6th Annual

Department of Medicine Research Day

Celebrating Research Efforts Across the Department

ABSTRACT BOOK

October 17, 2013
Cox Hall Ballroom
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Oral Presentations

8:15 am

Anti-HIV Immunotherapy Delivered by AAV-Mediated Gene Transfer in Rhesus Macaques

Trible RP, Palesch D, Kirchoff F, Silvestri G.

The ability to induce sustained expression of anti-HIV antibodies that suppress viremia would revolutionize HIV therapy. Studies have shown that passive immunization of acutely HIV-infected persons with anti-HIV antibodies could transiently control viremia. Recent technological advances have allowed the cloning of neutralizing anti-HIV antibodies whose breadth and potency far exceed those utilized in previous studies. In addition, vector delivery systems now permit long-term expression of transgenes within primates, including a recent demonstration that vector-mediated expression of anti-SIV antibody activity within rhesus macaques (RMs) protects against SIV challenge. We chose to expand upon these findings to determine if vector-mediated expression of a combination of potent, broadly neutralizing antibody activities can lower viremia in stably infected non-human primates. Fourteen RMs were infected with SHIVsf163p3n8 and allowed to establish stable viremia. Eight animals were then inoculated with a cocktail of adeno-associated viruses (AAVs), each containing an antibody-like immunoadhesin transgene encoding the variable region sequence from one of six different broadly-neutralizing antibodies fused to the simian IgG-Fc receptor coding region. Plasma and sera samples were collected and analyzed for SHIV viral load and immunoadhesin (anti-HIV) activity. To date, 7 of 8 treated animals have maintained stable viremia, while one animal developed viremic control immediately after AAV inoculation and is likely a spontaneous controller. Neutralization assays have revealed low anti-HIV activity within the sera of treated animals, suggesting limited expression of the immunoadhesins or host rejection of AAV-delivered immunoadhesins. Additional studies are underway to better determine the expression level and functional effects of the immunoadhesins.

8:30 am

Efficacy of Multicomponent Behavioral Therapy, Alpha-adrenergic Antagonist, and Combined Therapy in Men with Nocturia: the BEDTIME Trial


Background: Nocturia, awakening from sleep to void, is highly prevalent and bothersome. Behavioral treatments are recommended for many urinary symptoms, but have not been specifically evaluated for efficacy in men with nocturia.

Methods: This study represents a two-site, RCT in male Veterans with nocturia (≥ 2 nightly episodes). We evaluated changes in nocturia, sleep, and quality-of-life resulting from a novel multicomponent behavioral and exercise therapy (M-BET); alpha-adrenergic antagonist therapy (α-blocker, tamsulosin 0.4 mg); and a combination of α-blocker therapy and M-BET. M-BET included pelvic floor muscle training, delayed voiding, and urge suppression techniques. After recording voiding and sleep information at baseline, eligible participants had four visits over twelve weeks (in-person or telephone). Outcomes were assessed via participant-completed seven-day bladder diaries, wrist actigraphy, and questionnaires.

Results and Limitations: Participants included 72 men (mean, 65.8 years). At baseline, mean episodes of nocturia were similar across groups. At 12-weeks, M-BET changed mean nocturia by -1.39 episodes/night (p=0.0004), α-blocker therapy by -0.59 episodes/night (p=0.0042), and combination by -1.03 episodes/night (p=0.0015). These effect size differences were not statistically significant (p=0.41). All treatments showed statistical reduction in self-reported nocturia and improved global satisfaction. M-BET showed statistical improvements in the Pittsburgh Sleep Quality Index, quality of life, and nocturia bother.

Conclusions: Behavioral therapy in men, alone or with α-blocker therapy, showed clinically significant reductions in nocturia and favorable effects on quality-of-life, sleep, and bother. Behavioral therapy has not been previously demonstrated as efficacious for nocturia, yet it provides a meaningful treatment option.

8:45 am

High-fat Diet, Not Genetic Obesity, Induces Enteric Neuronal Loss, Delayed Colonic Motility and Altered miRNA-375 Expression

Lee J, Anitha M, Mwangi, S, Nezami B, Srivinasan S.

Background: Obesity has been associated with gastrointestinal dysmotility. The link between obesity and colonic motility is not fully understood. Studies looking at Ob/Ob mice with genetic obesity suggest a genetic component while high-fat diet (HFD) has also been investigated. Our aim was to compare the effects of HFD and genetic obesity on colonic motility.

Methods: Studies were performed on 20-week old Ob/Ob mice (n=4) and their wild type mates (n=4) and 8-week old mice fed with a HFD (n=12) or regular diet (RD)(n=12) for 11 weeks. Our study assessed colonic motility with stool characteristics and conducted morphological assessment of damage to enteric nervous system in proximal colon sections. miRNA was isolated from enteric ganglia by Laser-Capture-Microscopy.

Results: Ob/Ob mice and HFD-mice had significantly higher weights at the end of the study compared to their respective controls. The stool of HFD-mice showed constipation phenotype, including decrease in stool frequency (P<0.001), wet weight(P<0.001), dry weight (P<0.01), water content (P<0.001) and water percentage (P<0.001) compared with RD-
mice (Figure 1). Total colonic enteric neurons and nNOS neurons per field were lower in HFD-mice (Figure 2). HFD-mice had increased enteric neuronal apoptosis with associated increase in the pro-apoptotic miRNA-375 and consequent decrease in its downstream target PDK1, assessed by Real time-PCR. These changes seen in HFD-mice were not present in Ob/Ob mice compared to WT.

Conclusions: Our results revealed colonic dysmotility, enteric neuronal cell loss, and altered miRNA-375 expression induced by HFD. Ob/Ob mice failed to demonstrate same effects. Therefore, this study suggests that HFD induces delayed gastrointestinal motility rather than genetic obesity.

A Composite Risk Score of Biomarkers of Inflammation, Thrombosis, and Cell Stress Pathways Significantly Predict Risk of Adverse Cardiovascular Outcomes

Ghasemzadeh Nima, Hatem Al Kassem, Danny Eapen, Mohamed Khayata, Ayman Samman Tahhan, Pankaj Manocha, Bushra Alzaraneh, Ravi Nanjundappa, Emory Univ, Atlanta, GA; Christian W Thorball, Copenhagen Univ Hosp, Copenhagen, Denmark; Riyaz Patel, Stamatios Lerakis, Laurence Sperling, Tomasz Pielak, Virogates, Sergey Sikora, Stephen E Epstein, MD; Arshed A Quyyumi.

Background: An aggregate biomarker risk score of C-reactive protein (CRP), heat shock protein (HSP)-70, and fibrin degradation products (FDP) significantly predicts adverse outcomes in patients with CAD. Soluble Urokinase Plasminogen Activator Receptor (suPAR) is an inflammatory biomarker that has been associated with incident CVD. We sought to investigate the value of adding plasma suPAR to the 3 original biomarkers in predicting risk of outcomes. Methods: 3,280 patients (aged: 62±11, 64% with CAD ≥50%) undergoing diagnostic angiography were followed for death and/or MI over median 2.3 yrs. Plasma suPAR and serum CRP, HSP-70, and FDP were measured using ELISA. Cox proportional survival was performed adjusting for traditional risk factors, ejection fraction, serum creatinine, and Gensini. Results: Patients with suPAR level >3.5ng/ml had a greater CAD burden (Gensini: 49±68 vs. 41±64, p=0.003). Plasma suPAR > 3.5 also predicted death/MI (HR=1.76, p<0.001) after adjustment for all covariates and the 3 biomarkers, CRP, HSP-70, and FDP; the C-statistic improved (0.69 to 0.71, p=0.01). A 4 biomarker aggregate score composed of suPAR, CRP, FDP, and HSP-70 significantly predicted risk of death/MI. Thus, compared to those with 0 biomarkers, HR for those with 1, 2, 3, or 4 positive biomarkers were: 1.5, 2.1, 4.4, and 7.3 respectively (P<0.001). Addition of the 4-marker score significantly improved the C-statistic compared to a model of traditional factors (0.68 to 0.75, p=0.004). Conclusion: An aggregate risk score of CRP, HSP-70,FDP, and suPAR representing inflammatory, thrombotic, and cell stress pathways, identifies CAD patients at low and very high risk of death/MI.

10:15 am

Purification of cardiomyocytes from differentiating pluripotent stem cells using molecular beacons targeting cardiomyocyte-specific mRNA


Background: While methods for generating cardiomyocytes (CMs) from pluripotent stem cells (PSCs) have been reported, current methods produce heterogeneous mixtures of CMs and non-CM cells. Here, we report an entirely novel system in which PSC-derived CMs are purified by CM-specific molecular beacons (MBs). MBs are nano-scale probes that emit a fluorescence signal when hybridized to target mRNAs.

Method and Results: Five MBs targeting mRNAs of either cardiac troponin T or myosin heavy chain 6/7 were generated. Among five MBs, a MB targeting myosin heavy chain 6/7 mRNA (MHC1-MB) identified up to 99% of HL-1 CMs, a mouse CM cell line, but < 3% of four non-CM cell types in flow cytometry analysis, indicating that MHC1-MB is specific for identifying CMs. We delivered MHC1-MB into cardiomyogenically differentiated PSCs through nucleofection. The detection rate of CMs was similar to the percentages of cardiac troponin T (TNNT2) or cardiac troponin I (TNNI3)-positive CMs, supporting the specificity of MBs. Finally, MHC1-
MB-positive cells were FACS-sorted from mouse and human PSC differentiating cultures and ~97% cells expressed TNNT2- or TNNI3 determined by flow cytometry. These MB-based sorted cells maintained their CM characteristics verified by spontaneous beating, electrophysiologic studies, and expression of cardiac proteins. When transplanted in a myocardial infarction model, MB-based purified CMs improved cardiac function and demonstrated significant engraftment for 4 weeks without forming tumors.

Conclusion: We developed a novel CM selection system that allows production of highly purified CMs. These purified CMs and this system can be valuable for cell therapy and drug discovery.

10:30 am

High volume, moderate intensity adapted tango benefits mobility and dual tasking ability in Parkinson's disease. Hackney ME, Revill KP.

