CPG OLIGODEOXYNUCLEOTIDES DIRECTLY SUPPRESS THE GROWTH OF AGGRESSIVE HUMAN B-CELL LYMPHOMAS

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Background: Immunostimulatory CpG oligodeoxynucleotides (CpG ODN) are potent activators of T cell immunity and ADCC, and under study as immunotherapeutic agents for a variety of cancers, including B cell lymphomas. Recently, anti-CD20 antibody-CpG conjugates have been shown to eradicate rituximab-resistant B cell lymphoma in a syngeneic murine lymphoma model (D. Betting et al, ASCO 2009). CpGs strongly stimulate the proliferation of normal B cells. Paradoxically, CpG markedly inhibits the in vitro growth of the murine B-cell lymphoma A20, prompting us to investigate the direct effects of CpGs on the growth of human B cell lymphomas.

Methods: A panel of 12 human lymphoma cell lines (DLBCL, Burkitt's, mantle cell) were cultured in the presence or absence of varying concentrations of CpGs of A, B, or C classes (50, 10, or 2 ug/ml) or control ODN. Proliferation was measured by 3H-thymidine incorporation in quadruplicate 72-hour cultures, and apoptosis measured by annexin V flow cytometry.

Results: CpG ODNs strongly stimulated the proliferation of normal peripheral blood B cells (stimulation index for class B 27.5 at 5 ug/ml). In contrast, the proliferation of all 12 lymphoma lines were inhibited by CpGs. The strongest inhibitory effects were seen with CpG 7909, a class B CpG under clinical development for cancer therapy (Pfizer, PF-3512676). Raji cells were inhibited by 77.9%, 40.7%, and 8.8% at CpG concentrations of 50, 10, and 2 ug/ml, respectively ($p \leq 0.01$ for all comparisons vs. media alone). Among the 12 tested cell lines, the percentage growth inhibition using 50 ug/ml CpG 7909 was 61.2-80.4% for germinal center-type DLBCL (SUDHL-4, SUDHL-6, OCI-Ly19), 50-59.5% for activated B cell-type DLBCL (SUDHL-2, OCI-Ly3, OCI-Ly10), 56.4-79.3% for Burkitt's lymphomas (Raji, Ramos, Daudi, BJAB), and 69.6-69.9% for mantle cell lymphomas (Jeko-1, Granta-519). CpG 7909 also induced significant levels of apoptosis in Raji and Jeko-1 cells.

Conclusions: We report here for the first time on the ability of CpGs to directly inhibit the proliferation of a large panel of human B-cell lymphomas representing the majority of aggressive histologies. These results provide a novel mechanism of action for CpGs as therapeutic agents for B-cell lymphomas.

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CARDIOMYOCYTES UNDERGO DIVISION POSTNATALLY TO GENERATE NEW CARDIOMYOCYTES IN MOUSE 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 neonatal mouse can regenerate its ventricle if the apex is resected within the first week of birth. However, 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)) to identify which cell type(s) generate cardiomyocytes.

We made two triple-transgenic mouse strains: actinCreER;MADMGT/TG and Myh6CreERT2;MADMGT/TG. The former model allows us to identify postnatal cardiogenesis from any cell type, whereas with the latter model we identify postnatal cardiomyocytes that arise from existing alpha-myosin heavy chain-expressing cardiomyocytes. Our studies demonstrate 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, in both models. The rate of generation of cardiomycoytes is very similar in both models, suggesting that the postnatal cardiomyocyte division accounts for all postnatal cardiomyocyte birth. In the injury model, we provide evidence for cell division in the infarcted heart of a myocardial infarction model, but this rate of division is not greater than in the sham animal, suggesting that the inflammatory environment following an MI does not provide additional pro-mitotic signals.

Our data suggests that, as in teleost fish such as zebrafish that demonstrate robust myocardial regeneration even in adulthood, the cell of origin for mammalian postnatal cardiogenesis is a differentiated cell – the cardiomyocyte – rather than a multipotent progenitor cell. It remains to be explored whether all cardiomyocyte are able to divide postnatally or whether this phenomenon is restricted to a subpopulation of cardiomyocytes.

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VITAMIN D DEFICIENCY AND SKIN AGING IN MIDDLE-AGED WHITE WOMEN

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Skin aging is a complex process influenced by genetic and environmental factors. Environmental exposure to ultraviolet radiation (UVR) promotes skin aging and photoprotection (sunscreen, shade use) has been shown to reduce skin aging. However, limiting sun exposure may also reduce vitamin D levels as UVR stimulates vitamin D production in keratinocytes. Because the face is an oft-exposed area and facial skin aging is an easily visible phenotype, we investigated whether individuals with less facial aging due to photoprotection are more likely to have low vitamin D, as measured by 25(OH)D levels.

To investigate the relationship between UV-induced skin photodamage and 25(OH) vitamin D levels, we performed a cross-sectional study in 45 female subjects aged >40. Menopausal status, smoking status, skin cancer history, oral supplement use, and season of blood draw were recorded and serum 25(OH)D measured. A single-blinded, dermatologist evaluated standardized digital facial images for overall photodamage, erythema/telangiectasias, hyperpigmentation, number of lentigines, and wrinkling. Adjusting for age and season of blood collection, women with lower photodamage scores were associated with a 5-fold increased odds of being vitamin D insufficient (OR 5.0, 95% CI: 1.1, 23). Low scores for specific photodamage parameters including erythema/telangiectasias, hyperpigmentation, and wrinkling were also significantly associated with vitamin D insufficiency.

Our results suggest an association between skin aging and 25(OH)D levels. However, given the study design, we cannot establish causality or determine the direction of the observed association. Prospective studies are needed to characterize the relationship between skin aging and vitamin D levels.

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FACTORS ASSOCIATED WITH POOR HEALING IN CHRONIC SKIN ULCERS: AN EXPLORATORY RETROSPECTIVE COHORT STUDY USING THE STRIDE DATABASE SYSTEM

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Chronic wounds such as venous stasis ulcers and diabetic foot ulcers cause a heavy burden of disease in the adult population of the United States. Although risk factors for the development of skin ulcers have been identified, clinical indicators of poor wound healing are less well-studied.

We employed the Stanford Translational Research Integrated Database Environment (STRIDE) system to construct a cohort of 637 patients with chronic skin ulcers based on EMR data from Stanford University Hospital and Clinics. A combination of MySQL queries and manual chart extraction using STRIDE's Data Review Tool was used to extract demographic and clinical patient data. Patients were included who had incident cases of skin ulcer between January 1, 2002 and January 1, 2008 as determined by ICD-9 codes for skin ulcers or wounds. Exclusion criteria included surgical wounds, pressure ulcers, primary cellulitis, mucosal ulcer, malignancy within wound, no documentation of ulcer on chart review. A poor outcome was defined as non-healing ulcer present for greater than 6 months or requiring surgical reconstruction or amputation.

Preliminary data analysis (n=251) shows that 46% of the patients had a poor outcome. Factors significantly associated ($p<0.05$) with poor outcome included age > 85, diabetes mellitus, chronic kidney disease and concurrent malignancy. Patients with poor outcomes tended to be anemic (Hct <32%) and have low albumin (<3 g/dL) but these trends were non-significant. There was no significant gender differential with outcome or association with smoking.

There are limitations of the present study including the use of ICD-9 codes as criteria for inclusion, lack of specific follow-up for study outcome due to nature of EMR, confounding due to observational design, limited power to adjust for multiple factors that influence wound healing. Ongoing work will involve the analysis of greater number of patients and multivariate analysis for subset of predictors.

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THE EFFECT OF UNILATERAL HEARING LOSS ON SPEECH AND LANGUAGE DEVELOPMENT

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Studies in children with unilateral sensorineural hearing loss demonstrate risk for developmental delays, citing increased difficulty in academic settings and poorer scores on language and speech recognition tests. However, little has been studied on the difficulties that children with unilateral conductive hearing loss may face. We evaluated children with canal atresia - a condition in which one ear canal maldevelops and remains closed, resulting in a single-sided hearing loss - to examine the relationship between unilateral conductive hearing loss and speech delay. To further elucidate risk factors for speech delay, we also assessed whether the sidedness of the hearing loss impacts speech development.

Children with a moderate to severe unilateral conductive hearing loss due to canal atresia (N = 60) were included from a database of children from LPCH spanning 19 years. 32% (N = 19) had left-sided canal atresia, and 68% (N = 41) had right-sided canal atresia. Speech and language delay were measured as evidenced by the need and referral for speech therapy. A t-test was performed to assess the relationship between hearing loss sidedness and the outcome of speech delay. We adjusted for confounders in a step-wise manner. All statistical tests were two-sided and a p-value <0.05 was considered significant.

Preliminary results show that 33% of our population with unilateral aural atresia have speech delay. No relationship was seen between hearing loss sidedness and speech delay (p-value = .99), even after adjusting for confounders. Although the sidedness of the hearing loss does not correlate with speech delay, children with unilateral conductive hearing loss are at increased risk for speech and language delays. Future studies include elucidating how other factors, such as insurance status, bilingualism, and socioeconomic level, affect speech delay. Results from this study help define the risks of unilateral hearing loss and delineate standards of care for children at risk for speech delay so that they can receive the proper intervention necessary during a critical developmental time.

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CLASSIFYING AYAHUASCA: THE ROLE OF SUBJECTIVE EXPERIENCE IN PSYCHIATRIC RESEARCH WITH PSYCHEDELICS

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Ayahuasca is a psychedelic brew made from Amazonian plants and which is ritually consumed in the ceremonies of urban religions practiced throughout Brazil, the United States and Europe. Recently, neuropsychiatric studies with ayahuasca have been initiated by a small group of researchers in Brazil. Their research alternatively portrays the modified state of consciousness induced by ayahuasca as psychopathological, psychotherapeutic or spiritual by, respectively, using ayahuasca to model psychosis, to treat depression, and to induce religious visions. Through interviews with the scientists doing this research I develop a case study of how these researchers' own subjective experiences with ayahuasca, as well as the experiences of religious ayahuasca users, shape the researchers' classifications and representations of the ayahuasca experience.

The inclusion of subjective experiences in considerations about the nature of the ayahuasca experience lends itself to establishing a complex understanding of the brew's effects that is often at odds with conventional psychiatric understandings of psychedelic drug's effects, particularly the categorical delimitations between what is considered psychopathological, psychotherapeutic and spiritual. The alternative understanding of ayahuasca's effects that is examined here is shown to result from one psychiatrists' unexpected degree of ontological flexibility; from structural factors like the legality of ritual ayahuasca use; and from novel modes of experimental practice and data representation—all consequences of the researchers' own deliberate use of ayahuasca.

This case study problematizes the relationship between subjective experience and the construction of controversial psychiatric categories—a relationship that in this case has significant medical, social and political consequences not only in Brazil, but now increasingly so in the United States and elsewhere.

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TREATMENT ADHERENCE AMONG HIV/AIDS PATIENTS AT ALERT HOSPITAL IN ETHIOPIA

Hiwot Araya, Ehete Bahiru, Corinna Haberland M.D., M.S., Carol Harris M.D.

HIV/AIDS is the second leading cause of death in Ethiopia next to malaria. Although there were an estimated 1.4 million people in the country who were infected with the virus as of 2009, close to 60% of the population is still without access to anti-retroviral treatment. ALERT hospital provides a longitudinal care, free of charge, to HIV/AIDS patients. No study to date has characterized adherence to treatment in this patient population. Such a study is necessary since there are a range of factors that can influence adherence to treatment, in resource-poor settings like that of ALERT, even when cost is not a factor.

Using a retrospective chart review, we examined general characteristics and self- reported adherence of 276 patients admitted to ALERT in 2008. Of the 276 patients in the study, 37% (n=102) were not on ART despite a mean CD4 count of 128 cells/μl with a range of 1 - 686 cells/μl. 71% of those on treatment (total = 174) reported good adherence. The remaining 29% were non-adherent. We found that chronic diarrhea is significantly associated with poor adherence (P=.009). Age less that 36, male gender, patients without an anemia diagnosis, and patients without a pulmonary TB diagnoses showed non-significant trends in association with poor adherence. We found a higher mortality rate in those who haven't initiated therapy and similar mortality rates between the adherent group and the non-adherent group, suggesting that patients are likely to initiate and take their medications with optimal adherence during the late stages of the disease.

In addition to developing a reliable system to better assess adherence accurately, a strong focus in education and counseling for newly diagnosed patients and those initiating therapy would be beneficial in achieving optimal levels of adherence early on so patients can benefit from the treatment in the long run.

