compared with S (p=0.03) or CRT (p=0.02) alone. There was no significant difference between CRT-S and S-CRT (p=0.21). The Cox proportional hazard model showed that compared to S (hazard = 1.0), the hazard ratios for the other groups were: 1) CRT- 0.56 (p=0.06), 2) CRT-S - 0.43 (p=0.03), and 3) S-CRT- 0.17 (p<0.01).

For locally advanced esophageal carcinoma, combined modality therapy with surgery and chemoradiation improved survival over surgery or chemoradiation alone. No significant difference was seen between preoperative versus postoperative chemoradiation.

Quantitative and Reproducible Murine Model of Frostbite

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Frostbite occurs when exposure to cold results in the freezing of tissue and subsequent ischemia/reperfusion injury, leading to severe tissue loss. The mainstays of treatment to decrease tissue loss and necrosis are limited. Here we describe a novel murine model of frostbite that is reproducible, quantifiable, and accurately mimics human frostbite injury. This model of frostbite will allow insight into the pathophysiology of frostbite as well as other mechanisms of cutaneous injury that involve ischemia/reperfusion, and will provide a reliable animal model for development and evaluation of novel treatment modalities.

Frostbite injury was created on the dorsum of C57Bl/6 mice with two ceramic magnets frozen in dry ice. Both single applications of the magnets and repeated freeze-thaw cycles were assessed. Wound edges were tattooed to assess wound contraction and tissue loss. Digital photographs were taken prior to injury and every other day thereafter until completely healed. H&E histology of the model was conducted 1, 3, and 7 days after injury. Immunohistochemistry quantification of inflammatory cell migration (F4/80 for macrophages, myeloperoxidase for neutrophils) and apoptosis (TUNEL) staining were also determined. This model effectively created frostbite injury, demonstrating full thickness tissue necrosis, tissue edema, and inflammatory leukocyte infiltration. Wound healing was shown to occur primarily through granulation and re-epithelialization. The kinetics of wound healing were assessed by measuring the mean area of the original wound size and by measuring re-epithelialization, wound contraction, and tissue loss. Similar to human frostbite, multiple cycles of freezing and thawing resulted in a higher degree of injury.

This model effectively replicates human frostbite injury, specifically the histologic pathology and molecular mechanisms. We believe that this model will be useful for the study of frostbite and ischemia/reperfusion injury mechanisms, as well as for the evaluation of new therapeutic modalities.