Adapted tango (AT) has improved mobility in individuals with Parkinson Disease (PD) over 10-12 weeks. This study examined the effects of 3 weeks of high volume, moderate intensity AT, on balance, spatial cognition and dual task ability. In a pre-post, repeated baseline measures, with removed-treatment design, 20 participants with PD (age: 64.5(13.2); Hoehn & Yahr stage: 2.3(4.4); PD duration: 5.9(3.8) y) were assigned to AT (fifteen, 1.5h classes). Participants were tested while "ON" medications 3 weeks before (n=7), immediately before and after (n=20), and 3 weeks after (n=13) for: disease severity (Unified Parkinson Disease Rating Scale), dynamic postural control (Dynamic Gait Index), balance (Berg Balance, Fullerton Advanced Balance), endurance (six-minute walk test), cognitive and manual dual tasking (Timed Up&Go cognitive and manual), leg strength (30-s chair stand, two-footed jump), spatial ability (Corsi blocks, Brooks task), and balance confidence (Activities-specific Balance Confidence). Forward, backward and fast gait speed and cadence were measured. Measures were stable pre-intervention in these participants (ICC>0.813). All participants completed 15 classes in 3 weeks. Significant improvements from pretest were noted on dynamic postural control (p=0.004), balance (p=0.001), advanced balance (p<0.001), forward cadence (p=0.03), fast gait speed (p=0.04), fast cadence (p=0.01), two-footed jump (p=0.006), leg strength (p=0.05), and cognitive dual tasking (p=0.05). No changes were noted in balance confidence or cognitive measures. Improvements were maintained at follow-up (post to follow-up; p>0.106). High volume AT is feasible, and may improve mobility and dual tasking ability in those with PD.

10:45 am

Insulin and LPA restore intestinal fluid homeostasis in type 1 diabetes via activation of NHE3 He P, Zhu L, Srinivasan S, Yun CC.

Diarrhea is a troublesome intestinal complication of diabetes with a prevalence of over 20%. The cause of diabetic diarrhea is complex and remains elusive. The goal of this study is to determine whether Na+/H+ exchanger NHE3, the membrane transporter that mediates back of Na+ and fluid absorption in the intestine, contributes to diabetic diarrhea. We found that induction of diabetes in CF-1 mice by streptozotocin resulted in a significant decrease in net fluid absorption and NHE3 activity in mouse ileum. Immunoblotting analysis revealed that expression of NHE3 and its binding proteins IRBIT and NHERF1-2 was decreased in the brush border membrane (BBM). Reintroduction of insulin (i.p.) stimulated NHE3 transport activity and partially restored intestinal fluid absorption in mice. By using intestinal Caco-2bb and SK cells, we determined the mechanisms of NHE3 activation by insulin. Insulin activated PI3K-PDK2 signaling which, in turn, increased phosphorylation of IRBIT and Ezrin and their association with NHE3. Insulin thus promoted the formation of a macromolecular complex that involves IRBIT, Ezrin, NHERF1, and NHE3. More importantly, knockdown of either IRBIT or NHERF1 or expression of dominant negative Ezrin abrogated insulin-induced exocytosis and activation of NHE3. Previously we demonstrated that lysosphatidic acid (LPA) stimulates NHE3-dependent intestinal fluid absorption. Here we found that LPA (gavage) increased intestinal NHE3 activity and fluid absorption in diabetic mice. In conclusion, these findings highlight that NHE3 contributes to diabetes-induced defects in fluid absorption and implicate that insulin and LPA can ameliorate diabetic diarrhea through their stimulatory effects on NHE3 protein.

11:00 am


Background: Avoiding the use of potentially inappropriate medications (PIMs) is an important, simple and effective strategy in reducing medication-related problems in older adults.

Methods: EQUIPPED is a quality improvement initiative aimed at changing prescribing practices of Emergency Department (ED) providers at the Atlanta VA Medical Center. Specifically, the goal is to decrease the number of PIMs prescribed to Veterans 65 and older at time of ED discharge. Interventions include: provider education; electronic decision support and individual performance feedback. Prescription data for the ED were analyzed monthly from February – July 2013 and compared to May 2012 as baseline.

Results: At baseline (May 2012) ED staff providers (n=16) prescribed 51 PIMs out of 346 total prescriptions (14.7%) to discharged Veterans 65 and older. In July 2013 (post all
Conclusions: The EQUIPPED intervention led to a significant reduction of ED provider prescribed PIMs in the elderly that was sustained over 6 months. Longer follow-up and larger sample sizes are needed to assess impact on patient health outcomes and savings. This model is being disseminated to other VA EDs and other sites of geriatric care within VA.

2:00 pm

Cardiomyocytes do not terminally differentiate in the neonatal period

Naqui N, Li M, Calvert J, Tejada T, Lefer DJ, Graham RM, Husain A.

It is widely believed that within a couple of days after birth, cardiomyocytes of mammals permanently withdraw from the cell cycle (that is, terminally differentiate) restricting postnatal heart growth to cardiomyocyte hypertrophy. Cardiomyocyte terminal differentiation requires stem cell factor receptor (c-kit) signaling. Here we show that murine heart grows a remarkable ~400% in the preadolescent period resulting in an ~300% increase in stroke volume. Sequential analysis of cardiomyocyte numbers, volume and nucleation state, and cardiac cell cycle gene expression, indicate that this mass change results from a discrete temporal burst of cardiomyocyte proliferation leading to an ~40% increase in ventricular cardiomyocyte numbers between postnatal days 14 and 18, flanked by periods of cardiomyocyte enlargement. This hyperplastic burst, associated with a marked increase in serum 3,5,3'-triiodothyronine (T3) levels, was abolished by anti-thyroid treatment with propylthiouracil. Further, we show that until after the proliferative burst, the number of ventricular cardiomyocytes in wild type mice is similar to that in transgenic mice in which cardiomyocyte terminal differentiation is permanently inactivated through c-kit inhibition. Thereafter, at postnatal day 35, we find ~20% more cardiomyocytes in the latter. This and differential in vitro proliferation rates of postnatal day 35 cardiomyocytes isolated from wild-type and dominant-negative c-kit transgenic mice indicate that cardiomyocytes do not terminally differentiate until around puberty. These findings suggest a previously unknown cellular mechanism for preadolescent heart growth and suggest that therapeutic strategies may be developed to harness cardiac regeneration for children with congenital heart diseases before the onset of puberty.

2:15 pm

Efficacy and Safety of High-Dose Vitamin D3 For Treatment of Pulmonary Tuberculosis: A Randomized Controlled Trial

N Tukvadze, E Sandikidze, M Kipiani, G Hebbar, RR Kempler, JK Frediani, C DelRío, V Tangpricha, HM Blumberg, TR Ziegler.

Background: Patients with tuberculosis (TB) are commonly vitamin D deficient, which may impact immunity. Design/Methods: Double blind, randomized, controlled, intent-to-treat (ITT) trial in adults with pulmonary TB in Tbilisi. Subjects were randomized to oral vitamin D3 (1,400,000 IU in divided doses) or placebo over 16 weeks, concomitant with anti-TB drugs. Sputum AFB cultures (LJ) were performed for 16 weeks. Cox proportional analysis was used to assess time to culture conversion. Results: Of 199 patients enrolled, 100 received vitamin D3 and 99 placebo; baseline characteristics were similar. Most subjects (> 85%) were vitamin D deficient at entry [serum 25-hydroxyvitamin D [25(OH)D] < 20 ng/mL]. With vitamin D3, serum 25(OH)D levels peaked at ~ 100 ng/mL at 8 weeks and decreased to ~ 60 ng/mL by week 16. High-dose vitamin D treatment was safe, with similar adverse events and serum calcium levels between groups. 192 subjects with confirmed TB infection were included in modified ITT efficacy analysis. 23 (12%) had confirmed multi-drug resistant TB (MDR-TB; 12 vitamin D, 11 placebo). Culture conversion rates were similar between the vitamin D and placebo groups. However, MDR-TB subjects given vitamin D3 exhibited enhanced culture conversion at 8 weeks (88% vs. placebo 40%; p=0.03) and over time (p=0.02). Conclusions: High-dose vitamin D3 treatment was safe but did not improve TB clearance in the overall study cohort. However, patients with MDR-TB randomized to vitamin D demonstrated a shorter time to TB culture conversion. Additional trials on the efficacy of high-dose vitamin D3 in MDR-TB patients are warranted.

3:15 pm

Hydrogen Peroxide Regulates Osteopontin Expression through Activation of Transcriptional and Translational Pathways


Recent in vivo studies establish that osteopontin (OPN) expression is hydrogen peroxide (H2O2)-dependent; however, the mechanism(s) by which this occurs remain poorly defined. Therefore, further investigation into the signaling events responsible for H2O2-dependent OPN expression is needed. We found that OPN protein expression increases in an unusual bi-phasic pattern (6h 96.9%±1.5, p<0.001; 18h 234.0%±3.6, p<0.001). To investigate if H2O2-induced increases in OPN were transcriptional and/or translational, smooth muscle cells stimulated with 50μM H2O2 were used as an in vitro system. Early protein increases at 6 hours were not preceded by
increased mRNA, whereas late increases (18h 120.2%±5.2, p<0.005) were, suggesting multiple mechanisms of regulation by H2O2. Polysomal fractionation assays established that early increases (6h) in OPN expression are due to increased translation, which occurs through phosphorylation of 4E-BP1 at the ROS-sensitive Ser-65. This allows for release and activation (89.4%±6.1, p<0.05) of eukaryotic initiation factor elf4E (139.2%±3.9, p<0.05) and subsequent OPN translation. This early increase (6h) in OPN was blunted by a phospho-deficient 4E-BP1 mutant. H2O2 stimulation increased rat OPN promoter activity at 8h (2.1±0.3 fold, p<0.05) and 18h (5.0±0.8 fold, p<0.001) and promoter truncation studies established that promoter region -2284 to -795 is crucial for H2O2-dependent OPN transcription. Chromatin immunoprecipitation (ChiP) studies determined that H2O2-dependent transcription is mediated by the ROS-dependent transcription factors NF-κB and AP-1. In conclusion, H2O2 increases OPN expression through translation- and transcription-dependent mechanisms. As a potential therapeutic target, it is critical to understand how OPN expression is regulated in disease pathologies with underlying increases in H2O2.

3:30 pm

Influence of age on durability of initial combined antiretroviral therapy in the established antiretroviral era


Background: The HIV epidemic in the 21st century has spread to the younger and the older population. We present the effect of age on durability of first antiretroviral regimen (ART).