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ANALYSIS OF THE MOST PREVALENT OPPORTUNISTIC INFECTIONS, CAUSES OF MORTALITY AND CHARACTERISTICS OF THE INPATIENT HIV/AIDS PATIENT POPULATION ADMITTED IN 2008

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Background/Methods: Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) is the second leading cause of death in Ethiopia, after Malaria. Although there were an estimated 1.4 million people who were infected with the virus as of 2009, close to 60% of the country's population is still without access to ARV treatment. The purpose of this study was to characterize the study population by stratifying the data by age, gender, and opportunistic infections, and to identify predictors of mortality in the various groups. We reviewed a total of 276 charts of patients who were admitted in 2008 and gathered data both from inpatient and outpatient follow-ups. We characterized the study population based on variables such as age, gender, WHO stage of disease, CD4 count, and number of diagnoses.

Results: We found the top 5 most common prevalent opportunistic infections in the ALERT patient population studied to be Tuberculosis (TB), Central Nervous System (CNS) toxoplasmosis, Cryptococcus meningitis, and pneumonia. In our chi-squared analysis of our dependent variables, age, sex, WHO stage of disease, and ART treatment status we found out that the there was significant association between ART treatment status and mortality rate with a p value of 0.000 while WHO stage of disease and age came close to significance at p-value of 0.067. A univariate linear regression also showed presumed TB to be significantly associated with mortality with the highest odds ratio of 6.25. The mortality rate at ALERT is about 42% and the highest sub-group mortality rate was associated with tuberculosis.

Discussion: The study showed that the opportunistic infection that are most prevalent in the ALERT HIV/AIDS patient population are also the most common opportunistic infections that are associated with the disease both in fully resourced or resource limited settings. There is a general trend of patients presenting mostly at WHO Stage III or IV disease which partly contributes to the high mortality rate associated with the ALERT patient compared to the national mortality rate. The hospital could definitely use better diagnostic techniques and equipment in the attempt to improve patient outcomes. However, there are also areas among other things to improve in terms of initiating or devising ways to catch patients at earlier stages of the disease and investigating why those that qualify for treatment based on their CD4 count are not getting started on ARVs would be helpful in trying to improved the current existing system.

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EVALUATING KETOPROFEN AS PREVENTIVE PHARMACOTHERAPY OF ACQUIRED LYMPHEDEMA

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Acquired lymphedema is a common and disabling state of vascular insufficiency lacking satisfactory pharmacotherapeutics. 400,000 Americans suffer from lymphedema of the upper extremity alone, primarily due to lymph node dissection and radiation therapy secondary to cancer treatment. We are accruing evidence that acquired lymphedema is, at its inception, an inflammatory process and that abrogating inflammation through specific pharmacologic mechanisms may invoke reversal of the architectural tissue pathology previously thought to be irreversible.

Based upon findings of our lab demonstrating the ability of ketoprofen to ameliorate lymphatic vascular insufficiency in a murine tail lymphedema model mimicking human acquired lymphedema (Nakamura et al, PLoS 2009), we are currently conducting phase II studies on human subjects to study the drug in treating secondary lymphedema. Simultaneously, our lab is exploring potential prophylactic properties of ketoprofen in similar mouse models. In addition, we are exploring whether the treatment effect previously noted is ketoprofen-specific or is noted in other therapeutic agents with overlapping characteristics.

Our studies suggest potential for prophylactic therapy for high-risk patients. Treated specimens demonstrated qualitative histological improvement of the tissue and decreased tail volumes compared to controls, similar to the response seen in human lymphedema skin biopsy specimens. Data comparing ketoprofen to ibuprofen, another nonselective COX1/COX2 inhibitor, suggests the treatment effect seen is specific to ketoprofen. Studies with similar agents are planned. The repurposing of a safe and inexpensive drug such as ketoprofen could revolutionize current treatment approaches.

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PROXIMITY LIGATION ASSAY TO DETERMINE SERUM CTGF/CCN2 IN PANCREATIC CANCER PATIENTS PRE-/POST-TREATMENT WITH mAb FG-3019

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Pancreatic cancer is an aggressive disease which suffers from lack of early diagnostic tools and effective treatments, resulting in 5-year survival rates in the 5% range. Recent advancements have implicated a role for a cleavable, multi-modular protein CTGF/CCN2. CTGF/CCN2 mediates *in vitro* anchorage-independent growth in cell culture studies and *in vivo* growth advantage by increasing proliferation rates and decreasing apoptosis in pancreatic cancer xenograft models. Use of FG-3019, a mAb against CTGF/CCN2, in animal models injected with human pancreatic cancer lines that endogenously expresses CTGF/CCN2 has reduced tumor size, and is, in fact, currently in Phase I-II clinical trials. In this study, we investigated if there were any correlation between serum levels of CTGF/CCN2 in pancreatic cancer patients with FG-3019 treatment and survival data.

To further understand the role of CTGF/CCN2 in disease states, we used a technology called Proximity Ligation Assay (PLA), which consists of polyclonal antibodies conjugated to DNA oligonucleotides as probes. When two probes bind to the same antigen in close proximity, the two oligonucleotides are brought in close proximity to permit hybridization to a third oligonucleotide sequence, called a “splint.” The DNA is then ligated, amplified and quantified by RT-qPCR. So far, we have created several domain-specific probes against CTGF/CCN2. The probes generated a linear signal across four orders of magnitude, using cross-species Abs against these probes. However, we experienced difficulties in reliably detecting specific domains across the same concentration gradient, using purified recombinant human CTGF/CCN2. This result can be due either to the lack of epitope availability with the polyclonal probes encountering steric hindrance or the probes have a low binding affinity. To investigate whether epitope availability was an issue, we performed studies where we combined probes for different domains. These combinatorial probes performed well against cross-species controls but encountered the same problem with the recombinant antigen, suggesting an issue with either the binding affinity or the pure antigen itself. To address this issue, we plan to look for other CTGF/CCN2 Abs and/or recombinant antigen.

In addition, we tested the probes on patient plasma pre- and post- multiple anti-CTGF/CCN2 monoclonal antibody treatments and observed a decline in CTGF/CCN2 levels in those with high expression pre-treatment. Preliminary data suggests the need for either a more robust pAb against CTGF/CCN2 or a better recombinant full-length human CTGF/CCN2 protein to achieve better controls. However, the serum results are promising. We anticipate that the results of this study will generate important hypotheses regarding the role of CTGF/CCN2 in pancreatic cancer therapy and will aid in the design of future clinical studies.

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WRINKLES IN REPORTING: A COMPARATIVE STUDY OF STEM CELL NEWSPAPER REPORTING AND POLICY IN FOUR COUNTRIES

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Newspaper coverage of stem cells in different geographic regions of the world produce newspaper articles that vary greatly in balance, subject, and stakeholder. We wish to see if there is any relationship between such variance in coverage and the differing policies that governs stem cell research and treatment. In what kinds of articles is the term "stem cell" used in each region? Are the issues brought up in conjunction with stem cells being addressed by policies set there?

Methods include content analysis of articles from national and regional newspapers and tabloids from the United States, Canada, the United Kingdom, and Australia from January 1st, 2011 to June 30th, 2011 using searches for the term "stem cell" in databases like LexisNexis and Proquest. The newspapers span liberal, mainstream, and conservative political leanings in order to represent a full gamut of views. The coding schemes focus on article tone, subject matter, and stakeholders portrayed in each article and verified through intercoder reliability. Our current results reveal that stem cell newspaper coverage in the UK, Canada, and Australia are more positive than coverage in the US, which tends to have more equivocal coverage.

After we fully analyze the dataset, we plan to look at the regulatory environment of each region and see if the issues brought up in the articles about stem cells are being addressed by these policies. We hope that our analysis will enable us to identify the main issues associated with stem cells, as highlighted by the media, in order to help inform and direct public policy.

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CHARACTERIZING THE ROLE OF THE NOTCH PATHWAY IN HEAD AND NECK SQUAMOUS CELL CARCINOMA PROGRESSION

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Squamous cell carcinoma (SCC) is a malignancy of squamous epithelium and a significant health problem. Because risk for metastasis of head and neck SCC is high, neck dissection of lymph nodes is commonly performed. There is an increasing need to identify molecular markers of prognostic value such that in the future, unnecessary procedures can be avoided. This is an exploratory study to test a candidate gene, Notch, as a marker for SCC progression. Notch's role in cancer biology is dependent on cell context. Conflicting data has emerged regarding the role of Notch in keratinocyte-derived SCC. Murine data suggest that Notch1 loss-of-function increases SCC tumorigenesis. On the other hand, several clinical papers suggest that Notch upregulation is associated with cancer progression.

This research project assesses the hypothesis that tumors with high Notch expression are characterized by more aggressive behavior in the form of lymph node metastasis. This will be performed via immunohistochemistry on a tissue microarray of patient tumors. A retrospective chart review and statistical analyses will be performed. Second, this project evalutes the notion that dysregulation of negative EGFR signaling on the Notch pathway explains heterogeneity in Notch expression levels. Chemical and molecular perturbations of this relationship and subsequent analysis of Notch gene expression will elucidate this possibility. The outcomes of this study have the potential to bear direct impact on clinical practice as well as providing insight into the field of cancer biology at large.

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COMPLICATIONS OF INTERSPINOUS SPACER (X-STOP) VERSUS LAMINECTOMY FOR THE TREATMENT OF LUMBAR STENOSIS: A PROPENSITY SCORE MODEL MATCHED COMPARISON

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Lumbar spinal stenosis is a common cause of back pain, neurogenic claudication, radiculopathy and functional impairment. The X-Stop spacer is an FDA-approved alternative to laminectomy for treatment of stenosis. X-Stop has been shown to be more effective than conservative management. However, no previous studies have directly compared X-Stop to laminectomy using matched cohort analysis.

The Nationwide Inpatient Sample was searched to identify patients who received X-Stop or laminectomy in 2007-2009. In-hospital complications, mortality, cost, and length of stay were compared between two matched cohorts created with propensity score modeling.

The database search identified 1,300 X-Stop patients and 27,676 laminectomy patients. For all patients, X-Stop patients were more likely to be elderly and female. Propensity score matching was used to produce new cohorts that were balanced across treatment groups on these variables. Analysis of the matched cohorts showed that when compared to laminectomy, X-Stop was associated with a lower complication rate (4.27% vs. 10.86%, $p < 0.0001$) and had shorter hospital stays (1.76 vs. 2.72 days, $p < 0.0001$), but incurred greater total costs ($21,562 vs. $10,690 dollars, $p < 0.0001$).

Nationwide, patients who receive X-stop tend to be older. After balancing for age, sex and comorbidity, X-Stop patients were found to have fewer complications and a shorter hospital stay than laminectomy. Further studies are needed to assess the cost-effectiveness of X-Stop, particularly in the inpatient setting.

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ENCEPHALOPATHY IN CANCER PATIENTS RECEIVING CAPECITABINE AS A SINGLE AGENT WITH OR WITHOUT RADIOTHERAPY

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Capecitabine is an orally administered chemotherapy used to treat a variety of cancers including: breast, esophageal, gastric, pancreatic, colon, and rectal cancers. Carboxylesterases in the liver convert capecitabine into its active 5-fluorouracil (5-FU) form, where it acts to inhibit DNA synthesis. Though side effects of capecitabine are relatively mild, a poorly understood but serious side effect of chemotherapy is encephalopathy. Clinically, patients may present with memory loss, subtle personality changes, and an inability to concentrate. Severe encephalopathy has been reported as a rare complication of 5-FU. We propose that for some patients, administration of capecitabine in the context of a partial urea cycle defect may be responsible for hyperammonemia-induced encephalopathy. The condition may be under-diagnosed and more common than previously thought, particularly because capecitabine, unlike intravenous 5-FU, is typically given over a protracted period of time.

Our index patient is a 67 year old female seen by Dr. Gilbert Chu for the treatment of gastric cancer. After the administration of 3 cycles of capecitabine, the patient experienced symptoms of confusion, lethargy, and drowsiness. On the fourth cycle, she ingested a daily dose of 1mg folic acid, which is a co-factor for 5-FU's inhibition of thymidylate synthetase. She then experienced delirium and severe hyperammonemia (>100ng/mL). Genetic analysis revealed that the patient was a carrier for several genes involved in the urea cycle. Our current on-going project follows patients who are receiving capecitabine as a single agent, measuring plasma ammonia levels before and after a cycle of treatment. Verbal Mini-Mental Status Exams (MMSEs) and a self-reported questionnaire assesses mental cognition and correlates ammonia levels with encephalopathy.