Methods: This is a retrospective chart review of adult patients (older than 18 years old) starting ART at the Ponce de Leon Clinic from 6/1/2004 to 12/31/2011. Demographic characteristics along with relevant HIV-related measures were recorded. Patients were grouped into young (18-39), middle aged (40-49), and elderly (50 years or older). Log-rank tests were used to compare regimen change according to baseline clinical characteristics. Univariate and multivariate Cox proportional hazards regression analyses were performed.

Results: Most of the 967 HIV-1 naïve adult patients were male (76%), African-American (83%), with a mean age at HIV diagnosis of 37 years (SD 10.6). The overall cumulative regimen change (RC) was 34.6% (95% CI: 31.5%, 37.7%) at 12 months and 62.3% (95% CI: 58.6, 66.0) at 48 months. RC was lower in older individuals (p=0.0009). The adjusted risk for RC decreased approximately 14% per 10-year-increase in baseline age. (HR 0.86; 95% CI: 0.79 to 0.94). Other risk factors independently associated with better durability of cART included Hispanic ethnicity (p=0.003), baseline BMI ≥ 25 kg/m2 (p=0.02) and once daily regimen (p=0.0015). Coinfection with Hepatitis C increased the risk of RC by 40% (p=0.03).

Conclusion: In our retrospective cohort, the predictors of first ART durability were older age group, Hispanic ethnicity, higher BMI, and once daily regimen. HCV coinfection negatively impact the ART duration.

Poster Presentations

In order of poster number. See index for list of presenting authors.

Morning poster session 9:15 – 10:00 AM

Posters (1-10)

1

Frequency and Temporal Patterns of ICD therapies in patients with left ventricular assist devices

Appelbaum JM, Weragoda R, Lloyd M, Langberg J, Hoskins M.

Background: Left ventricular assist device (LVAD) use is becoming increasingly common in patients with end-stage heart failure. These patients are at high risk for ventricular arrhythmias. The pattern of ICD therapies in these patients is not well characterized.

Methods: Our retrospective cohort included 86 patients with ICDs and LVADs. Data collected included frequency of ICD therapies, type of therapy, and time-course.

Results: 36 patients (42%) received ICD therapy during a mean follow-up period of 13.6 months. There were 105 episodes (2.9 episodes/patient) during which ICD therapies were delivered, with a total of 379 arrhythmias treated (3.6 arrhythmias per episode of therapy). 61.4% of therapies were appropriate, 8.4% were inappropriate, and 31.6% were unknown. Of the 79 episodes treated with appropriate therapy, 36 episodes (46%) required ICD shocks, whereas 43 episodes (54%) were treated with ATP alone. Overall, 33% of the cohort and 81% of patients who received therapy received at least one ICD shock. Twenty-two patients (61%) received therapy 1-3 times, while the remainder (14 patients, 39%) received therapy 3-10 times. Eighteen (17%) episodes occurred within the first month after LVAD implantation, with 10 (10%) occurring 1-3 months, 53 (50%) occurring 3-12 months, and 24 (23%) occurring more than one year after implantation.

Conclusion: Analysis revealed: 1. ICD therapy is very common (42%) in the first year after LVAD implantation; 2. Ventricular arrhythmias are highly clustered in this cohort; 3. ATP is frequently effective. These findings have important implications on device follow-up and programming for this expanding patient population.

2

The Prevalence of Subclinical Iron Deficiency in Patients with Irritable Bowel Syndrome

Yarandi S, Christie J.

Background: Iron is a modulator of intestinal motility and iron deficiency may be involved in the pathophysiology of irritable
The prevalence of subclinical iron deficiency in the general population is 9 to 15%, but this prevalence is unknown in IBS patients.

Methods: Patients diagnosed by a gastroenterologist according to Rome criteria with IBS from 2000 to 2013 who had iron studies were included. Patients with concurrent GI disorders other than gastroesophageal reflux and history of gastrointestinal surgery were excluded. We used a serum ferritin level <26 ng/mL, which predicts depletion of iron stores with high sensitivity and specificity, and normal hemoglobin as definition of subclinical iron deficiency.

Results: A total of 107 patients were included, 93 (87%) females and 14(13%) males with mean age of 39 and 35 years old, respectively. Six patients were postmenopausal. Thirty-six patients were African-American (34%) and 71 were Caucasians (66%). Forty patients (37%) had IBS-D, 27 (26%) had IBS-C and 40(37%) had mixed symptoms. The mean of serum ferritin level was 31.23 ng/dL in females and 121.5 ng/dL in males. Sixty-one (57%) females and 2(2%) males had serum ferritin level <26 ng/dL. Of these patients, 55 (51%) fulfilled the criteria for subclinical iron deficiency. Mean serum ferritin level in these patients was 11.53 ng/dL. ANOVA showed no significant difference between IBS subtypes (p= 0.741) or race (p=0.375).

Conclusion: This data suggests that the prevalence of subclinical iron deficiency is high in patients with IBS, regardless of race or subtype.

3 OutFoxOing Skeletal Muscle Atrophy with miR-182

Skeletal muscle atrophy occurs in response to a variety of conditions including diabetes, chronic kidney disease, cancer, mechanical ventilation, and HIV/AIDS. Previous studies have demonstrated that activation of the Forkhead box O (FoxO) transcription factors results in skeletal muscle atrophy in patients, animals and cultured cells. The FoxO proteins cause muscle wasting by increasing the expression of components of the ubiquitin-proteasome and autophagy-lysosome proteolytic systems. To identify potential modulators of the atrophy process, an in silico target scan analysis of known microRNAs (miRs) was performed, and miR-182 was predicted to target the FoxO mRNAs. To test whether miR-182 regulates expression of the FoxOs, C2C12 myotubes were transfected with miR-182 and levels of FoxO1 and FoxO3 proteins were evaluated. miR-182 reduced the amount of FoxO3 but not FoxO1. Treatment of C2C12 myotubes with dexamethasone (1 μM, 6 hr) to induce muscle atrophy decreased miR-182 expression by 63% (P<0.05). Transfection of miR-182 into myotubes prevented the glucocorticoid-induced upregulation of multiple FoxO3 target mRNAs including MAFbx/Athrogen-1, ATG12, Cathepsin L, and LC3. To determine if miR-182 is altered in an in vivo model of muscle atrophy, miR-182 was measured in the gastrocnemius muscle of rats with acute diabetes (3 d) induced by streptozotocin. miR-182 was decreased 44% (P<0.05) by diabetes. These data identify miR-182 as a new and important regulator of FoxO3-mediated signaling during muscle atrophy induced by catabolic disease states. Source of funding: NIH T32 DK007656 (M.B.H.) and NIH R01DK95610, AHA GRNT7660020, and VA Merit (S.R.P.)

4 Outcomes of Octogenerians after Cardiac Resynchronization Therapy
Mohamed Kelli H, Merchant F, Mengistu A, Hoskins M, El Chami M.

Background
Cardiac resynchronization therapy (CRT) reduces mortality and morbidity in patients with left bundle branch block and symptomatic systolic heart failure (HF). Data on procedural safety of CRT in octogenerians and intermediate-term survival of this group of patients after CRT is limited.

Methods
We identified 96 consecutive patients ≥ 80 years of age who underwent an initial implant or an upgrade to a CRT system (both CRT-defibrillators or pacemakers) at our institution between January 2003 and July 2008. Then we randomly selected 177 patients < 80 years of age who underwent an initial implant or an upgrade to a CRT system during the same time at our institution. Baseline clinical and demographic patients’ characteristics were collected. Follow-up data included, long-term mortality and rate of appropriate and inappropriate ICD shocks.

Results
The characteristics of the 2 cohorts are summarized in table 1. The rate of appropriate shocks were higher in the control vs octogenerian group (27% vs. 14%, p=0.024) while the rate of inappropriate shocks were similar (6% vs. 3%, p=0.55). The 3 year mortality of the 2 cohorts was also similar (8% vs. 11%, p=0.381)(figure)

Conclusion
Octogenerians who are candidates for CRT have similar periprocedural outcomes and similar intermediate-term mortality to a younger cohort of HF patients receiving CRT. Candidates for CRT should be offered this therapy even if they are of advanced age.

5 The Prevalence of Vitamin D Deficiency in Patients with Irritable Bowel Syndrome
Yarandi S, Christie J.

Background: Irritable bowel syndrome (IBS) is associated with a higher prevalence of osteoporosis. Vitamin D has been used...
in the treatment of IBS with variable success but the prevalence of vitamin D deficiency in patients with IBS is unknown.

Methods: Patients diagnosed by a gastroenterologist according to Rome criteria with IBS from 2000 to 2013 who had vitamin D level documented were included. Patients with concurrent GI disorders other than gastroesophageal reflux, history of gastrointestinal surgery and chronic kidney disease were excluded. We used a serum 25-hydroxy vitamin D level <30 nmol/L as the definition of vitamin D deficiency.

Results: One hundred patients were included, 93 (93%) females and 7(7%) males with mean age of 43 and 48 years old, respectively. Twenty-nine patients were African-American (29%) and 71 were Caucasians (71%). Twenty-nine patients (29%) had IBS-D, 38 (38%) had IBS-C and 33(33%) had mixed symptoms. The mean of vitamin D level was 25.05 nmol/L. There was no significant difference based on sex (p=0.17) or IBS subtype (p=0.77) while Caucasians had higher levels of vitamin D (26.94 vs. 20.43 p=0.008). Seventy-two (72%) females and 3(3) males had vitamin D deficiency. ANOVA in this sub-group showed no significant difference based on IBS subtype (p= 0.399) but showed higher levels in Caucasians (20.91 vs. 16.81, p = 0.005).

Conclusion: This data suggests that the prevalence of vitamin D deficiency is high in patients with IBS, regardless of the predominant symptom. African Americans with IBS have lower vitamin D levels compared to Caucasians.

6 Resistance exercise prevents muscle wasting in mice with chronic kidney disease by increasing microRNA-23
Zhang C, Wang XH.

There are 23 individual microRNAs that are significantly changed in muscle of chronic kidney disease (CKD) mice (Wang et al, JASN 2010). This study identifies in silico analysis that microRNA-23 (miR-23) targets several proteins associated with muscle atrophy.

Since atrogin-1, MuRF-1, YY1 and PTEN are putative targets of miR-23, and each of them can impact muscle wasting, we verified whether miR-23 changes the expression of each target using 3’-UTR-luciferase reporter assays. Overexpression of miR-23 in cultured muscle cells inhibits the reporter gene activities of atrogin-1 and MuRF1, suggesting that miR-23 attenuates the expression of its predicted targets. Exercise significantly increased the level of miR-23 in muscle of CKD mice vs. unexercised CKD mice. Consistent with the response, exercise decreased PTEN protein and increased Akt phosphorylation (2.3-fold, P<0.001) which would limit muscle atrophy. Exercise increased MyoD, myogenin and eMyHC, myogenic proteins that are linked to YY1 transcription factor in CKD mice. Finally, mRNAs for the muscle-specific E3 ubiquitin ligases, atrogin-1 and MuRF-1, were attenuated by exercise in CKD mice. Other muscle-specific miRs (i.e., miR-1, miR-133b and miR-206) were unchanged by exercise in muscle of CKD mice, indicating specificity of the miR-23 response.

Conclusions: Resistance exercise can prevent CKD-induced muscle loss by increasing miR-23. This leads to a suppression of multiple atrophy-related target proteins that may contribute to the muscle-sparing effects of exercise in CKD.

7 Phosphodiesterase inhibition increases PGC-1α in muscle cells
Rahner JA, Zheng B, Price SR.

Muscle wasting associated with chronic diseases such as type I diabetes (DM) can be countered by restoring the reduced expression of the transcriptional coactivator PGC-1α. Previous reports indicate that PGC-1α transcription can be regulated by CREB in a process that requires nuclear localization of CRTC (CREB-regulated transcription coactivator). We recently found that CRTC protein is reduced in skeletal muscle of diabetic rats whereas CREB is highly phosphorylated (i.e., activated). Nuclear translocation of CRTC is promoted by dephosphorylation via calcineurin (CaN) whereas nuclear export occurs by phosphorylation via salt-inducible kinase (SIK). Inhibition of phosphodiesterases (PDE), which raise cyclic AMP (cAMP) levels, results in increased PGC-1α mRNA expression. cAMP also inhibits SIK suggesting this pathway may contribute to CRTC localization and thus PGC-1α activity. Our experiments tested the hypothesis that inhibition of PDE increases the amount of nuclear CRTC and PGC-1α transcription. Myotubes were transfected with luciferase reporter plasmids (Luc) and treated with the general PDE inhibitor IBMX (500uM) for 6 hours. IBMX increased PGC-1α-Luc promoter activity as well as the transcriptional activity of CREB. Deletion of the CRE site in PGC-1α-Luc prevents the increase in luciferase activity suggesting IBMX increases PGC-1α activity via CREB. In addition, protein analysis indicates IBMX increases the level of nuclear CRTC. Together, these data imply that raising cAMP levels by inhibiting PDEs results in increased PGC-1α transcriptional activity by a mechanism involving CREB and its coactivator, CRTC. Supported by NIH RO1 DK95610 and VA merit.

8 Hemin-Induced miR-27a Reduces PPARγ Expression in Sickle Cell Disease with Pulmonary Hypertension

The hallmark of sickle cell disease (SCD) is hemolysis, vasoocclusion, and oxidative stress. Pulmonary hypertension (PH) is a serious complication of SCD that causes significant morbidity and mortality. We previously demonstrated that: 1) activation of the nuclear hormone receptor, peroxisome proliferator-activated receptor gamma (PPARγ), attenuated...
hypoxygen-induced Nox4 and endothelin-1 (ET-1) expression and PH in mice and 2) that levels of Nox4 and ET-1 were increased in the lungs of 12 weeks old SCD transgenic mice compared to controls whereas PPARγ levels were reduced. The current study further examines mechanisms of PH in SCD. Human pulmonary arterial endothelial cells (HPAECs) were treated with the hemolysis product, hemin (5 μM), for 72 h. Hemin reduced PPARγ and increased Nox4 and ET-1 expression and HPAEC proliferation. MicroRNA-27a (miR-27a), which negatively regulates PPARγ, was increased in hemintreated HPAECs. In contrast, treating HPAECs with the PPARγ ligand, rosiglitazone (10 μM) for the final 24 h of hemin exposure attenuated increased HPAEC proliferation and miR-27a levels. These findings suggest that hemin increases miR-27a to reduce PPARγ and increase Nox4, ET-1, and HPAEC proliferation. These results suggest novel pathways for SCD-PH pathogenesis and therapy.

9

Echocardiography for Right Ventricular Failure Prediction After Implantation of Left Ventricular Assist Devices: Preliminary Results From a Prospective Cohort Study

Introduction: Right ventricular failure (RVF) occurs in 20%-40% of left ventricular assist device (LVAD) recipients. Preoperative clinical scores inadequately predict RVF risk in these patients.

Methods: Beginning 6/2012, all adults with INTERMACS ≥2 profile scheduled for LVAD implantation, without planned right ventricular (RV) support, were prospectively enrolled in a study evaluating standard and speckle-tracking echocardiographic parameters of RV function for RVF prediction. Preoperative echocardiograms were performed 5±5 days prior to LVAD implantation by study protocol. The primary endpoint was RVF, defined (by INTERMACS) as need for (1) inotropes or pulmonary vasodilators any time past 7 days post-LVAD implant with concomitant evidence of high central venous pressure and low cardiac index or (2) mechanical RV support. The secondary endpoint was RVF or death from any cause.

Results: As of 5/2013, 26 patients have been enrolled. Preoperative clinical and hemodynamic characteristics are presented in Table 1. Overall, 7 patients (26.9%) developed RVF and 3 patients (11.5%) died at 30 days. Intensive care length of stay (LoS) was 8.0±3.6 days and total LoS was 16.3±9.5 days. Among echocardiographic parameters (Table 2), RV global longitudinal strain (GLS) was the strongest predictor of RVF with a C statistic of 0.86 (95% CI, 0.60 to 0.99). At a cut-off -6.0%, sensitivity and specificity were 86% and 79%, respectively. RV GLS was predictive of combined death or RVF. Standard RV function parameters were weak outcome predictors.

Conclusion: Among preoperative echocardiographic parameters, RV GLS by speckle tracking appears to be the strongest predictor of post-LVAD RVF.

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Chronic Calcineurin Inhibition Impairs Both Recovery from Isoflurane Anesthesia and Visuospatial Learning Performance
Ma CM, Fidler J, Garcia PS, Gooch JL.

Patients suffering from diseases that chronically impair neurocognitive function, such as dementia, depression, or schizophrenia, are at greater risk for a complicated postoperative recovery of executive function. The pathogenesis of these and other neurocognitive conditions is associated with abnormal activity of the protein phosphatase, calcineurin. For decades calcineurin inhibitors, such as cyclosporine, have been a mainstay for post-transplant immunosuppression, but the potential for chronic administration of these drugs to adversely affect cognition warrants investigation. We explore the role of this ubiquitous enzyme in modifying recovery from general anesthesia. Young adult male wild type C57/Bi6 mice (n=20) were exposed to either 30 minutes of isoflurane anesthesia or sham procedure. These experiments were repeated in animals treated with 12 days of cyclosporine (20mg/kg/d) prior to the anesthesia/sham challenge. Ambulatory activity was recorded via a cage-mounted automated monitoring system. Visuospatial learning was assessed via the water radial arm maze (WRAM). The data show that chronic calcineurin inhibition impairs both cognitive recovery from general anesthesia and visuospatial learning in our model. Whereas exposure to isoflurane alone causes a modest decrease in exploratory activity, there is an amplification of adverse neurocognitive sequelae post-anesthesia when combined with chronic cyclosporine treatment. These results suggest that chronic calcineurin inhibition adversely affects learning and exploratory behavior and accelerates the adverse cognitive consequences of isoflurane anesthesia. Further work is necessary to characterize the role of this enzyme in cognitive problems and to optimize the anesthetic for patients receiving cyclosporine therapy.

11

Integrated redox proteomics and metabolomics to identify mechanisms of Cd toxicity
Young-Mi G, Orr M, Jones DP

Cadmium (Cd) exposure contributes to human diseases affecting liver, kidney, lung and other organ systems, but mechanisms underlying the pleotropic nature of these toxicities are poorly understood. Cd accumulates in humans from dietary, environmental (including cigarette smoke), and occupational sources, and has a twenty-year biologic half-life. Our previous mouse and cell studies showed that environmental low dose Cd exposure altered protein redox states resulting in stimulation of
inflammatory signaling and disruption of actin cytoskeleton system, suggesting that Cd could impact multiple mechanisms of disease. In the current study, we investigated effects of acute Cd exposure on the redox proteome and metabolome of mouse liver mitochondria. To identify redox-sensitive liver proteins and gain insight into associated toxicological mechanism and functions, we analyzed redox states of liver mitochondrial proteins by redox proteomics using ICAT (isotope coded affinity tag)-combined mass spectrometry. Redox ICAT identified 2687 cysteine-containing peptides (peptidyl Cys). Of these, 48% (1302 peptidyl Cys) were oxidized more than 1.5-fold relative to controls. Bioinformatics analysis using MetaCore software showed that oxidized peptidyl Cys by Cd affected 89 pathways, including 24 Cys in proteins functioning in branched chain amino acid and 4 proteins functioning in fatty acid (acylcarnitine/carnitine) metabolism. Consistently, high-resolution metabolomics data showed that Cd treatment elevated levels of branched chain amino acids and carnitine metabolites. Together, these results show that mitochondrial protein redox and metabolites are targets in Cd-induced hepatotoxicity. The results indicate that redox proteomics and metabolomics can be used in an integrated systems approach to investigate complex disease mechanisms.

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Relaxin attenuates Angiotensin II induced proinflammatory signaling in vascular smooth muscle cells
Vukelic S, Lassegue B, Griendling K.