Unfortunately 8 out of 8 patients who received single agent capecitabine did not show a significant change in ammonia levels before and after chemotherapy treatment. It is possible that most of these patients, who have received multiple rounds of capecitabine in the past, have already adapted to the effects of the drug or may not be affected by capecitabine at all. The incidence of capecitabine-induced encephalopathy may also be too low for detection in a small population. A concurrent study on the effects of capecitabine-induced encephalopathy in the context of multiple drug combinations have preliminarily shown some increases in ammonia levels following treatment. A larger study will be required to gain a better understanding of the incidence of hyperammonemia in the setting of capecitabine treatment.

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EFFECTS OF TGF- β1 ON MARFAN VASCULAR SMOOTH MUSCLE CELLS REFLECT PATHOPHYSIOLOGY OF EARLY AORTIC ROOT ANEURYSM

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The greatest cause of mortality in Marfan syndrome (MFS) is dissection and rupture of aortic root (AR) aneurysms. While fibrillin-1 mutations result in dysregulation of transforming growth factor-β (TGF-β), it is unclear how excessive TGF-β causes aneurysms. We investigate effects of TGF-β1 on Marfan aortic smooth muscle cells (SMCs), in context of the pathology of AR aneurysms in a mouse model of MFS (*Fbn1*$^{C1039G/+}$). Elastin and immunofluorescence staining were performed on cryosections of 2, 4 week old Marfan and wild type (WT) aorta. SMCs, from 4 week old Marfan and WT aorta, were treated with TGF-β1 for 48 hours. Activity of matrix metalloproteinases (MMPs) secreted by SMCs were measured with zymography. Cell lysate proteins were used to assess apoptosis.

We previously showed aortic dilation beginning at 2 weeks in Marfan mice. Here we observe elastin fragmentation at 2 weeks localized only to the Marfan AR. *In vitro* TGF- β1 treatment increases MMP-2, -9 activity in the media of Marfan SMCs 7.08±0.05 fold (p=0.00128) and 6.73±0.15 fold (p=0.0015), respectively, over that of WT SMCs. These *in vitro* results are in line with *in vivo* data showing increased MMP -2,-9 activity and gene expression in the AR of young Marfan mice. We also demonstrate increased apoptotic SMCs in the AR of 2, 4 week old Marfan mice. *In vitro*, TGF- β1 treatment increases caspase-3 activity in Marfan SMCs 12.51±0.17 fold (p=0.0001) over WT SMCs.

We demonstrate for the first time TGF-β1 increases MMP activity and increases apoptosis in SMCs from young Marfan aorta. This novel finding potentially explains the pathology we observe in the AR of young Marfan mice, namely increased elastin fragmentation and SMC apoptosis at 2 and 4 weeks. Therefore, more research is needed to elucidate the role of SMC in aneurysm development in MFS.

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INTEREST AND CONCERNS REGARDING PRE-IMPLANTATION GENETIC DIAGNOSIS IN FEMALE BRCA1/2 MUTATION CARRIERS

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BRCA1/2 carriers may face difficult decisions about their treatment and reproductive options. In addition to their increased risk of breast and ovarian cancer, they have a 50% chance of transmitting this mutation to their children. In a previous study, BRCA1/2 carriers were surveyed about contraception, prophylactic surgery, and family planning choices. Although 88% of respondents were frequently or extremely worried about passing on the BRCA1/2 mutation to their children, only 13% said they would likely consider using pre-implantation genetic diagnosis (PGD). The objective of this current study was to explore BRCA1/2 carriers' understanding and concerns about PGD.

A web-based questionnaire was used to ask BRCA1/2 carriers about their knowledge and potential use of PGD. We collaborated with an advocacy group for hereditary breast and ovarian cancer, Facing Our Risk of Cancer Empowered (FORCE). Of survey respondents intending to have more children (49 of 77), 32% were not interested in PGD, 29% said they might be interested but needed more information, and 30% were definitely interested. Twenty-six percent of respondents reported knowing a great deal about PGD, 36% said they knew only some, and 38% reported knowing nothing. Cost of PGD procedure was the highest reported concern, followed by uncertainty that the procedure would result in pregnancy.

Most respondents showed some interest in PGD, but 74% reported limited or no knowledge about PGD. Our study indicates that many BRCA1/2 carriers lack information about PGD, and education about this option should be incorporated into their treatment and reproductive counseling.

SYSTEMATIC ASSESSMENT OF ADVERSE EVENTS ASSOCIATED WITH OFF-LABEL USE OF HUMAN RECOMBINANT FACTOR VIIA

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Objectives/Methods: Human recombinant factor VIIa (rFVIIa; NovoSeven, Novo Nordisk, Bagsvaerd, Denmark) is US Food and Drug Administration (FDA) approved for the prevention and treatment of bleeding episodes in patients with hemophilia and inhibitory allo-antibodies. In recent years, rFVIIa has been increasingly utilized for many diverse off-label uses including prevention of intracranial bleeding and hemorrhagic shock from trauma. The patient populations that now receive rFVIIa differ significantly from the initial clinical trial populations for which rFVIIa was approved. Currently, information is limited on the extent of harm associated with off-label usage for intracranial hemorrhage and brain and body trauma. To identify relevant randomized clinical trials (RCTs) and observational studies on rFVIIa, we searched: PubMed, Cochrane Database of Systematic Reviews, ACP Journal Club, DARE, CCTR, CMR, HTA, NHSEED, EMBASE and BIOSIS, as well as sources of grey literature, through August 4, 2009. We collected non-comparative harms data from registries and cohorts. The Premier database was also analyzed to determine trends and patterns of rFVIIa use for off-label indications in-hospital.

Results: 28 studies met our inclusion criteria. We focused our assessment on mortality and thromboembolic events as key direct outcomes. Across clinical indications, the mortality rate and thromboembolic event rate ranged widely depending on the clinical indication for use of rFVIIa, age, dose, and study design. For intracranial hemorrhage mortality ranged from 0 to 0.43 and total thromboembolic events ranged from 0 to 0.33. For body trauma, rates for mortality ranged from 0.07 to 0.58 and thromboembolism ranged from 0 to 0.12. Across both indications, mortality rates among patients in the Premier database were uniformly higher than the mortality rates in the RCTs.

Conclusions: The trend toward higher mortality rates in the Premier database emphasizes that patients receiving rFVIIa in practice may differ in important ways from those in the included trials. Such distinctions may alter the risk-benefit profile of rFVIIa administration for real-world populations.

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NANOMEDICINE AT THE FOREFRONT OF MEDICAL DIAGNOSTICS

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Nanotechnology-based biosensors are well suited for rapid and sensitive protein detection due to their ability to transduce molecular events into signals measurable by macroscopic instrumentation. Translation of nanosensor applications from the research setting to clinical use, however, has been limited by the inherent heterogeneity of biological samples. In the research setting, experimental samples are often controlled to minimize the "matrix effect" whereby pH, ionic strength, temperature, and autofluorescence often distort the desired signal or add background noise.

In this work, we present a magnetic nanosensing platform capable of exceptionally sensitive multiplex protein detection that is not limited by this matrix effect. Even the most complex biological samples lack a magnetic background signal and do not interfere with the magnetic transduction mechanism. Accordingly, a magnetic field-based detection platform is ideally suited for exceptionally sensitive protein detection in diverse clinical samples. With our technology, we demonstrated the ability to detect on the order of 100 unique proteins simultaneously at concentrations down to the femtomolar (10^{-15}) regime with equally quantifiable detection in phosphate buffers, serum, cell lysates, urine, and saliva samples. We have applied our technology to multiplex cancer diagnostics and sensitive tumor marker profiling that is orders of magnitude more sensitive than the gold standard protein detection platform. In addition, we have miniaturized the detection platform into a hand-held device and developed a novel autoassembly immunoassay capable of point-of-care diagnostics in a cost-effective and easy-to-use (e.g. wash-free) process that can make a significant contribution to global health.

In conclusion, arrays of magnetically responsive nanosensors offer great promise in diverse applications such as medical diagnostics, response to therapy monitoring and global health.

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THE EFFECT OF MINDFULNESS-BASED STRESS REDUCTION ON ANGINAL SYMPTOMS AND VASCULAR FUNCTION IN WOMEN WITH NON-OBSTRUCTIVE CORONARY ARTERY DISEASE

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Introduction: The prevalence of angina is higher among women than men, but women are less likely to have obstructive coronary artery disease on angiography. This phenomenon of non-obstructive coronary artery disease (NOCAD), often secondary to a vascular function abnormality, such as endothelial dysfunction, can result in angina associated with physical exertion or emotional stress. In this clinical trial, we tested the hypothesis that mindfulness-based stress reduction (MBSR) training would reduce anginal symptoms and improve vascular function in women with NOCAD.

Methods: We conducted a randomized, non-blinded, cross-over pilot study. Nine patients were randomized to 8 weeks of treatment (5 patients, MBSR + usual care) vs. waitlist-control (4 patients, usual care alone). At baseline, and after each 8 week period of MBSR training or waitlist, subjects underwent testing of vascular function (brachial artery flow-mediated dilation, peripheral arterial tonometry); physiologic stress (high-sensitivity C-reactive protein, interleukin-6); self-reported physical symptoms (Seattle Angina Questionnaire, SF-36); and self-reported mental health (Perceived Stress Scale, Beck Depression Inventory-II, State-Trait Anxiety Inventory, Social Support Scale); as well as a structured psychiatric diagnostic interview.

Results: Compared with usual care, MBSR resulted in reduced anginal frequency ($p=0.06$), and positive trends in physical limitations due to angina ($p=0.02$), self-reported general health ($p=0.035$), self-esteem ($p=0.007$), stress ($p=0.03$), anxiety ($p=0.3$), and mindful describing ($p=0.02$). On psychiatric interview, there was resolution of numerous anxiety-related conditions, including generalized anxiety disorder, complicated bereavement, and hypochondriasis. There were no significant changes in markers of vascular function or physiologic stress.

Conclusions: In women with NOCAD and angina associated with emotional stress, MBSR training results in a significant reduction in angina, and positive trends in physical and psychological disability. These results are based on interim pilot data and enrollment continues. Future research will investigate the effect of MBSR on the broader population of patients with angina.

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VALIDATION OF VIRTUAL REALITY SURGICAL SIMULATION

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The time spent in the operating room per trainee is decreasing for many reasons including work hour restrictions, an increase in specialization, and the ethics of medical education with patients. Trainees now have to look to other places to improve their skills. Simulation has repeatedly been suggested as the answers to this problem but its acceptance has been slow. Virtual Reality (VR) simulation has been shown to improve skills and time efficacy in the operating room on the first surgery after training. But what happens on the second or twentieth surgery? How long does the difference last? The goal of this study is to test the hypothesis that surgeons trained on VR simulators have a shorter learning curve in real procedures than those using a traditional training approach.

A prospective randomized control trial is being conducted comparing residents trained with a VR simulation curriculum versus no extra training. After the training period, they will be assessed in the OR on the length of the surgery, the quality of the procedure, as well as clinical outcomes of the patients. At this time 24 out of 30 residents have been recruited. The basic demographic information of the control and intervention group is similar with the simulation group tending to be younger. The learning curve constructed from the first 17 surgeries with time as the outcome fits a logarithmic curve.

The study is currently still recruiting residents and collecting data on laparoscopic cholecystectomies. The learning curve will be assessed using non-linear regression and implications about simulation effectiveness can be made. Using the initial information from the randomized trial, a back of the envelope cost assessment of the simulation training will be done. If the data suggest it, a further true cost-effectiveness study would be undertaken.

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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 α3 chain is a domain whose function, both in normal tissue and carcinomas, is not yet known. This project investigates domain IIIa of laminin-332 α3 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 α3 domain IIIa. This construct has been produced by PCR using full length human cDNAs coding for human laminin α3 chain as template. In order to prepare the antibody, laminin α3 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 α3 chain domain IIIa deletions (ΔIIIa cells), we will be doing the following. We recently produced a mutant cDNA construct coding for laminin α3with a deletion of the laminin α3 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 α3 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.