Relaxin 2 (RLX), a member of the insulin-like family of peptides, was traditionally considered as a reproductive hormone. However, recent studies indicate that it also has potent short-term vasodilatory and long-term arterial remodeling and anti-fibrotic effects. Increased secretion of the monocyte chemoattractant protein-1 (MCP-1) and Interleukin 6 (IL-6) are initial steps in vascular inflammation, an important early step in the development of cardiovascular disease. Angiotensin II (Ang II), the principal hormone of the renin-angiotensin system, has been suggested as one of the crucial mediators of vascular inflammation. In the present study, we tested the hypothesis that RLX can attenuate the Ang II effect on vascular inflammation. The effect of RLX cotreatment on Ang II stimulated secretion of MCP-1 and IL-6 by human and mouse aortic smooth muscle cells was quantified by ELISA. RLX abolished the Ang II-induced increase in the MCP-1 and IL-6 secretion. This effect is possibly a consequence of RLX inhibition of the Ang II signaling through the MAPK/ERK pathway, as evidenced by decreased phosphorylation of ERK on western blotting. This inhibitory effect of RLX on secretion of the proinflammatory cytokine IL-6 and the chemokine MCP-1 can lead to decreased monocyte recruitment and activation in the vascular wall, and consequently attenuated vascular inflammation and ECM remodeling, endpoints to be tested later in this study. This effect may be relevant for the therapeutic use of RLX in the treatment of pro-inflammatory cardiovascular conditions such as acute heart failure, atherosclerosis, aortic dissection and aneurysms.

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Docosahexaenoic acid counteracts palmitate-induced proteolytic signaling in myotubes

Dyslipidemia is a comorbidity common to illnesses like diabetes and chronic kidney disease which contributes to debilitating muscle atrophy. The saturated fatty acid palmitate (PA) induces muscle insulin resistance and disrupts protein metabolism in cultured myotubes, whereas the omega-3 fatty acid docosahexaenoic acid (DHA) has beneficial metabolic effects. We evaluated the effects of PA and DHA on atrophy-related signaling in skeletal muscle cells, with the hypothesis that DHA prevents PA-induced myotube atrophy by counteracting the stimulatory effects of PA on protein degradation. C2C12 myotubes were treated with 500µM PA and/or 100µM DHA for up to 28h. PA increased the rate of protein degradation, while co-treatment with DHA completely prevented the response. Akt is a key modulator of protein balance that inhibits the FoxO3 transcription factors which regulate "atrogene" expression. PA reduced the activation state of Akt (phospho:total Akt) as well as increased the level of nuclear FoxO3 protein while decreasing cytosolic FoxO3. Accordingly, PA also increased the mRNA levels of two FoxO3 atrogen targets, the E3 ubiquitin ligase atrogin-1/MAFbx and the autophagy mediator Bnip3. DHA reversed the effects of PA on Akt, FoxO3, and both atrogenes. These data indicate that PA induces myotube atrophy by inducing components of the ubiquitin-proteasome and autophagic proteolytic systems and that DHA counters the catabolic effects of PA by improving Akt signaling.

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Cardiac remodeling and hypertension in elite Division I-A football players: A longitudinal pilot study
Kim JH, Patton J, Williams BR, Sperling L, Quyyumi AA.

Previous data has demonstrated a high prevalence of prehypertension and hypertension in elite, professional American-style football (ASF) players. There is little known regarding the presence of hypertension in collegiate ASF players. Additionally, it remains unknown the mechanism(s) responsible for the development of hypertension in elite ASF players, and whether specific underlying clinical or phenotypic factors are associated with hypertensive football players. We conducted a pilot, longitudinal study evaluating 16 incoming freshmen football players at the Georgia Institute of Technology who were followed from the pre-season through the end of the
The Melatonin in nighttime blood pressure in African-Americans (MAP) trials: design and baseline characteristics

Rahbani-Oskoui F, Bruckman AM, Abramson J, Bliwise D, Chapman A.

Background: Previous studies have shown a positive role of melatonin in reducing nighttime blood pressure. The magnitude of this effect in the high risk group for cardiovascular events of African-Americans with nocturnal hypertension is unknown. It is also unclear whether the blood pressure effect is due to a better quality of sleep or independent of it.

Methods: In a phase II, randomized, double blind, cross over design, forty patients with essential hypertension, on less than 3 drugs, were randomized to be exposed to 4 weeks of immediate release oral melatonin or placebo at a daily dose of 8 mg at bedtime followed by 4 additional weeks of exposure to the other agent. Another overlapping group of 40 patients were exposed to 24 mg daily dose with the same design. Routine laboratory tests, overnight polysomnography (PSG) sleep studies, and 24h ambulatory blood pressure monitoring was performed at baseline and after each arm of treatment. Additional tests included the measurement of plasma p-selectin, e-selectin, and catecholamine levels and urinary 6-sulfatoxymelatonin as a marker of compliance.

Results: Since the study is ongoing for 2 more months, we are only presenting the baseline characteristics of the studied cohorts.

Conclusion: We have successfully completed the recruitment for the MAP trials and are presenting the design and baseline characteristics of the MAP trials. Final results should be available in Oct 2013.

Mast Cell Chymase: An Angiotensin II (Ang II)-Independent Therapeutic Target In The Post-Myocardial Infarction Heart

Tejada T, Zlatopolosky M, Bhushan S, Naqvi N, Abrik M, Pfeijer G, Lefer D, Husain A

Mast cell chymase (CHY) is a multifunctional protease with Ang II-forming activity. VALIANT trial combined an AT1 Ang II receptor blocker (ARB) to an ACEi post-myocardial infarction (MI) but showed no additional benefit, suggesting CHY is unimportant post-MI. However, the role of CHY, independent of its Ang II-forming activity, remains unaddressed. We evaluated this by blocking the effects of ACE+CHY-generated Ang II using an ACEi+ARB+AT2 receptor blocker combination (AAA), and compared this to ACEi monotherapy in wild-type (WT) and chymase (MMCP-4)-deficient mice. WT and KO mice underwent sham or MI surgery, and were treated daily with vehicle (Veh), ACEi, or AAA 24h post reperfusion. In WT mice post MI, ACEi vs Veh resulted in improved ejection fraction (EF) (34±1.8% vs 28±1.7%; p<0.05) and reduced LV end-diastolic dimension (LVEDD) (4.2±0.9 mm vs 4.7±1.0 mm; p<0.05). However, AAA did not result in further improvements in either parameter as compared to ACEi, suggesting that CHY-generated Ang II is unimportant post-MI, confirming VALIANT. While the 24-h post-MI infarct sizes were similar between genotypes (p=0.6), EF was superior in KO vs WT mice in all treatment groups (36±3% vs 28±2% (Veh); 47±3% vs 34±2% (ACEi); 49±3% vs 36±3% (AAA); p<0.05). Further, LV dilatation was also more pronounced in WT mice (LVEDD: 4.7±0.4 mm, WT-Veh vs 4.3±0.5 mm, KO-Veh; p<0.05). ACEi decreased mean arterial pressure post-MI (p<0.01), but not differentially in WT vs KO. Thus, we conclude that CHY, independent of Ang II, is a potential therapeutic target for the treatment of the post-MI heart.

Measuring domestic violence among married women in India: development of the Indian Family Violence & Control Scale

Kalokhe AS, del Rio C, Sahay S

The high prevalence of domestic violence (DV) in India and its association with poor mental, physical, and sexual health underscore the need for enhanced DV prevention strategies and an instrument to accurately measure prevalence and the efficacy of such interventions. We hypothesized that Western scales fall short in measuring DV in the Indian context because of differences in Indian family structure, in readily-available tools to inflict physical DV, and the normalization and acceptance of DV. We thus aimed to develop and validate a culturally-tailored scale to effectively measure DV among married women in India. This mixed-methods study utilized a formative phase (a systematic literature review of the prior decade of Indian DV literature, key informant interviews and focus groups, and field pre-testing) to inform the development
of a culturally-tailored 63-question item pool. In the quantitative phase, this item pool was tested in a randomly-selected, developmental sample of 630 married women in Pune, India. Analysis is currently underway to reduce the items to yield the Indian Family Violence and Control Scale (IFVCS) and assess the scale's psychometrics (internal consistency and construct validity).

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Poldip2 knockout results in perinatal lethality and reduced cellular growth of mouse embryonic fibroblasts

Brown DI, Lassagne B, Zafari R, Long JS, Saavedra HI, Griendling KK.

Poldip2 is an understudied protein, originally described as a binding partner of polymerase delta and PCNA. Numerous roles for Poldip2 have been proposed, including mitochondrial elongation, DNA replication and ROS production via Nox4. In this study, we have identified a role for Poldip2 in the cell cycle. We used a Poldip2 gene-trap mouse and found that homozygous animals die around the time of birth. Poldip2/- embryos are significantly smaller than Poldip2+/- or Poldip2+/+ embryos. We found that Poldip2/- MEFs exhibit reduced growth by population doubling and growth curves. This effect is not due to senescence, as measured by p16 and p19 expression. Measurement of DNA content revealed more Poldip2/- cells in the G1 and G2/M phases of the cell cycle, accompanied by a decrease in S-phase cells. Cdk1 and CyclinA2 are downregulated in Poldip2/- cells, and these changes are reversed by SV40 large T-antigen, suggesting that Poldip2 may target the E2F pathway. In contrast, p21 expression is unaffected by SV40 transfection. Overall, these results reveal that Poldip2 is an essential protein in development, and underline its importance in cell viability and proliferation. Poldip2 may be a novel target for treating proliferative diseases such as cancer and restenosis.

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PPARγ Depletion Promotes H2O2 Generation and Proliferation of Human Pulmonary Artery Smooth Muscle Cells via ERK1/2-NF-kB-Nox4 Pathway

Bijli KB, Murphy TC, Adesina SB, Kang B-Y, Hart CM.

Hypoxia stimulates pulmonary hypertension (PH) in part by increasing the proliferation of pulmonary vascular wall cells. Recent evidence suggests that signaling events involved in hypoxia-induced cell proliferation include sustained Nuclear factor-kappaB (NF-kB) activation, increased NADPH oxidase4 (Nox4) expression, and downregulation of peroxisome proliferator-activated receptor gamma (PPARγ) levels. To further understand the role of reduced PPARγ levels in PH pathobiology, siRNA approach was employed to reduce PPARγ levels in human pulmonary artery smooth muscle cells (HPASMC) in vitro under normoxic conditions. By this approach, PPARγ protein levels were reduced to levels comparable to those observed under hypoxic conditions. Depletion of PPARγ for 24 to 72 hours resulted in the activation of mitogen-activated protein kinase, ERK 1/2. It resulted in the activation of NF-kB as determined by its phosphorylation at Ser536 and degradation of its inhibitory protein IkappaB-alpha (IkBα). Depletion of PPARγ induced the expression of NF-kB target gene, Nox4 and increased HPASMC proliferation as determined by MTT assay. PPARγ depletion resulted in generation of H2O2, and furthermore, treatment with PEG-catalase attenuated PPARγ depletion-mediated HPASMC proliferation. Additionally, pharmacological inhibition of ERK 1/2 prevented NF-kB activation caused by PPARγ depletion. Taken together these findings provide novel evidence that reductions in PPARγ levels are sufficient to promote HPASMC proliferation via an ERK1/2-NF-kB-Nox4 dependent mechanism.

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Enteric Neuropeptide Y (NPY) Promotes Colitis-associated Carcinoma by Enhancing Epithelial Cell Survival and Proliferation

Chandrasekharan B, Jeppsson S & Srinivasan S.

Rationale: Patients with inflammatory bowel disease (IBD) are highly susceptible to colitis-associated cancer (CAC). The neuropeptides produced by the enteric nervous system are powerful regulators of inflammatory responses in IBD, yet their role in the pathogenesis of CAC is unclear. We investigated the role of neuropeptide Y (NPY), a 36-amino acid peptide that regulates diverse biological functions like food intake, anxiety and sedation, in CAC. Methods: Chronic dextran sodium sulfate (DSS) model (3 cycles of 3 % DSS @ 7 days per cycle, with a recovery period of 2 weeks of water between DSS cycles), was utilized to induce CAC in WT and NPY knockout mice (NPY -/-). Tumor burden was assessed in mice after 65 days. Epithelial proliferation was assessed by ki67 immunostaining in colonic sections. Alterations in β-catenin protein (western blotting) and localization (immunostaining) were assessed. NPY-treated (10-7 M) T84 and Caco2-BBE cells were assessed for epithelial survival (pAkt) and proliferation (PCNA, proliferating cell nuclear antigen; and phospho-β-catenin) by western blotting. Results: NPY -/- mice had less tumor burden and tumor size compared to WT (p < 0.05). There was a significant increase in percent ki67 cells/ crypt in tumors from WT mice compared to NPY -/- (p < 0.05). WT mice depicted significant increase in PCNA and β-catenin compared to NPY -/- mice (p < 0.05). NPY-treated epithelia exhibited enhanced pAkt, PCNA and β-catenin indicating enhanced proliferation (p< 0.05). Conclusions: NPY-epithelial interactions are crucial in the pathophysiology of CAC; hence NPY maybe a potential therapeutic target in CAC.

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miRNA-mediated Regulation of Endothelial Gene Expression in a Mouse Model of Unidirectional and Oscillatory Shear Stress

Shear stress forces of blood flow are potent regulators of function. In this study we examine shear stress forces, EC gene expression and miR-155 in mouse aorta. We used two mouse models for shear stress forces: the aortic arch of wild type mice and abdominal aorta of mice subjected to coarctation. The latter model induces an artificial narrowing in the lumen of the aorta, thereby acutely creating an area of disturbed, low flow (oscillatory shear stress or OSS) downstream of the clip. Expression of miR-155 in the EC isolated from the lower curvature of the aortic arch (OSS) was decreased compared to that in the EC from thoracic aorta, where ECs are exposed to unidirectional shear stress (USS). En face staining showed differences in EC in segments exposed to OSS compared to USS, including F-actin staining. We observed similar shear stress-associated changes in EC phenotype in aortas of mice subjected to aortic coarctation. ECs in aortic segments above the coarctation were aligned with flow, whereas ECs below the coarctation show no clear orientation. Furthermore, we found that ECs exposed to OSS had increased F-actin staining. MiR-155 targets RhoA and MYLK, two important regulators of the actin cytoskeleton. In both models, we found a significant decrease in RhoA and MYLK in areas exposed to OSS compared to USS areas. In conclusion, our findings suggest that different shear stress forces in vivo, either chronic (aortic arch model) or acute (abdominal coarctation model) alter the EC phenotype by modulating miR-155 expression and regulation of cytoskeletal signaling pathways.

A high phosphate environment induces angiogenesis which is mediated by FOXC2

Lin Y, Garneys L, Bennett K, Beck GR.

Recent studies in both rodents and humans suggest that elevated serum phosphate, in the context of normal renal function, potentiates or exacerbates pathologies associated with cardiovascular disease, bone metabolism, and cancer. Our recent microarray studies identified the potent stimulation of pro-angiogenic genes such as Forkhead box protein C2 (FOXC2), osteopontin, and Vegf, among others in response to elevated inorganic phosphate (Pi). Increased angiogenesis and neovascularization are important events in tumor growth and the progression to malignancy and therefore in this study we addressed the possibility that a high Pi environment would increase the angiogenic potential of cancer cells. Here, our in vitro studies utilized lung and colon cancer cell lines in combination with the human umbilical vascular endothelial cell (HUVEC) vessel formation model to better understand the mechanism(s) by which a high Pi environment might alter cancer progression. Exposure of cancer cells to elevated Pi stimulated expression of FOXC2 and conditioned medium from the Pi-stimulated cancer cells stimulated migration and tube formation in the HUVEC model. Mechanistically, we define the requirement of FOXC2 regulated OPN expression and secretion from cancer cells as necessary for the angiogenic response. These studies reveal for the first time that cancer cells grown in a high Pi environment promote migration of endothelial cells and tube formation and in so doing identify a novel potential therapeutic target to alter tumor progression.

BTBD4 Is a Sensitive Transcription Regressor in Endothelial Cells

Rezvan A, Sun H, Monfared S, Kim CW, Kumar S, Jo H

Background: BTBD4, also known as ZBTB46 or zDC, is a member of the BTB-ZF protein family which comprises a diverse group of transcription factors. This protein has not been widely studied and was recently suggested as a specific marker for classical dendritic cells to differentiate cDCs from other peripheral blood cells. In DCs, BTBD4 is primarily a negative regulator of gene expression, its deficiency up-regulates activation pathways including MHCII and enhances production of VEGF and is down-regulated by TLR activation. BTBD4 was also noted to be expressed in endothelial cells, however its function and regulation in endothelial cells has not been studied. Endothelial cell activation in areas of disturbed flow is a critical step in initiation of atherosclerosis, leading to heart attacks and stroke. Hypothesis: BTBD4 is expressed in endothelial cells under laminar shear conditions and is down-regulated by low and oscillatory shear stress patterns as seen in areas of disturbed flow, leading to endothelial cell activation.

Methods: We use the mouse partial carotid ligation to assess shear responsiveness in vivo and a cone and plate model to assess shear responsiveness in vitro. Results: We confirm that BTBD4 is expressed in endothelial cells both by mRNA analysis via qPCR and protein analysis via immunofluorescence. We also show that BTBD4 is down-regulated under low and oscillatory shear conditions both in vivo and in vitro. Further functional studies including monocyte adhesion and migration assays as well as mechanistic studies assessing upstream and downstream pathways are currently underway.

Use of Recombinant Adenovirus, Ad5-PK4, as a Malaria Vaccine Platform in Heterologous and Homologous Prime Boosting Experiments in Mice

Elgin IL, Hoffman T, Cabrera-Mora M, Moreno A.

Many studies have shown adenoviruses have great potential for cancer gene therapy and vaccine development. Adenoviruses are attractive for vaccine use given their ability to stimulate cellular and humoral responses against expressed transgenes. The Adenovirus genome is able to stimulate several arms of the innate immune response. Several promising malaria studies have been carried out with, Adenovirus 5, (Ad5) and Plasmodium antigens in vaccine development. Ad5 expressing P. falciparum CSP, promoted robust CS protein specific lymphocyte activation and antibody titers. The primary receptor
for Ad5 is the Coxsackie adenovirus receptor (CAR). Ad5-PK4 is a recombinant adenovirus which contains tandem carbohydrate binding domains (CBD) from the fiber protein of porcine adenovirus type 4 (PAdV-4). These carbohydrate binding domains can direct CAR-independent infection of cells using glycosylated cell surface molecules as primary attachment sites, which can promote infectivity of different cell types. For our experiments, we compared the immunogenicity of Ad5-PK4 containing P.yoelli lpc/rcmc and Ad5 lpc/rcmc in heterologous and homologous prime boosting experiments in mice. Lpc/rcmc represents a hybrid chimeric recombinant protein that incorporates linear epitopes from P.yoelli (CSP) and distinct modules derived P. yoelli merozoite surface protein 1 (MSP-1). First, mice were immunized with Ad5-PK4 and Ad5- lpc/rcmc recombinant adenoviruses. The cellular and humoral immune response was assessed by using tetramer staining, FACS sorting, and ELISA. The mice were then challenged with Plasmodium yoelli sporozoites and protection was assessed by examining infectivity and parasitemias in the mice. It is our hope that Ad5-PK4 may represent a novel platform for malaria vaccine development.

Detection of Bacterial Device Infection of Cardiac Devices in a Rodent Model Using a Novel PET Imaging Probe and a Fluorescent Imaging Probe


Background: Implantable cardiac devices have problems with device infection. There is often difficulty in making a definitive diagnosis of device infection. To this end, we have developed a new approach to specifically detect bacteria using a novel PET imaging probe. Maltohexaose is taken up by bacteria and is internalized as a major source of glucose. Mammalian tissues do not take up maltohexaose. In this study, we evaluated whether maltohexaose labeled with fluorine-18 (F-18 FMH) is useful as a PET tracer to detect bacterial device infection in a rat model. We also evaluate whether IR-786 conjugated maltohexaose dye accumulated specifically in the infected device in this model.

Methods and results: We implanted surgical grade stainless steel device mock-ups in Sprague-Dawley rats. In the device infection group, rats were injected Methicillin sensitive Staphylococcus aureus (MSSA) around the mock-ups on post-operative day four. Two days later, rats were injected with F-18 FMH and were scanned with micro PET/CT. The ratio of the signal in the region of interest (ROI) around the mock-up area and normal skin area was measured. The ratio of ROI in the device infection group was significantly increased compared to that in the control group. The fluorescent dye also accumulated around the infected device, but the control group had low signal around the device.

Conclusion: F-18 FMH shows great promise as a PET imaging tracer for the specific diagnosis of bacterial infections of implanted cardiovascular devices.

Chromatin Binding in SLE Patients Correlates with the Intensity of Apoptotic Binding by 9G4+ B Cells

Chida AS, Li QZ, Mohan C, Wang Y, Jenks S, Hartson L and Sanz I.