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RADIOGRAPHIC OUTCOMES OF AVM EMBOLIZATION PRIOR TO STEREOTACTIC RADIOSURGERY USING 3D VOLUMETRIC DIGITAL SUBTRACTION ANGIOGRAPHY AND CYBERKNIFE PLANNING SYSTEM

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Brain AVMs (AVMs) are direct communication of arteries to abnormally tortuous and dilated veins without interposing capillaries. Management strategies include single or combined therapy with microsurgery, endovascular embolization, or stereotactic radiosurgery (SRS). Current indications for embolization are divided into presurgical embolization in large cortical AVMs and embolization prior to SRS to reduce AVM nidus size. Controversy continues to exist over the true reduction in AVM nidus induced by pre-SRS embolization. The current project quantified the benefit provided by AVM embolization prior to Cyberknife (CK) SRS in terms of volumetric reduction.

All patients were treated according to an approved IRB protocol at Stanford. Angiograms and endovascular embolization procedures were performed by the cerebrovascular/interventional neuroradiology team. CK planning contouring was conducted by neurosurgeons. Data concerning treated nidus, radiation dose, and volume were stored for project analysis.

During the project's duration, we *prospectively* recruited 11 patients, nine of which received further analysis. Most patients presented with clinical symptoms consistent with the location of a ruptured AVM, such as hemi-paresis, headache, and seizure. Seven of the eleven AVMs were localized to the left hemisphere, seven were located in eloquent parenchymal tissue, and the average nidus size was 3.1 ± 1.4 cm. One lesion received a Spetzler-Martin (SM) grade of one, four lesions were SM grade two, four lesions were SM grade three, one lesion was SM four, and one lesion was SM five. Preliminary data is available regarding the volumetric reduction achieved by pre-SRS embolization using 3-Dimensional DSA combined with the Cyberknife Surgical Planning System. Of the volumes available, it was demonstrated that pre-SRS embolization resulted in a 60 – 72 % reduction in AVM volume.

Further studies could investigate the *clinical outcome* compared to those AVMs that do not undergo pre-SRS embolization. While it is well known that smaller AVMs obtain superior clinical outcomes, future studies could examine whether embolization will reduce large AVM volumes to replicate the more impressive clinical outcomes of smaller AVMs.

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EVALUATING THE EFFECTS OF A NEW MODE OF MECHANICAL VENTILATION, AIRWAY PRESSURE RESEARCH VENTILATION, IN CHILDREN WITH ACUTE RESPIRATORY FAILURE

Swetha Kambhampati, Solomon Messing, Tracey Roberts, Jason Arimura, Julie Williamson, Saraswati Kache

Background: Acute hypoxemic respiratory failure is a significant problem in the pediatric population, with an overall morality rate of 43% and a mortality rate of 51% in those patients diagnosed with ARDS. Although Airway Pressure Release Ventilation (APRV) has been used for many years in adults with acute respiratory distress syndrome (ARDS), there is little in the literature to inform its growing use in children. It is hypothesized that in ARDS, APRV allows improvement in oxygenation consistent with a lung protective strategy of ventilation and does not cause adverse cardiovascular and renal effects. This retrospective case review surveys the safety and utility of this mode in children in ARDS ventilated with the APRV mode for greater than 24 hours between April 2010 and November 2011.

Methods This pilot APRV study was conducted at the Lucille Packard's Children Hospital at Stanford. Any pediatric patient (under age 18) who was placed on the mode for greater than 24 hours and was not placed on ECMO was studied. The primary measures assessed were the respiratory parameters PaO2/FiO2 ratio and the Oxygenation Index (OI) (measured at 24 and 2 hours prior to and after transition to APRV mode). The secondary measures studied were cardiac parameters (one hour prior to and after conversion to APRV mode), renal parameters (24 hours prior to and after conversion), and sedation dosing requirements (48 hours prior to and after conversion). A paired t-test was used to compare parameters over time and a mixed linear model with a random effect was used to test for significant differences over time for the respiratory paramters.

Results The respiratory parameters P/F ratio and Oxygenation Index have improved after conversion to APRV. The P/F ratio increased by a mean difference of 28.490 (CI 3.44 to 58.51) indicating an improvement in ventilation. While the mean difference of 3.569 (CI -3.78 to 9.62) in Oxygenation Index appears as if the OI has increased upon conversion to APRV, tracking the change of OI shows a steady decline upon conversion to APRV. The systolic blood pressure, diastolic blood pressure, mean arterial pressure, renal function (creatinine and BUN), urine output, and level of analgesia and sedation all remained stable.

Conclusion The rise in P/F indicates an improvement in oxygenation and ventilation in critically ill children changed to the APRV mode of ventilation. The drop in Oxygenation Index upon switching to APRV indicates a decline in the amount of ventilatory support needed to achieve the same level of oxygenation. The cardiac and renal parameters indicate stable hemodynamics upon conversion to APRV with absence of hypovelemia or acute renal failure. The total analgesia-sedation doses also decreased when transitioned to APRV. This retrospective study of APRV demonstrates safety and efficacy in a small pilot population of children with the acute respiratory distress syndrome. A prospective randomized controlled study will need to be performed to assess the efficacy of this mode of ventilation to improve the outcomes of critically ill children.

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AUTOMATED PERFUSION IMAGING FOR THE EVALUATION OF TRANSIENT ISCHEMIC ATTACK

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Transient ischemic attacks (TIA) affect 250,000 patients per year, and over 10,000 will experience a stroke within a few days. Urgent management appears to reduce the risk of further stroke by up to 80%, although this requires an accurate diagnosis of TIA. Diffusion weighted imaging (DWI) is recommended for the evaluation of transient ischemic attack (TIA) and perfusion imaging may increase the yield of MRI in TIA. We evaluated automated bolus perfusion (TMax and MTT) and arterial spin labeling (ASL) sequences for the detection of ischemic lesions in TIA patients. This is the first report of ASL for use in TIA.

We enrolled consecutive patients evaluated for suspicion of acute TIA by multimodal MRI within 36 hrs of symptom onset. Two independent raters assessed the presence and location of ischemic lesions blinded to the clinical presentation. The prevalence of ischemic lesions and the inter-rater agreement were assessed. From January 2010 to 2011, 93 patients were enrolled and 90 underwent perfusion imaging (69 bolus perfusion and 76 ASL). Overall, 25/93 patients (27%) were DWI positive, and 14 (15%) were perfusion positive but DWI negative (ASL n=9; TMax n=9; MTT n=2). MTT revealed an ischemic lesion in fewer patients than TMax (7 vs. 20, p=0.004). Raters agreed on 89% of DWI cases, 89% of TMax, 87% of MTT, and 90% of ASL cases. The inter-rater agreement was good for DWI, TMax, and ASL (κ=0.73, 0.72 & 0.74 respectively), and fair for MTT (κ=0.43). Diffusion and/or perfusion were positive in 39/69 (57%) patients with a discharge diagnosis of possible ischemic event.

Our results suggest that in patients referred for suspicion of TIA, automated TMax is more sensitive than MTT, and both ASL and TMax increase the yield of MRI for the detection of ischemic lesions.

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INTRACEREBRAL HEMORRHAGE-ASSOCAITED ISCHEMIA MAY BE CAUSED BY BLOOD PRESSURE LOWERING

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Optimal blood pressure (BP) control following intracerebral hemorrhage (ICH) remains controversial. Blood pressure lowering may limit hematoma expansion, but may also cause hypoperfusion and ischemia. We investigated the relationship between blood pressure (BP) lowering in the first 24 hours and the presence of diffusion weighted imaging (DWI) lesions on MRI.

We prospectively enrolled consecutive patients presenting with an acute spontaneous ICH. Brain MRIs were obtained at 37 hours (IQR 25-75) after symptom onset and reviewed for the presence of ischemia in the hemisphere ipsilateral to the hematoma. Blood pressures were recorded on admission, and at 6, 12, 18, and 24 hours thereafter. Of 160 patients, 136 met inclusion criteria; age: 63±17 years; hematoma volume: 10 mL (IQR 4-33); and admission NIHSS: 6 (IQR 2-16). DWI lesions were detected in 50% of patients. Patients with DWI lesions had larger hematoma volumes (32 vs. 12mL, $p < 0.001$); higher admission mean arterial pressures (MAP) (125 vs. 113mmHg, $p=0.006$); and greater average MAP reductions (25 vs. 17%, $p=0.006$). The risk of DWI lesions increased with the percent of MAP reduction in a dose dependent fashion. After controlling for ICH volume using logistic regression, each 10% reduction in MAP increased the risk of the presence of DWI lesions (OR 1.3, 95% CI 1.01-1.69). Patients with >30mmHg of MAP reduction had the highest risk of DWI lesions (OR 2.3, 95% CI 1.09-4.6). In patients with ICH <10mL (N=70), DWI lesions were not associated with ICH volume (4.1 vs. 4.8mL, $p=0.40$) but were associated with: maximum MAP reduction (45 vs. 31 mmHg, $p=0.03$); and maximum percent MAP reduction (34 vs. 25%, $p=0.03$)

Ischemic brain lesions in patients with spontaneous ICH are very common and associated with hematoma volume and blood pressure lowering. Aggressive BP lowering may contribute to ICH associated ischemic lesions and needs further study.

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SEQ-ING GENETIC SIGNATURES THAT PRODUCE AUTISM: A TRANSCRIPTOME ANALYSIS 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 differentiated induced pluripotent stem cells (iPSC) from patients with Phelan- McDermid syndrome and from unaffected controls into cortical neurons. We then applied whole transcriptome shotgun sequencing or RNA-seq analyses to examine the transcriptional signatures and cellular phenotypes associated with Phelan-McDermid syndrome. In addition, we used single-cell PCR arrays to study alterations in the subpopulations of neurons produced from IPSC derived from patients. The power of this approach is that it allows us to examine transcriptional differences in large groups of neurons via RNA-seq and then further localize them to neuronal sub-populations via single-cell PCR.

Fibroblasts were previously reprogrammed into iPSC through infection with retroviruses expressing Sox2, Oct3/4, Klf4, and c-Myc. We 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 43 of neural differentiation, we harvested RNA and single cells and performed RNA-seq analyses. The single cell PCR data from 216 genes in hundreds of cells has been analyzed using Matlab. I will present key differences in gene expression apparent between human neurons with Phelan-McDermid syndrome versus control and transcriptional alterations found in neuronal subpopulations expressing Shank3, a gene hypothesized to account for some of the neuropsychiatric features of PM.

This approach represents an unbiased means of identifying putative therapeutic targets, neuronal subpopulations, and key molecular pathways involved in PM, which may be indicative of more general mechanisms that lead to autism. The ultimate goal of this work is to use genetic signatures to develop cell based screens to discover treatments for Phelan-McDermid syndrome and perhaps for autism.

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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, WHO and COG response criteria.

Materials and Methods
We retrospectively analyzed MR scans of sixty-nine patients with EFST who were treated at Stanford, UCSF and Hopital Timone Medical Centers. Tumor size was assessed on T2-weighted sequences and postcontrast T1-weighted sequences before, during and after therapy. Two readers independently made tumor measurements: a medical student and board-certified radiologist. Tumor measurements were obtained according to the RECIST 1.1 (longest single diameter), WHO (byproduct of the longest perpendicular diameters) and COG criteria (cubic volume). All three guidelines share the same four response categories: progressive disease (>20% increase RECIST/COG; > 25% WHO), stable disease (neither PR nor PD), partial response (< 30% decrease RECIST/COG; <50% decrease WHO) and complete response (100% decrease). Concordance between systems was assessed using Cohen's kappa (κ) coefficient.

Results
The κ statistic for concordance between the two readers was 0.775, indicating "good" agreement. Kappa for RECIST/COG and V/COG were 0.375 and 0.845 respectively.

Conclusions
This study demonstrates a fair level of agreement, by the κ statistic, between the RECIST 1.1 and COG response criteria, strong agreement between cubic volume and effective volume measurements 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.

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MATERNAL GLUCOSE RESPONSE TO BETAMETHASONE ADMINISTRATION

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Antenatal corticosteroids are routinely given to women who are at risk for preterm birth secondary to the well-established neonatal benefit. While the effect of antenatal corticosteroids on maternal glucose control has been investigated by others using glucose testing at various time points after steroid administration, few studies have fully characterized the maternal glucose response. Therefore, we designed a prospective observational trial to better describe the pattern of maternal glucose response to betamethasone (BMZ) administration using a continuous glucose monitoring system.