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterised by production of autoantibodies. IgG antibodies expressing the 9G4 idiotype are specifically expanded in SLE and provide a unique model to understand the participation of different autoantigens in the pathogenesis. 9G4 autoreactivity may be directed against different lupus antigens and accounts for significant fraction of anti-apoptotic cell binding (APCB), of lupus serum. Here we sought to understand the contribution of anti-nucleosome reactivity to APCB. A panel of 9G4+ monoclonal antibodies was generated from IgD-CD27+ memory cells of SLE patients. APCB was determined by flow and anti-Chromatin reactivity by ELISA. Three representative antibodies with strong APCB activity were also tested by antigen microarray. Our preliminary data demonstrate a strong correlation of apoptotic binding with chromatin. Out of 37 9G4+ monoclonals tested, 10 were positive for apoptotic binding and out of these 9 were positive for chromatin (p<0.001; fisher exact test). Glomerular microarray analysis identified reactivity with Chromatin, histones H2A, H2B, H3 and H4 in different patterns for the individual antibodies. Levels of reactivity against chromatin, H2A, H2B, H3, and U1-Sn-RNP-68 correlated with disease activity. This may suggest that SLE-specific 9G4 antibodies recognize nucleosomal antigens (chromatin and individual histones) and may be polyreactive against components of the snRNP complex. Both these types of antigens are expressed at high density on apoptotic cells which are highly immunogenic sources of self-antigens in SLE. Determination of 9G4 antibodies against apoptotic cells and/or nucleosomal antigens might provide a useful test for the diagnosis and monitoring of SLE.

TNF-α Alters the Cell-to-Cell Transfer of miRNAs by Microvesicles

Alexy T, Weber M, Searles CD.

MiRNAs are short, non-coding RNAs that post-transcriptionally regulate intracellular gene expression. Recently, it has become recognized that activated ECs export miRNAs packaged in microvesicles (MVs), including microparticles (MPs) and apoptotic bodies (ABs). We believe that miRNA export is a process that serves as a means of cell-to-cell communication. Here, we examined the export of miR-126, -21 and -155 by human aortic endothelial cells (HAECs) in response to TNF-α.

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and their subsequent transfer to recipient cells. We hypothesize that: 1) miRNA export is selective and occurs primarily through MPs; 2) MPs but not ABs transfer miRNAs to recipient cells. HAECs were treated with TNF-α and an apoptosis inhibitor (block AB formation) or a ROCK inhibitor (block MP formation). MPs were isolated and added to separate recipient HAECs. TNF-α induced a decrease in intracellular miR-126 and -21 expression but increased miR-155 levels dramatically. The abundance of all three miRNAs was increased in MPs and significantly reduced in ABs after TNF-α treatment. Inhibition of MP formation increased intracellular miRNA levels but did not alter export via ABs. MVs from TNF-α-treated cells had reduced miRNA transfer to recipient cells compared to control MVs; this reduction was abrogated when AB formation was inhibited. These findings indicate that cell-to-cell transfer of miRNA is primarily mediated through MP and not AB.

Conclusions: TNF-α regulates miRNA export through enhanced loading of MPs. In addition, cell-to-cell transfer of miRNA in response to TNF-α is mediated through MPs rather than ABs, underscoring their importance as miRNA delivery vehicles in health and disease.

Depression Screening in Patients with Systemic Lupus Erythematosus From The Southeastern United States: Missing Opportunities for Early Diagnosis and Treatment
Drenkard, C., Dunlop-Thomas, CM, Bao, G, Lim, SS.

Background: Depression is reported in 20-75% of systemic lupus erythematosus (SLE) patients. Depression increases the risk for disease activity, poor quality of life, poor treatment adherence and health system utilization in SLE patients. Depression screening, a service recommended by the US Preventive Services Task Force (USPSTF) for adults at risk, has shown improved outcomes when combined with further treatment. However, no study has examined this provision in SLE. We assessed gaps in depression screening service in a high-risk SLE cohort.

Methods: We examined cross-sectional data from the Georgians Organized Against Lupus Cohort (GOAL), a large population-based cohort of SLE patients from Atlanta, GA. GOAL participants of the full sociodemographic spectrum respond to an annual survey on health care utilization and health outcomes. We assessed the proportion of patients screened for depression in the past year using two screening questions recommended by USPSTF, and the prevalence of depression using the Hospital Anxiety and Depression Scale.

Results: Among SLE 519 respondents, 163 (31%) had depression. Only 306 (59%) respondents were screened for depression. Among 213 patients not screened, 18% reported symptoms compatible with depressive disorders. Patient factors associated with being screened for depression were black race, lower education, unemployment, and poor health. Patients who visited a cardiologist, the emergency department, or were hospitalized in the past year were more likely screened for depression.

Conclusion: Although SLE patients from socioeconomic disadvantaged groups were more likely to be screened; a substantial number of patients missed the opportunity of early depression diagnosis and proper care.

Heterogeneity of Antibody Secreting Cell Subsets in Blood after Vaccination and Bone Marrow in Healthy Adults: Identification of Human Long-lived Plasma Cells
F. Eun-Hyung Lee, J Halliley, C Tipton, E. Walsh, A Falsey, J Liesveld, D Kaminiski, A Rosenberg, C Fucile, E Ramos, R Burack, M Slifka, E Hammarlund, I Sanz

Generation of persistent serum protective antibodies is the ultimate goal of vaccination and is mediated by terminally differentiated long-lived antibody-secreting plasma cells (LLPC) that reside in the human bone marrow (BM). In contrast, early protection post-immunization is provided by circulating proliferative antibody secreting cells (ASC). Whether these early ASC differentiate into LLPC and the programs involved in such differentiation remain unclear. In this study, we identified 5 phenotypically distinct ASC subsets in circulation after tetanus vaccination using CD19, CD38 and CD138. Nearly all blood ASC subsets express Ki67+ staining (including CD19-CD138+ ASC) demonstrating ongoing in vivo proliferation, and all subsets contain tetanus-specific ASC suggesting that each subset participates in the new vaccine response. The same markers identified 4 novel ASC subsets in steady-state BM. One unique, non-proliferative subset, (CD19-CD38hiCD138hi) was morphologically distinct from the other fractions and contained specificities to long-lived viral antigens (measles and mumps) from exposures that occurred 40-50 years ago; thereby identifying a novel candidate phenotype for long-lived plasma cells. Clonal relationships using next gen sequencing showed in blood, 73% of the clones were interconnected. In contrast, only 8% of the clones were shared among BM subsets. Unique transcriptome signatures were identified in BM LLPC compared to the other BM and blood ASC. Overall, this study demonstrates the heterogeneity of human ASC in the blood and BM, and for the first time, identifies a discrete population in the human BM that represents the LLPC compartment. This knowledge will facilitate vaccine design for early blood biomarkers of LLPC as well as insights to LLPC gene targets for vaccine adjuvants.

Identification of IL-10 producing plasma cells in human and its deficiency in systemic lupus erythematosus patients
Wang X, Roger J, Sanz I.

Background: Regulatory B cells are active participants in down-regulating inflammation and autoimmunity, through the
production of IL-10. The purpose of this study is to explore the development of regulatory B cells either in healthy individuals or in patients with SLE.

Methods: Healthy controls (HC) and SLE patients fulfilling the American College of Rheumatology revised classification criteria were included in this study.

Results: In vitro and ex vivo analysis determined for the first time, that human plasma cells (PC) and plasmablasts (PB) are major sources of IL-10. This cytokine was secreted from PB and PC generated in vitro from cultured memory cells using ELispot assay and intracellular flow cytometry. Similarly, ex vivo analysis of unstimulated B cell subsets sorted 7 days after flu vaccination of HC identified PB and PC as the only source of IL-10. In contrast, circulating PB and PC spontaneously expanded in the circulation of active SLE patients failed to produce IL-10 ex vivo. In vitro stimulation of purified B cell subsets identified the non-switched memory B-cell as the main IL-10-producing precursor B cell. Of interest, IL-10 production from stimulated B cells was significantly lower in SLE patient in correlation with the reduction in non-switched memory cells of this disease.

Conclusion: We provide the first description of spontaneous IL-10 production by human PB and PC in HC and its deficiency in SLE. These findings suggest that IL-10 could negatively regulate antibody secreting cells in an autocrine fashion that would be deficient in SLE thereby enhancing autoantibody production.

**Endothelial Function Is Not Affected by Fresh or Storage-aged Autologous Blood Transfusions in Healthy Individuals**

_Ashra Kf, Smith G, Sher S, Neuman R, Roback J, Quyyumi A._

Background: The transfusion of storage-aged packed red blood cells (pRBCs) is associated with worse outcomes. We have recently demonstrated a decrease in endothelial function in anemic patients receiving aged compared to fresh blood transfusions, suggesting impairment of nitric oxide (NO) signaling. To further investigate this phenomenon, we tested endothelial function during transfusion of aged and fresh autologous pRBCs in healthy subjects, with the hypothesis that aged pRBCs will impair endothelial function. Methods: 16 healthy participants (aged 31±10 years, 38% female) were transfused with 1 unit of fresh (aged 3-7 days) autologous pRBCs and subsequently with aged (35-42 days) autologous pRBCs. Endothelial function was assessed using flow mediated dilatation (FMD) of the brachial artery before, 1 hour, and 24 hours after transfusion. Data are presented as mean ±SD and were analyzed using a repeated measures ANOVA test. Results: Hemoglobin was similar before both transfusions (p=0.066) and increased by 1.02±1.0 g/dL and 1.2±1.2 g/dL with fresh and storage-aged transfusions, respectively. Pre-transfusion FMD was similar before transfusions. There were no changes in FMD with transfusions; mean FMD at 1 hour and at 24 hours-post fresh transfusion and at 1 hour- and at 24 hours-post storage-aged transfusion. Conclusions: NO-mediated vascular reactivity is not impaired by transfusion of fresh or storage-aged pRBCs in healthy individuals, unlike findings in anemic hospitalized patients. This may be due to robust NO signaling in healthy participants or muted response due to the use of autologous blood. Further research on populations with underlying endothelial dysfunction is needed to better understand the effects of blood storage age on vascular function.