The study was conducted among women receiving clinically indicated BMZ between 24 and 34 weeks of pregnancy. Data was available for 15 patients (11 non-diabetic and 4 diabetic women), and was compared with data from five additional non-diabetic women who did not receive BMZ (controls). At the time of initial BMZ administration, a Dexcom continuous glucose monitoring system was inserted which monitored tissue glucose levels every five minutes for up to 7 days or time of hospital discharge or delivery. Both diabetic and non-diabetic women had the highest recorded blood glucose readings between 24-48 hours after the first injection of BMZ. In that period, non-diabetic women spent 73%, 40%, and 17% of the time with blood glucose levels above the 110, 144, and 180 mg/dL thresholds, respectively. These thresholds were chosen as they have been associated with neonatal hypoglycemia in women in labor. During a 24-hour monitoring period, controls spent 27%, 9%, and 0.2% above the same thresholds. At each glucose threshold, the difference between non-diabetics who did and did not receive BMZ was statistically significant (P=0.003, P=0.03, P=0.01, respectively).

We observed significant elevations in maternal glucose levels among non-diabetic women receiving BMZ. While it is not clear that a short period of hyperglycemia has any long-term impact on women or newborns, for those women who are progressing in preterm labor, this trend may significantly impact neonatal care and the risk of neonatal hypoglycemia. The observations in this study suggest that non-diabetic women likely to delivery within 72 hours of receiving betamethasone may warrant glucose monitoring in labor similar to protocols used to monitor diabetic women in labor.

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ROLE OF MACROPHAGES AND CD47 IN THE REGULATION OF RADIATION INJURY

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Radiotherapy is an important treatment for a number of malignancies, but applications are limited by its toxicity to normal tissues. Radiation injury is modulated in part by the resulting inflammatory response, and it has recently been shown that macrophages may regulate the radiation sensitivity and response of healthy and malignant tissues. Moreover, others have shown that the phagocyte regulatory molecule CD47 modulates radiation responses, as CD47-/- knockout mice are resistant to partial body irradiation.

We studied the role of macrophages in the response of healthy tissues to radiation injury. CD47-/- mice which received partial body (hind limb) radiation had delayed loss of hair and skin injury, decreased overall injury, as well as more rapid recovery than WT controls. However, in preliminary experiments CD47-/- mice receiving total body irradiation had accelerated mortality compared with control mice.

Depletion of phagocytes *in vivo* with liposomal clodronate (a bisphosphonate) resulted in both earlier and more severe damage in our partial body irradiation models. These animals also showed systemic signs of injury, including increased kyphosis and decreased activity. We characterized the importance of phagocytes in the peri-radiation period by treating with clodronate beginning 5 to 7 days before irradiation, beginning on day of irradiation, or beginning day 2 after radiation. In these experiments, we found that the sensitizing effect remains partially intact even when clodronate administration is delayed.

In additional experiments, we have shown that after single dose lethal total body irradiation, treatment with clodronate accelerates mortality (median survival of 7 vs. 12 days). This may suggest that macrophage depletion via clodronate causes impaired survival duration after radiation injury.

We conclude that macrophages and CD47 may play a role in response to radiation injury, potentially in an organ-specific manner. Thus, CD47 and macrophages may represent potential candidates for modulating the response to irradiation in healthy and malignant tissues.

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REGULATION OF DEVELOPMENT AND TUMORIGENESIS BY THE NFI FAMILY OF TRANSCRIPTION FACTORS: CONVERGENCE WITH HEDGEHOG SIGNALING

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Medulloblastoma (MB) is the most frequent pediatric brain cancer and little improvement has been made in its treatment. The disease is often fatal, and for the survivors the long term sequelae can be severely debilitating. A prime cause of MB is genetic damage to the Hedgehog (Hh) signaling pathway. Defects in Hh signaling have also been implicated in other cancers. The role of the Hh pathway in MB reflects its normal role in development. Sonic hedgehog (SHH) ligand is a potent mitogen for granule precursor cells (GNPs) in the developing cerebellum, but this stimulating effect is restrained by negative regulatory components in the Hh pathway. Mutations that reduce those restraints lead to MB. GLI transcription factors are regulated by SHH signaling in many tissues and organs, including the proliferation of GNPs in the developing cerebellum and during the growth of MBs. This raises an important question: how changes in GLI actions contribute to the difference between normal GNP development and MB growth.

Recent research in the Scott lab has examined GLI1 binding to chromosomes in normally developing GNPs, and in MBs. In studying the DNA sequences associated with GLI factors, they found frequent nearby binding sites for a family of transcription factors called Nuclear factor I (NFI) proteins. NFI proteins are therefore candidates for cofactors that work together with GLI proteins to control transcription in response to Hh signals. NFI proteins are required for the development of many organs and tissues, including the cerebellum. In order to further explore the role of NFI in GLI1 transcription regulation, we have used cell lines to measure the ability of the four NFI proteins to control gene expression through enhancer motifs upon which GLI1 also acts. We found that NFIB is an activator while NFIX is a repressor when cotransfected with GLI1. NFI does not bind GLI1 directly in co-immunoprecipitation experiments, so the two proteins act in parallel but separately. The possible role of NFI proteins in cancer is supported by the recent discovery of mutations in NFIB in MBs. We have tested two mutant forms and found that they may increase luciferase activity even further.

To further examine the role of NFIB in Hedgehog-associated MB tumorigenesis, we will investigate several enhancers associated with SHH signaling and perform functional assays on stable clones with NFIB mutants. We will use ChIP-seq analysis as well as verification with ChIP-qPCR to assess the occupation of the enhancers by GLI and NFIB transcription factors in vivo. Discovery of cofactors that modulate the actions of GLI proteins in development and cancer will make provide insights into target gene selection and control. The work will generate new candidates for targeted therapy and help us better understand MB and other diseases associated with SHH deregulation.

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Nuclease Enhanced Homologous Recombination in Human ES Cells for a Gene Therapy Approach to Sickle Cell Disease

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Sickle cell disease (SCD) is an autosomal recessive disorder caused by a point mutation in the beta-globin gene that results in abnormal hemoglobin polymerization. Previous research has demonstrated the viability of combining embryonic stem (ES) cells and gene therapy to cure SCD in mice. Our objective was to translate these methods into the human system in order to (1) create an efficient system utilizing nuclease enhanced homologous recombination for gene correction of the B-globin mutation in hES/iPS cells and (2) produce novel hES reporter cell lines to study definitive erythropoiesis.

Our research team constructed three targeting vectors (TVs). Two were designed with homology to the B-globin gene locus containing either a GFP reporter insert or the Wild-Type B-globin cDNA with a P140MGMT selection marker. The last TV was designed against the gamma-globin locus containing a TdTomato reporter insert with a Neomycin-Resistance selection marker. Next, we constructed Zinc Finger Nuclease (ZFN) and TALEN pairs that targeted gene sites near the ATG start codon of both the beta-globin and gamma-globin genes. Using the Cel-I assay in K562 cells, we identified two nuclease pairs that efficiently initiated double stranded breaks at the intended target sites, including L4.2/R4.2 (23.9% cut) against beta-globin and L2/R2 (35.0% cut) against gamma-globin. Finally, we nucleofected these reagants into H9 ES cells and achieved efficient targeting of the gamma globin locus with 66% of Neomycin-selected clones containing the TdTomato insert. We have not yet successfully targeted the beta-globin locus.

Our study has produced nucleases that effectively initiate double stranded breaks at the beta globin and gamma globin locuses. Utilizing these tools, we have produced one of the first transgenic hES cell lines ever created. Improving the efficiency of the beta-globin targeting will allow us to create additional cell lines containing either single or dual reporter systems for beta-globin and gamma-globin expression. These lines could be utilized for further research including the study of definitive erythropoiesis from hES cells and high-throughput drug screening to identify novel pharmaceutical compounds for treating SCD patients.

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HEALTH AND HEALING IN A POLYGAMIST COMMUNITY OF SOUTEHRN UTAH

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Short Creek is a largely closed and isolated community on the border between Utah and Arizona, made up of the sister towns of Hildale, Utah, and Colorado City, Arizona. Beginning from childhood, the 6,000 or so members of the Fundamentalist Church of Jesus Christ of Latter-Day Saints (FLDS) are brought up in a lifestyle of plural marriage, meaning a marriage among one man and more than one woman, and are surrounded by their peers in "the covenant."

A lifestyle of plural marriage is likely to affect the health of community members, but its effects have not been studied because of the community's isolation and distrust of outsiders. This paper addresses several questions that arise in contemplating the health of the Short Creek community: What are the health beliefs in this community, and what are their historical bases? Where do families seek medical care, and for what or at what threshold of illness or injury? What is the attitude of care providers serving this community, and how are the providers viewed by the community? More broadly, this paper examines the ways in which polygamy configures health.

In order to meet this objective, this paper aims first to provide a brief account of this community's history and demographic profile, followed by a discussion of healthcare in this community and how it is affected by the practice of plural marriage, with the data comprised of qualitative interviews with healthcare providers to the community. The goal of this project is to gain a rich, historically nuanced understanding of the health of community members, and to identify directions for further academic and policy research. Our findings indicate that health in this community is shaped by limited resources, an attitude of health fatalism, and a profound insularity and corresponding isolation from the outside world.

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PACING-INITIATED VENTRICULAR TACHYCARDIA IN PATIENTS WITH CARDIAC RESYNCHRONIZATION THERAPY VERSUS IMPLANTABLE CARDIOVERTER DEFIBRILLATORS

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Implantable cardioverter defibrillators (ICD) with single-chamber pacemakers can be programmed to provide ventricular pacing when normal conduction fails. Studies suggest that ICD pacing after a pause in native conduction may be paradoxically proarrhythmic by inducing short-long-short sequences (SLS) that initiate ventricular tachycardia (Sweeney et al, JACC, 2007). Compared to ICD, cardiac resynchronization therapy (CRT) delivers mandatory pacing, and thus, it is hypothesized that CRT may also be proarrhythmic. The purpose of this study was to compare the proportion of monomorphic ventricular tachycardia (VT) episodes initiated by pacing with CRT versus ICD.

VT episodes occurring in subjects enrolled in the MADIT-CRT trial were analyzed. Subjects in this trial with NYHA class I or II heart failure had been randomized to receive either CRT-ICD or ICD alone in a 3:2 ratio. Each electrogram recording a VT episode was reviewed by two investigators who were blinded to pacing treatment (NM, KC). VT episodes initiated by SLS sequences were classified as pacing permitted (SLS, not paced) or pacing facilitated (SLS, paced). The proportion of VT episodes initiated by pacing in each group was then compared using the chi-square statistic. A total of 338 episodes of VT were reviewed from 129 subjects. Of these subjects, 83 had CRT, 45 had ICD, and one crossed over between the treatment arms. There was no significant difference between the treatment groups in the mean VT cycle length (324 msec in CRT v. 328 msec in ICD, p=ns). As expected, most of the subjects with CRT were paced at baseline prior to the onset of VT. However, the proportion of VT episodes initiated by pacing-facilitated onset was similar in both groups (9.5% in CRT v. 9.4% in ICD, p=ns)

Both CRT and ICD therapies initiate VT by inducing SLS sequences. Surprisingly, given its high-volume pacing, CRT was not associated with an increased proportion of pacing-facilitated VT, suggesting that it is not more proarrhythmic than ICD therapy.

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DRIVERS OF HOSPITAL LENGTH OF STAY AFTER SURGERY IN THE STANFORD HOSPITAL

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The unsustainable rise in health care expenditure has led to a greater push to understand the drivers of healthcare costs and hospital utilization. Hospital length of stay is an important metric that helps quantify resource utilization within the hospital. The ability to understand the drivers and accurately predict length of stay is valuable for effective management of resources and improved patient care. Using regression analysis, we evaluated a wide range of parameters and their effects on hospital length of stay ("LOS") after orthopedic operations at the Stanford Hospital.

Data was obtained from the Stanford's enterprise data warehouse - Stanford Translational Research Integrated Database Environment. We analyzed data for a total of 3,321 patients that underwent hip, knee or shoulder surgery from 2008 -2011. Our results showed that Age (beta coefficient =0.005), BMI (0.008), Depression (0.29), Anxiety (0.10), Pain (0.21), Operation duration (0.19), Outpatient oxycodone use (0.32), Gabapentin administration (-0.27), General (0.14) and Regional (-0.58) anesthetic use are statistically significant drivers of hospital LOS (t-stat greater than 2 and p value less than 0.05). On the other hand, Anxiety, Sex and Outpatient hydrocodone use were not statistically significant. The goodness of fit (R squared value) was 8.5%.

Given the fact that the variables in the regression accounted for only 8.5% of changes in LOS, additional variables are needed to increase the robustness of our analysis. Additional variables such as details of operative complications, hospital-acquired infections should be further investigated. Due to the low R squared (8.5%), our regression is limited as a predictive model. However, we will continue to investigate other parameters.

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DOES WAIST CIRCUMFERENCE PREDICT BARIATRIC SURGERY OUTCOMES?

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Morbid obesity is an emerging national health issue. In 2008, over 200,000 bariatric surgeries were performed in the United States and there has been a 10- fold increase in the procedure in the last decade. With this rapidly increasing use of bariatric surgery, it is important to understand the predictors of risks and benefits in order to counsel patients effectively and accurately. In prior research, waist circumference correlates strongly with levels of visceral adipocyte tissue, this hormonal tissue is under intense research to understand its inflammatory role and impact on chronic diseases such as cardiovascular disease, diabetes, and metabolic syndrome. We analyzed preoperative waist circumference as a predictor of postoperative success in patients after bariatric surgery.

Data were obtained from a retrospective chart review of 562 patients >18 years old in a multidisciplinary, obesity clinic. The effect of preoperative waist circumference on postoperative outcomes including percent excess weight loss, postoperative complications and cardiometabolic risk factors were analyzed using a multivariate linear regression model. Three hundred twenty-nine patients met the inclusion criteria (69 men and 260 women). The mean age was 47 years, and the mean body mass index (BMI) was 46 kg/m2. Comorbid disease includes diabetes (41%), hypertension (55%), sleep apnea (43%) coronary artery disease (10%), depression (26%), and musculoskeletal disease (56.8%). Procedures performed included roux en y gastric bypass and laparoscopic adjustable gastric bands. Preoperative mean waist circumference was 52cm. Preoperative waist circumference predicted percent excess weight loss ($R=-0.84$, $p=0.046$) as well as absolute weight loss ($R=2.16$, $p=0.00$), but did not significantly predict changes in postoperative complications or cardiometabolic risk factors.

Preoperative waist circumference predicts weight loss, but does not accurately predict changes in postoperative complications or cardiovascular risk factors. More research is required to understand the preoperative variables that help predict success following bariatric surgery.

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NO REDUCTION IN NO SHOW RATES USING A NOVEL INTERVENTION OF "SAD CHILD" POSTERS AT LUCILE PACKARD CHILDREN'S HOSPITAL SUBSPECIALTY CLINICS

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Patients who fail to keep their appointments, without rescheduling or canceling, are considered "No-Show" (NS) patients and incur large direct and indirect healthcare costs. Interventions like reminder calls have aimed to reduce the NS rates.

The objective of our study was to determine the effectiveness of Lucile Packard Children's Hospital's (LPCH) novel intervention of displaying posters of a "sad child" with the message, "*If you don't plan on keeping your appointment, can I have it?*" in outpatient subspecialty clinics to reduce NS rates.

From a prior Stanford Medical Scholars project, a database of predictor variables for NS patients was generated from clinical encounters between 2006 and 2010 at 15 LPCH subspecialty outpatient clinics. The Sad Child Poster was placed in 10 LPCH subspecialty clinics from March 2010 to December 2010. A two-tailed homoscedastic student t-test was performed between the 2 six-month periods before and after the intervention. STATA Data Analysis and Statistical Software (College Station, TX) was used to run univariate and multivariate logistic regressions on patient demographics and predictor variables to determine differences in NS rates among patients seen before and after the intervention for each of the clinics.

From June 2009-Dec 2009, there were 12,393 total encounters, with an average NS rate of 10.32%. From June 2010-Dec 2010 there were 12,897 total encounters with an average NS rate of 11.46%. Two clinics reported a decline in NS rates, while four reported increased rates. One clinic reported no change in NS rates. The two-tailed homoscedastic student t-test showed no statistical difference in NS rates ($p = 0.1826$) before and after the intervention. Secondary analysis shows that higher NS rates were associated with patients less than 12 years old (before intervention: OR 2.11, 95% C.I. 1.22-3.65, $p = 0.007$) (after intervention: OR 1.92, 95%C.I. 1.00-3.67, $p =0.049$) and requiring an interpreter for language translation (before intervention: OR 1.42, 95% C.I. 1.16-1.74, $p =0.001$) (after intervention: OR 1.27, 95% C.I. 1.06-1.53, $p =0.009$).

1) The Sad Child Poster invention did not reduce outpatient subspecialty NS rates at LPCH (10.32% before vs. 11.46% after).
2) Younger age (< 12 years) and non-English speaking (interpreter-requiring) patients were significant predictors for higher NS rates.

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THE SWAN-GANZ CATHETER HISTORY AND CURRENT APPLICATIONS

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Background: The Swan-Ganz flow or pulmonary artery catheter (PAC) is a directed balloon-tipped catheter is used as a diagnostic tool to measure hemodynamic status in critically ill patients. Its use revolutionized medicine but it was introduced without the extensive clinical trials mandated today to assess safety and efficacy. After many years, investigators have identified potential increases in mortality associated with the use of pulmonary artery catheters but continue to use them in critically ill patients. This review will highlight the history of the Swan-Ganz, its current use and potential future utilization.

Research Findings: In 2005 a landmark study evaluated the efficacy of pulmonary artery catheterization in heart failure.[5] The ESCAPE Trial (Evaluation Study of CHF and Pulmonary Artery Catheterization Effectiveness) assessed various end-points like cumulative primary end point, days alive and out of hospital was determined to be identical for PAC plus clinical assessment compared to clinical assessment only.

Conclusion: Thus the PAC has left and continues to be used as a tool to better understand the hemodynamics of the heart and to dictate treatment. Although its use remains controversial, PACs are still being used and remain popular in monitoring cardiac patients.

MOLECULAR ENGINEERING OF SIRPα: A NEW STRATEGY IN CANCER IMMUNOTHERAPY

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The ability of tumors to evade the immune system is an emerging "hallmark of cancer," and a strategy of many current and experimental anti-tumor therapies is to activate immune responses against tumors. Recent studies have identified CD47 as an anti-phagocytic signal highly expressed on many disparate forms of cancer. Antibodies that block the interaction between CD47 and SIRPα, an inhibitory receptor on macrophages, greatly increase phagocytosis of cancer cells and offer a promising new axis in anti-tumor therapy. We sought to improve CD47-targeted therapy by engineering a soluble IgV domain of SIRPα as a high-affinity competitive antagonist of CD47. Using yeast surface display, we evolved high-affinity SIRPα variants that bound to CD47 with ~20,000-fold higher affinity than wild-type (WT) SIRPα (30 pM versus 500 nM). Affinity and kinetics measurements by surface plasmon resonance indicated the affinity gains were manifested by a dramatic decrease in off-rate, but the crystal structure of a high-affinity variant complexed to CD47 revealed its binding mode was nearly indistinguishable from the WT SIRPα:CD47 complex. Sequence analysis of several high affinity variants revealed a consensus set of mutations that endowed high affinity when grafted onto other naturally occurring SIRPα polymorphs. Thus, the SIRPα variants we obtained represent excellent candidate antagonists of the SIRPα:CD47 pathway and warrant further characterization as potential therapeutics for cancer.

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ANALYSIS OF THE EXISTING SURGICAL SAFETY MEASURES, DURATION OF EACH PROCEDURE AND PER-OPERATIVE COMPLICATIONS DURING AND UP TO 72 HOURS POST-PROCEDURE FOR PEDIATRIC SURGICAL CASES CONDUCTED UNDER GENERAL AND SPINAL ANESTHESIA

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Inadequate patient safety grew to become such a worldwide concern that the World Health Organization (WHO) adopted resolution WHA55.18 in 2002 asking countries to take proactive steps in improving safety of healthcare and monitoring systems. The second part of their campaign in 2007-2008 focused on surgical care safety leading to the inception of the WHO Surgical Safety Checklist. As of 2010, the Pediatric Surgery department of Chittagong Medical College Hospital (CMCH) have attempted implementing the WHO Safe Surgery Guidelines to the extent feasible with the resources at the team's disposal. In order to review their performance in terms of surgical outcomes under the given degree of compliance with the WHO Safe Surgery Checklist, such that the results may guide the department in deciding whether or not it is cost effective to develop a formal safety protocol incorporating all aspects of the WHO Safe Surgery Checklist comprehensively. To approach this we collected safety scores from 108 general anesthesia and spinal anesthesia cases using a 51-item checklist of detailed steps of the 'Sign-In' and 'Time-Out' sections of the Checklist, as well as the incidence of surgical complications during and up to 72-hrs post procedure by assigning a score of 1 for each complication, and the duration of each procedure. A regression analysis was conducted on the data using STATA 11.

The mean safety score, out of a total score of 51, was 28.3 with a SD of 3.0. Linear regression analysis revealed no statistically significant correlation between degree of safety and rate of complications. While no statistically significant correlation existed between degree of safety and duration of procedure, the correlation coefficient revealed an increase in overall procedure by 2 mins for each additional safety step observed.

The results of the study were raises important questions regarding the universal applicability of the WHO Safe Surgery Checklist. The adherence to a high degree of safety based on the checklist items were not found to correlate significantly with surgical outcome, while each additional safety step prolonged the procedure by 2mins. With almost 15-20 patients on the surgical roster for each 6hr shift, this can have a significant consequence on the number of patients that can be operated upon in a public teaching hospital setting like that at CMCH. The importance of safety regulations prior to, during and post-procedure can never be undermined in the field of surgery. However, what remains to be investigated further is a step-by-step risk analysis of each safety procedure to determine their absolute need in a resource-constrained surgical practice like that at CMCH, Bangladesh, which is the next stage of analysis to be conducted with the data collected from this study. The results must also be viewed in light of the limitations of the study. Given the low rate of incidence of surgical complications in general, even with a sample size of 108, the study had low power ($\beta=0.4$). A larger sample size will be required in order to be able to generate results with more confidence. The study design does not weigh the complications based on their degree of severity and assigns the same weight to each complication that arises. Analyzing an individual safety step with a specific outcome may help shed valuable information overlooking the equal weighing of each complication.

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VISUALIZING CELLULAR INTERACTIONS WITH A GENERALIZED PROXIMITY REPOTER

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A living animal is comprised of intricate networks of cells that communicate over many time and length scales. Physical contacts and other molecular interactions drive numerous cell behaviors, and breakdowns within cellular communication networks can initiate pathologies, including cancer progression. Despite the clear connections between cell-cell interactions and human health, there are no general methods to examine cell contacts on a global scale in tissues and live animals.

We report here a broadly applicable, longitudinal strategy for probing cellular interactions in living subjects. This approach relies on the generation of bioluminescent light when two cells come into close proximity. The intensity of the optical signal thus reflects the relative location of the distinct cell populations. We used this reporter strategy to gauge cell-cell proximity in culture models *in vitro* and *in vivo.* We also applied the method to analyze tumor-immune cell interactions in a murine breast cancer model. In these studies, our imaging strategy enabled the facile visualization of features that are difficult to observe with conventional imaging techniques: metastatic lesions and sites of tumor immunosurveillance. The proximity reporter described here will be applicable to probing numerous types of cell-cell interactions and will stimulate the development of similar strategies to detect rare events and pathologic processes in live animals.

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PATIENT ADHERENCE WITH POST-OPERATIVE RESTRICTIONS AFTER ROTATOR CUFF REPAIR

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For up to 6 weeks after rotator cuff repair orthopedic surgeons instruct their patients to restrict motion by wearing a sling. However, it is unclear how much shoulder activity patients actually engage in during the postoperative period. The purpose of this study was to elucidate how well patients adhered to their surgeons' recommendations, describe any correlation between adherence and post-surgical outcome, and identify any possible indicators of poor adherence. Fifty consecutive patients who underwent repair for rotator cuff tears were included and instructed to wear an abduction brace for 6 weeks after surgery. Functional evaluations, including simple shoulder test, American shoulder and elbow surgeons score, and UCLA shoulder score were made preoperatively and between 6 and 25 months postoperatively. Patients were asked to comment on their adherence using a medical adherence measurement questionnaire. Patient demographics were obtained by chart review.

Average adherence was 88% ±10%. There were no significant correlations between adherence and improvement in ASES, UCLA, or SST shoulder scores after rotator cuff repair ($P = 0.06245$, 0.5891, and 0.7688 respectively). Of the patient demographics analyzed, only smoking status had a positive effect on adherence ($P = 0.00432$, coefficient 9.867). All other demographics, including mechanism of injury, cohabitation and employment status, dominance of injured arm, comorbidities, and age had no significant effects on self-measured adherence to post-operative restrictions ($P = 0.5889$, 0.06086, 0.4171, 0.7876, 0.419 and 0.5402 respectively).

Patients' self-reported adherence did not have an effect on shoulder outcome as measured any of three functional outcome scores. Of the patient demographics assessed for correlation to self-reported adherence, smoking was identified as a possible correlate, which may or may not be relevant given the low volume of self-identified smokers. Further study should involve mechanical calculation of adherence and higher research subject volume.

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LESCH-NYHAN DISEASE: GENOTYPE-PHENOTYPE CORRELATION AND THE FUTURE OF TREATMENT

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Lesch-Nyhan disease (LND) is an X-linked metabolic disorder caused by the deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). This enzyme deficiency results in the buildup of uric acid in all body fluids and the brain. Clinical manifestations of the disease range from simple hyperuricemia (HRH), to hyperuricemia with neurologic manifestations (HRND), to the most severe form which includes self-mutilating behavior (LND). Research has made considerable headway in documenting disease-causing mutations; identifying greater than 300 mutations in the HPRT gene that lead to one of the aforementioned LND phenotypes. However, controversy still exists as to whether there, indeed, is a genotype-phenotype correlation. In addition, treatment for LND has been largely symptomatic with little therapy devoted to targeting the devastating neurological sequelae. The objective of this project was to analyze reported mutations in the HPRT gene to see if there is a pattern to the genotype-phenotype variability. Additionally, potential targets for therapy, including gene therapy, will be reviewed with regard to their feasibility both clinically and within the current political climate.

A literature review was conducted, covering all published articles through January 2012. Articles describing mutations in the *hprt* gene, LND therapy, and clinical trials in gene therapy were reviewed. With few exceptions, mutations leadings to deletions, insertions, inversions, or other larger changes in the *hprt* gene will invariably lead to the severe from of LND. With regard to non-conservative point-mutations, 139 distinct cases were found in the literature. While mutations leading to all three phenotypes were found in almost every exon, several “hot spots” were identified for each of the three phenotypes. For HRH codons 48-53 and 104-110 (corresponding to the active site of loops II); for HRND codons 186-188 and 193 (both corresponding to the active site of loops IV); and for LND codons 32-47 and 66-71 (corresponding to the active site of loops I). With regard to treatment, while HPRT was one of the first of 3 genes to be targeted for gene therapy, to date zero of >1500 clinic trials have actually addressed LND. While early research was halted due to difficulty specifically targeting the brain, recent advances have allowed for targeted drug delivery to this area, as seen by seven clinical trials (in either Phase I or Phase II) for the treatment of Parkinson’s Disease.

This paper compiles the largest single database of non-conservative point mutations leading to LND and LND-variants. The mutational “hot spots” identified not only provide insight into the various protein active sites, but allow for stratification of risk during genetic and prenatal counseling. In addition, because of the range of phenotypes associated with different mutations, the presence of general genotype-phenotype correlations could allow for more deliberate selection in future treatment trials.

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NEED FOR RECONSTRUCTIVE SURGERY IN DEVELOPING COUNTRIES: BURN INJURIES IN SIERRA LEONE

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Burn injuries result in significant morbidity and mortality worldwide. For survivors of burn injuries in developing countries, inadequate treatment and lack of rehabilitation further compound the challenges of recovery. In Sierra Leone, one of the world's poorest nations, there are less than 10 fully trained Sierra Leonean surgeons and no plastic surgeon for a population of over 6 million people. The purpose of this study was to provide data on the epidemiology of burn injuries in Sierra Leone in order to inform the development of programs to address the unmet need for reconstructive surgery in resource-poor settings.

This research was conducted as part of the Surgeons OverSeas Assessment of Surgical Need (SOSAS) study, which is a cross-sectional population cluster based survey. The nationwide survey was conducted in January- February 2012 in Sierra Leone, randomly selecting 75 clusters for a total sample of 1873 households stratified for rural and urban areas. Within each household two persons were assigned by a Random Calculator to be interviewed, aiming for a total of 3745 responses. Randomly selected household members underwent a verbal head to toe examination on burn injuries.

Preliminary results indicate that females and children under 15 years old have the highest prevalence of burn injuries. The extremities and upper torso were the most commonly involved body regions, and burns were most frequently caused by hot objects or liquids. Further data analysis will be conducted to ascertain the number of individuals currently affected by these injuries and the extent of associated disability. An estimate of the number of individuals who could potentially benefit from reconstructive surgery for burns and associated sequelae will also be determined. It is anticipated that this data will provide a valuable baseline by which to develop efforts to increase reconstructive surgical care in Sierra Leone and other developing countries.

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CHARACTERIZING THE EFFECTS OF ANTI-CD47 ANTIBODY THERAPY ON ANTIGEN PRESENTION IN AN IMMUNOCOMPETENT MOUSE TUMOR MODEL

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The molecule CD47 on tumor cells provides a “don’t eat me” signal that inhibits tumor phagocytosis by the innate immune system. Our laboratory has previously shown that treatment of xenografted human tumors with a CD47 blocking antibody leads to phagocytosis and elimination of tumors by macrophages, which express the CD47 ligand, SIRP-alpha. Phagocytosis is not only a mechanism by which macrophages engulf other cells, but it is also a mechanism by which professional antigen presenting cells (APCs), including macrophages and dendritic cells, take up antigens and present them to T cells.

We are testing the hypothesis that anti-CD47 antibody therapy allows tumor antigens to be presented by APCs to the adaptive immune system. Testing this hypothesis requires the use of an immunocompetent mouse tumor model rather than an immunodeficient mouse model. We have recently found that anti-CD47 antibody is effective in inhibiting tumor growth in the immunocompetent mouse setting using the MT1A2 mouse breast carcinoma. We have optimized a strategy for prospective isolation of candidate macrophages and dendritic cells from the mouse MT1A2 breast tumor model. We observed that both candidate tumor macrophage and dendritic cells phagocytose MT1A2 *in vivo* following anti-CD47 antibody treatment. Following anti-CD47 antibody treatment, there may be functional differences in how macrophages and dendritic cells respond to anti-CD47 treatment.

We will currently examining whether macrophages and dendritic cells can present tumor antigens to T cells using the ovalbumin model antigen. These studies will help us understand whether anti-CD47-mediated phagocytosis of tumor cells allows tumor antigens to be recognized by the adaptive immune system. This would provide new insight into therapeutic strategies for using anti-CD47 mediated therapy to mobilize not only the innate immune system, but also potentially the adaptive immune system, to eliminate cancer.

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IDENTIFICATION OF NOVEL REGULATORS OF THE IRE1-XBP1 PATHWAY OF THE UNFOLDED PROTEIN RESPONSE IN TUMOR CELLS

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The unfolded protein response (UPR) is an evolutionarily conserved pathway that functions to maintain endoplasmic reticulum (ER) homeostasis under conditions of chemical, synthetic or hypoxic stress, all of which are prevalent in the tumor microenvironment. This cell survival pathway has been shown to be important in tumor cell growth and survival. As such, a greater understanding of molecular signaling pathways regulating the UPR is needed. Previously, a genome-wide siRNA screen was conducted in HT1080 cells that identified hundreds of genes for which downregulation caused inhibition of the IRE1-XBP1 pathway of the UPR. The purpose of the project was to validate this screen by knocking down expression of two of the most promising genes and observing the phenotype.

Two genes that have not previously been linked to the UPR were selected. DELGEF is a guanine nucleotide exchange factor that is thought to regulate extracellular secretion of proteoglycans. Fbxo4 is a member of the F-box protein family that is thought to be a tumor suppressor that regulates cyclin D1 accumulation. To investigate a possible association, siRNA transfection was performed to verify that knockdown of these proteins caused decreased XBP1 splicing in HT1080 in response to chemical induction of ER stress compared to control. Following this verification, a lentiviral vector was used to create Panc1 cell lines that stably expressed an shRNA construct targeting DELGEF or Fbxo4. These cell lines show decreased XBP1 splicing in response to chemical or hypoxic ER stress.

This project has helped to validate the results of a novel, genome-wide screen conducted to identify regulators of the UPR. Because the UPR has been shown to be important for tumor survival, future work may identify new, targeted therapies for cancer treatment. The next step will be to further investigate the phenotype of cells with DELGEF or Fbxo4 knockdown.

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EXAMINATION OF THE ROLE OF THE LAMININ-332 α3 CHAIN, DOMAIN IIIa, IN EPITHELIAL CARCINOGENESIS

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Laminin-332 is a heterotrimeric glycoprotein component of the basement membrane essential for epithelial tissue adhesion and development. Disruption of normal laminin-332 functions has been implicated in the development of junctional epidermolysis bullosa and in squamous cell carcinoma (SCC). Whereas several laminin-332 domains have been shown to participate in pro-tumorigenic signaling in SCC, prior work on the N-terminal IIIa domain of the α3 chain is limited to the rare granulation tissue disorder laryngo-onycho-cutaneous syndrome (LOC) caused by a genetic truncation of the α3 chain at the IIIa domain. Given the role of other laminin-332 domains in tumorigenesis and the role of the IIIa domain in the LOC wound healing response, we have hypothesized a role for the α3 IIIa domain in promoting the development of SCC.

To study the function of this domain, the human laminin-332 α3 gene *LAMA3* was cloned with or without the IIIa domain (ΔIIIa) into mammalian expression vectors. Immortalized *LAMA3*-null human keratinocytes were retrovirally infected with the full-length or ΔIIIa gene constructs and examined for defects in laminin-332 deposition and cell adhesion. Immunofluoresence studies showed comparable levels of laminin-332 deposition between cells containing the full-length or ΔIIIa chain. Preliminary observations of the ΔIIIa cells indicate potential differences in cell morphology and increased sensitivity to trypsin dissociation, suggesting changes in cell adhesion to be further analyzed by confocal microscopy.

Future experiments will characterize the behavior and tumorigenic potential of the ΔIIIa keratinocytes. Cell migration, motility, and invasion will be assessed *in vitro*, and tumorigenicity and wound healing capacity will be evaluated *in vivo*. Development of an antibody to the IIIa domain for functional inhibition studies is also ongoing. Finally, we will attempt to identify the molecular mechanisms, including the potential induction of inflammatory cytokines, that underlie the observed role of the α3 IIIa domain in epithelial pathology.

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EVOLVING HIGH AFFINITY SIRPα VARIANTS AS IMMUNE-BASED THERAPIES FOR CANCER

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CD47 is a cell-surface molecule that acts as a "marker of self" to the host immune system. By signaling through SIRPα, a receptor on macrophages, CD47 inhibits phagocytosis. Many cancer cells upregulate CD47 expression, and antibodies that block the interaction between CD47 and SIRPα increase phagocytosis and eliminate tumors in pre-clinical models. Analogously, a soluble form of the SIRPα ectodomain could be used as a blocking reagent, but the weak interaction between native SIRPα and CD47 limits the potential of such a therapy.

To improve the pharmacokinetic and pharmacodynamic properties of the SIRPα ectodomain, we used a yeast-display system to bioengineer SIRPα mutants with increased binding affinity for human CD47. Monomeric and Fc-tagged high affinity SIRPα variants potently bound human tumor cells and blocked binding of wild-type SIRPα. In vitro, Fc-tagged high affinity SIRPα variants induced phagocytosis of tumor cells by human macrophages. High affinity SIRPα monomers produced only a marginal effect on macrophages, indicating that blocking the CD47:SIRPα interaction is not sufficient for maximal phagocytosis. However, high affinity SIRPα monomers substantially increased the efficacy of opsonizing monoclonal antibodies such as cetuximab and rituximab. Preliminary studies using orthotopic xenotransplantation mouse models of human cancer suggest high affinity SIRPα therapies are efficacious in vivo.

Since CD47 is a common mechanism that tumor cells use to evade the immune system, the molecules developed in this study could be effective immune-based treatments for many types of cancer, either as monotherapies or as adjuvants to monoclonal antibodies.

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BRAIN-DERIVED NEUROTROPHIC FACTOR INCREASES NEURAL STEM CELL ENGRAFTMENT AND ENHANCES CELL-BASED THERAPY FOR ISCHEMIC STROKE

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Increasing cell migration through upregulation of chemokine and adhesion molecule receptors could improve intravascular cell-based therapy for stroke. Brain-derived neurotrophic factor (BDNF) upregulates these pathways. Therefore, we tested whether exposing human neural progenitor cells (NPCs) to BDNF prior to delivery would improve cell migration, engraftment, viability, and functional recovery in an experimental stroke model.

NPCs pre-treated with BDNF for 5 hours and harboring a reporter gene construct containing renilla luciferase and eGFP, non-treated hES-derived NPCs, and media control with BDNF were delivered to the brain via the ipsilateral carotid artery at 3 days after hypoxic-ischemic stroke in immunodeficient mice. Cell engraftment was monitored by *in vivo* bioluminescence imaging (BLI). The ladder test was used to assess behavioral recovery throughout a 4 week time course. Brain homogenates from animals at 28 days were analyzed using RT-qPCR for common chemokines, adhesion molecules, and neurotrophins. Mechanisms of cell migration were evaluated by assessing cell receptor expression of chemokines and adhesion. Boyden-chamber migration assays were used to evaluate cell migratory potential *in vitro*.

Animals transplanted with BDNF-pretreated cells showed significantly higher BLI signal at 1 ($p=0.021$) and 7 days after transplantation ($p=0.002$). Histological analysis revealed engraftment of NPCs at 1 week after transplantation. The BDNF pre-treated group experienced better functional recovery throughout the 28 day time course (ANOVA, $p<0.05$). BDNF-pretreatment of NPCs upregulated CXCR4 expression 12.5-fold and led to significantly greater migration in response to CXCL12 (CXCR4 ligand) compared to untreated cells *in vitro*. At 28 days after transplantation, neurotrophic factors IL6, IL10, Ntrk1 were upregulated 3.3, 3.4, and 3.3-fold, respectively. Anti-angiogenic factor Adamts8 was also downregulated in the brains of animals transplanted with BDNF pre-treated cells. Lastly, MMP3, MMP8, and MMP9 were downregulated at 28 days after stroke indicating increased blood brain barrier integrity.

Exposing NPCs to BDNF enhances functional gains after an experimental model of ischemic stroke. This phenomenon may be mediated by increased engraftment of NPCs and promotion of a neuroprotective state. Future studies will determine if BDNF increases the efficacy of stem cell-based therapy for ischemic stroke in humans.

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THE TOBACCO INDUSTRY AND ANIMAL RESEARCH EXPERIMENTS: RABBIT-EYE EDEMA AND MEDICAL ADVERTISING

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The tobacco industry has funded animal research studies for nearly a century. These studies have been used towards the design of cigarettes and material for advertising campaigns. In many cases, animal research experiments were used to create scientific evidence against the harmful health effects of tobacco smoke. Currently, this area of history has not been widely studied. This project analyzed historical documents in the Legacy Tobacco Documents Library of the University of California: San Francisco and related advertisements in the archives of medical journals to understand how these experiments were performed and how the results were used to further the “tobacco agenda”.

To assess the irritating effects of a new formulation of cigarettes, Philip Morris began testing the effects of cigarette smoke condensate on rabbit eyes. The results of these studies were used in an advertisement campaign in 1943 where images of swollen rabbit eyes were published in medical journals. These ads claimed that Philip Morris cigarettes were “definitely less irritating” to smokers based on the rabbit eye edema tests. In response to these advertising claims, competing companies funded increasingly invasive rabbit experiments with the intent of debunking Philip Morris’ health claims.

This project has shown that cigarette companies have utilized animal research models to mitigate the negative health effects of tobacco. Further research is needed to understand the extent of this issue and its prevalence over the last century. Additionally, this research raises several ethical concerns about the use of animal research to mislead consumers and physicians on the negative health effects of cigarettes.

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MONITORING CANCER THERAPY WITH CERENKOV LUMINESCENCE IMAGING

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Cerenkov Luminescence Imaging (CLI) has emerged as a less expensive, easier-to-use and higher throughput alternative to nuclear imaging modalities such as positron emission tomography (PET). It is expected that CLI will find many applications in biomedical research such as cancer detection, probe development, and drug screening. In this study we explored the possibility of using CLI to monitor drug therapeutic effects and correlate with PET imaging. Bevacizumab (trade name Avastin®) was used to treat two mouse tumor models – large cell lung cancer cell line H460 and prostate cancer cell line PC3 – and two common radiotracers – 3'-deoxy-3'-^{18}F-fluorothymidine (^{18}F-FLT) and 2'-deoxy-2'-^{18}F-fluoro-D-glucose (^{18}F-FDG) – were used for both CLI and PET.

In ^{18}F-FLT scans both CLT and PET revealed significantly decreased signals from H460 xenografts in treated mice from pre-treatment to day 3. Moderately increased to unchanged signals were observed in untreated mice during the same time period. In ^{18}F-FDG scans both CLT and PET showed relatively unchanged signals from PC3 tumors in both treated and control groups. Quantifications of CLI and PET images showed that the two modalities had good correlations (R^2=0.9309 for ^{18}F-FLT probed treatment group; R^2>0.88 across all groups of mice studied)

CLI and PET exhibited good correlations across different tumor xenografts and radiotracers in both treated and untreated mice by Bevacizumab. The findings warrant further exploration and optimization of CLI as a less expensive, easier-to-use, and high throughput alternative to PET in preclinical therapeutic monitoring studies and drug screening processes.

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ZNF750 RESCUES DIFFERENTIATION DEFECTS DUE TO ANKYLOBLEPHARON, ECTODERMAL DYSPLASIA, AND CLEFTING (AEC) SYNDROME MUTANT P63

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The transcription factor p63 is essential for regulating cellular proliferation and differentiation in both the development and maintenance of stratified epithelial tissues, including the epidermis. Heterozygous dominant mutations affecting distinct domains of *TP63* are observed in a group of monogenic human syndromes that share the common feature of ectodermal dysplasia. Among these *TP63* genetic syndromes is Ankyloblepharon, Ectodermal Dysplasia, and Clefting (AEC) syndrome. AEC syndrome is distinguished by mutations in the p63 sterile alpha motif (SAM) domain and phenotypically with epidermal erosion. The mechanism by which AEC mutation disrupts epidermal differentiation is unknown.

To gain insight into the mechanisms whereby these mutants disrupt epidermal differentiation, we express a panel of AEC p63 mutants observed in human patients, including p63-C467W, -L459F, -I482T, and –R500P, in human keratinocytes. The AEC p63 mutants expressing cells were used to regenerate human epidermal tissue on intact human dermis. In our human AEC model, AEC p63 mutants down-regulated the expression of canonical epidermal differentiation genes, including *KRT1* and *FLG* and repressed transcriptional activators of epidermal differentiation, including *GRHL3, HOPX, KLF4,* and *ZNF750*. Among the panel of transcriptional activators, *ZNF750* was the first to be induced during normal differentiation. ChIP analysis of AEC mutants in differentiating keratinocytes showed that AEC p63 mutants remain bound to the *ZNF750* promoter despite repressing *ZNF750* induction, suggesting a dominant-negative role of the mutant p63 mutants. Remarkably, overexpression of *ZNF750* in AEC epidermal human tissue significantly rescued expression of epidermal differentiation genes and transcriptional activators of epidermal differentiation. These findings indicate that *ZNF750* repression by AEC p63 mutants block transcriptional activators required for epidermal differentiation.

Rescue of defective gene differentiation by *ZNF750* supports a model in which *ZNF750* repression by AEC p63 mutants is a preceding event in the pathogenesis of disrupted differentiation in AEC. These findings support the rationale for potential therapies to up-regulate *ZNF750* in the epidermis of AEC patients.

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ROLE OF MAST CELL-ASSOCIATED RABGEF1 IN IgE-DEPENDENT IMMUNE RESPONSES *IN VIVO*

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Mast cells are major effector cells in allergy and anaphylaxis, and a better understanding of the IgE-dependent signaling pathways involved in mast cell activation is needed for the development of better treatments for these disorders. RabGEF1 is a negative regulator of FcεRI-dependent mast cell activation and mast cells derived from RabGEF1-deficient (Rabgef1$^{-/-}$) mice exhibit delayed FcεRI receptor internalization, elevated and prolonged intracellular signaling events, and enhanced cytokine and mediator release in response to FcεRI aggregation.

In this study, we sought to investigate the role of mast cell-associated RabGEF1-mediated signaling in IgE-mediated skin inflammation and anaphylaxis *in vivo*. Using a Cre-lox system, we targeted RabGEF1 for deletion specifically in mast cells expressing the Cre recombinase under the mast cell-specific Mcpt5 promoter. We crossed C57BL/6 Mcpt5-Cre mice to C57BL/6 Rabgef1$^{fl/fl}$ mice to generate Mcpt5-Cre;Rabgef1$^{fl/fl}$ mice, which appeared normal, without increased mortality, morbidity or obvious gross phenotypic abnormalities. To assess whether *Rabgef1* gene was deleted in mast cells from Mcpt5-Cre+;Rabgef1$^{fl/fl}$ mice, we performed single cell sorting of different cell populations from Mcpt5-Cre-;Rabgef1$^{fl/fl}$ or Mcpt5-Cre+;Rabgef1$^{fl/fl}$ mice. Using single cell PCR, we showed that only peritoneal mast cells (PMCs) from Mcpt5-Cre+;Rabgef1$^{fl/fl}$ mice, but not other cell types from Mcpt5-Cre+;Rabgef1$^{fl/fl}$ mice or PMCs or other cell types from Mcpt5-Cre-;Rabgef1$^{fl/fl}$ mice, were completely devoid of detectable *Rabgef1* exon 2 genomic fragment. Animal models of IgE-dependent passive cutaneous anaphylaxis (PCA) and passive systemic anaphylaxis (PSA) were employed to assess whether IgE- and antigen-induced activation of mast cells *in vivo* was altered by their lack of RabGEF1 expression. When PCA reactions were quantified by measuring swelling of IgE anti-DNP-injected ear pinnae in response to i.v. injection of antigen (DNP-HSA), we found no significant differences in IgE-dependent ear swelling between Mcpt5-Cre+;Rabgef1$^{fl/fl}$ mice and their controls. PSA reactions were quantified by measuring the decrease in body temperature induced by IgE-DNP injection. Similar to what we had observed with PCA reactions, we did not find any significant differences in the decrease in body temperature between Mcpt5-Cre+;Rabgef1$^{fl/fl}$ mice and their controls in the PSA assays. These findings were unexpected as we had predicted that specific deletion of RabGEF1 in mast cells would have increased the severity of IgE-dependent PCA and PSA reactions, since mast cells are the main driver of such immune responses and *in vitro* derived *Rabgef1*$^{-/-}$ mast cells exhibit enhanced functional responses upon FcεRI aggregation.

In summary, we found that a specific and complete deletion of RabGEF1 expression in peritoneal mast cells did not appear to alter the severity of IgE-dependent PCA or PSA reactions. However, we have not yet determined whether complete deletion of RabGEF1 occurred in the skin, information needed to interpret the results of our *in vivo* experiments, since skin mast cells are responsible for IgE-dependent PCA and are thought to contribute to IgE-dependent PSA. Future experiments will be directed to determine, on the one hand, to what extent RabGEF1 deletion in skin mast cells is complete and, on the other hand, whether a complete deletion of RabGEF1 in both skin and peritoneal mast cells can influence the outcome of IgE-dependent PCA and PSA reactions.

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IDENTIFYING *CIS*-REGULATORY ELEMENTS IN DFNB1-RELATED HEARING LOSS

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Mutations at the DFNB1 locus are the major cause of non-syndromic sensorineural hearing loss (NSNHL) worldwide. This locus contains the genes *GJB2* and *GJB6*, which encode the gap junction proteins responsible for maintaining the endocochlear resting potential. In up to (depending on population) half of observed cases, recessive NSNHL is attributed to mutations in the coding region of *GJB2*. Truncations of *GJB6* are associated with NSNHL when homozygous or when heterozygous in *trans* with *GJB2* mutations, and it was previously hypothesized that the two connexin genes interacted via a digenic mechanism. However, recent *in vitro* experiments in the Schrijver lab point to the existence of several *cis*-acting regulatory elements in DFNB1 – presumably disrupted by the truncations of *GJB6* – that may activate *GJB2* expression. Moreover, computational analysis of the DFNB1 sequence predicts nine *cis*-regulatory elements that display conservation across species.

Using luciferase reporter assays in mammalian cell models, we are currently searching for *cis*-regulatory elements controlling *GJB2* expression by testing each of the nine computationally predicted enhancers for its level of *GJB2* promoter activation. If an element controlling *GJB2* expression is found, we plan to determine whether it functions in a cell-type-specific manner consistent with the non-syndromic nature of DFNB1-related hearing loss. The results of these studies will broaden our understanding of the genetics of hearing loss and could explain many cases of congenital hearing loss in patients with mutations outside the coding regions of *GJB2* and *GJB6*.

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