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**Healthcare Exposure as a Risk Factor for Hepatitis C Infection – An Analysis of Veterans With Documented Hepatitis C Seroconversion**

_Cartwright EJ, Rentsch C, Rimland D._

Background: Hepatitis C (HCV) infection is most commonly transmitted by injecting illicit drugs. However, lapses in infection control have resulted in HCV transmission in healthcare settings including hemodialysis centers.

Methods: A laboratory generated list of all HCV antibody tests performed at Atlanta VA Medical Center between 1992 and 2011 was used to identify cases and controls. Cases were those who converted from a negative to a positive HCV antibody positive, while controls had at least two negative tests; 1:1 matching using seroconversion date (within two weeks) was performed. Medical charts were abstracted using the electronic medical record.

Results: HCV seroconversion was identified in 130 veterans. There were no differences in age (mean age at time of testing: 55 years), race (51% African American), sex (91% male), alcohol abuse (38%), or cocaine use (16%) between cases and controls. More cases than controls were HIV positive (35% vs 18%, p=0.03), hepatitis B infected (11% vs 3%, p=0.01), and had used heroin (9% vs 2%, p=0.03). Cases were more likely to be on hemodialysis (13% vs 4%, p=0.008), and to reside in a nursing home (5% vs 0%, p=0.03). Hospital admissions, major surgeries, and invasive procedures were not different between cases and controls.

Conclusions: We identified 130 HCV seroconversion cases over a 20 year period with traditional and novel HCV risk factors. Hemodialysis and nursing home residence were associated with HCV seroconversion. Prospective investigation of seroconversion cases that lack behavioral risk factors may be useful to rule out HCV transmission in healthcare settings.

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**Induction of pluripotency in bone marrow mononuclear cells via polyketal nanoparticle-mediated delivery of mature microRNAs**
Since the successful generation of induced pluripotent stem cells (iPSC) from adult somatic cells using integrating-viral methods, various methods have been tried for iPSC generation using non-viral and non-integrating technique for clinical applications. Recently, various non-viral approaches such as protein, mRNA, microRNA, and small molecule transduction were developed to avoid genomic integration and generate stem cell-like cells from mouse and human fibroblasts. Despite these successes, there has been no successful generation of iPSC from bone marrow (BM)-derived hematopoietic cells derived using nonviral methods to date. Previous reports demonstrate the ability of polymeric micro and nanoparticles made from polyketals to deliver various molecules to macrophages. MicroRNA-loaded nanoparticles were created using the polyketal polymer PK3 (PK3-miR) and delivered to somatic cells for 6 days, resulting in the formation of colonies. Isolated cells from these colonies were assayed and substantial induction of the pluripotency markers Oct4, Sox2, and Nanog were detected. Moreover, colonies transferred to feeder layers also stained positive for pluripotency markers including SSEA-1. Here, we demonstrate successful activation of pluripotency-associated genes in mouse BM-mononuclear cells using embryonic stem cell (ESC)-specific microRNAs encapsulated in the acid sensitive polyketal PK3. These reprogramming results demonstrate that a polyketal-microRNA delivery vehicle can be used to generate various reprogrammed cells without permanent genetic manipulation in an efficient manner.

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Plasmablast and monoclonal antibody response to monovalent influenza H3N2 variant (H3N2v) vaccine


Infections and illnesses due to a novel swine-origin variant of influenza A virus subtype H3N2 (H3N2v) have recently increased in the United States, primarily among children who have little to no pre-existing immunity against the virus. Studies have shown recent formulations of seasonal influenza vaccine do not substantially improve sero-protection against this H3N2v. Therefore, a specific vaccine will be needed for control of H3N2v infection, particularly if this virus evolves to be efficiently transmitted human-to-human. In this study, we analyzed B-cell responses in 25 healthy adults immunized at the Hope Clinic of the Emory Vaccine Center with 2 doses of monovalent inactivated influenza H3N2v vaccine. In all cases, we found a rapid, predominantly IgG producing vaccine-specific plasmablast response which was cross-reactive to both ancestral and contemporary seasonal H3N2 strains. Analysis of the immunoglobulin heavy chain genes has thus far revealed a pauci-clonal response, and high levels of somatic hypermutation. These findings suggest the plasmablast response is derived from a memory B cell population. Work currently ongoing will further characterize cross-reactivity and epitope-specificity of the HA-specific monoclonal antibodies we are cloning from the vaccine-specific plasmablasts. This study will help us to understand if immunization with this strain, which is significantly different from currently circulating H3N2 virus strains, will increase the possibility of generating HA stalk-specific B cell responses, which provide heterosubtypic protection for influenza viral infection.

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HIV Testing Patterns among Black Men Who Have Sex with Men: A Qualitative Typology


Background: Black men who have sex with men (MSM) in the Southeastern United States are disproportionately affected by HIV. Black MSM are more likely to have unrecognized HIV infection, suggesting that testing may occur later and/or infrequently relative to current recommendations. The objective of this qualitative study was to explore the HIV testing behaviors of Black MSM in Atlanta, Georgia, who were participants in the HIV Prevention Trials Network Brothers Study (HPTN 061).

Methods and Findings: We conducted 29 in-depth interviews and four focus groups with a community-recruited sample. Modified grounded theory methodologies were used to guide our inductive analysis, which yielded a typology comprised of four distinct HIV testing patterns. Participants could be categorized as: (1) Maintenance Testers, who tested regularly as part of routine self-care; (2) Risk-Based Testers, whose testing depended on relationship status or sexual behavior; (3) Convenience Testers, who tested irregularly depending on what testing opportunities arose; or (4) Test Avoiders, who tested infrequently and/or failed to follow up on results. We further characterized these groups with respect to age, socioeconomic factors, identity, stigma and healthcare access.

Conclusions: Our findings highlight the heterogeneity of HIV testing patterns among Black MSM, and offer a framework for conceptualizing HIV testing in this group. Public health messaging must account for the diversity of Black MSM’s experiences, and multiple testing approaches should be developed and utilized to maximize outreach to different types of testers.

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SLE Flares are Characterized by Generalized Polyclonal Expansions of Antibody Secreting Cells without Preference for Autoimmune Responses

Ichikawa HT, Sanz I

Increased circulating antibody secreting cells (ASC) correlate with SLE activity and are prominent during Lupus flares. We tested whether this expansion is predominantly driven by typical
SLE autoreactivities including anti-dsDNA, -Sm and -Ro and 9G4+ antibodies.

Plasma cells and plasmablasts (PB/PC) frequencies increased up to 25-fold in SLE compared to healthy control. Of total IgG ASC, frequencies of each SLE specific IgG ASC (anti-dsDNA, anti-Sm and anti-Ro60) never exceeded 3.4% (mean ± SD, 0.25 ± 0.65%) in patients with high s. 9G4+ ASC, which include several SLE-specific autoreactivities including anti-dsDNA and anti-apoptotic cells, were the most abundant autoreactivity but did not exceed 6% (mean ± SD, 2.2 ± 1.7%). Combined, all the lupus–related autoreactivities accounted for <10% of all IgG ASC. No correlation was found between PB/PC frequencies and SLE specific or 9G4+ IgG ASC frequencies. Furthermore, anti-microbial ASC, usually not found in healthy PBL, were found in SLE PBL in frequency similar to autoreactive responses (0.06 ± 0.09% and 0.37 ± 0.12%, respectively). SLE specific and 9G4+ as well as anti-microbial IgG memory cells were present in SLE PBL.

Our results are consistent with a polyclonal expansion of ASC during lupus flares that is not predominantly driven by conventional autoantigens even in patients with high titers of serum antibodies. These studies have important implications for our understanding of the mechanisms underlying lupus flares and the contribution of different cellular compartments to the generation of serum autoantibodies at different times in the course of the disease.

Degree of plasma protein binding affects central nervous system penetration of the HIV-1 protease inhibitors atazanavir and darunavir


Background: HIV-associated neurocognitive disorders have been associated with suboptimal antiretroviral (ARV) central nervous system (CNS) penetration. Plasma protein binding could limit CNS entry of HIV-1 protease inhibitors (PIs) atazanavir (ATV), 86% bound, and darunavir (DRV), 95% bound, as primarily unbound (free) drug crosses blood-brain barriers.

Methods: A cross-sectional study was conducted with asymptomatic virologically suppressed HIV-1 infected adults receiving tenofovir/emtricitabine plus either ritonavir-boosted daily ATV or DRV for > 6 months. Paired trough plasma and cerebrospinal fluid (CSF) samples were collected. Free PI concentrations were measured using rapid equilibrium dialysis and liquid chromatography/tandem mass spectrometry. HIV-1 RNA and neopterin, an inflammatory biomarker, were measured by Ampliprep/COBAS® Taqman® 2.0 assay (Roche) and ELISA, respectively.

Results: Thirty subjects (15 per arm) were enrolled. CNS penetration, defined as CSF:plasma free drug ratio, was higher for ATV than DRV, 0.10 (95% CI 0.07-0.14) and 0.04 (95% CI 0.03-0.06), respectively. However, none of the free CSF ATV levels exceeded HIV wild-type IC50 compared to 14/15 (93.3%) in the DRV arm (p<0.001). Two of fifteen subjects (13.3%) on ATV and 4/15 (26.7%) on DRV had detectable CSF HIV-1 RNA. CSF neopterin was low and similar in both arms.

Conclusions: CSF:plasma free drug ratio was higher for ATV than DRV, likely due to lower ATV plasma protein binding, however absolute CSF concentrations of ATV were all below the IC50. Despite this, both groups had low levels of CSF HIV-1 RNA and neopterin, suggesting that drug concentrations achieved by these regimens were adequate for virologic and inflammatory control.

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Micronutrient status of pulmonary tuberculosis patients in Tbilisi, Georgia


Background: Several micronutrients are important in immunity and may influence tuberculosis (TB) outcomes; however, data on micronutrient status in patients with TB are limited. Methods: We obtained serial blood samples in a randomized trial of high-dose vitamin D treatment in pulmonary TB patients in Tbilisi, Georgia. Micronutrient levels in serum were determined by inductively coupled argon plasma spectrometry. Dietary intake of vitamins, minerals and trace elements was obtained using a validated instrument at baseline and 16 weeks after entry. Descriptive statistics and paired and unpaired t tests were used where appropriate. Results: A total of 189 TB subjects and 19 non-infected household contacts (controls) were studied (TB subjects mean age 35 y; 59% male; control mean age 40 y; 36% male), Baseline blood iron levels (24±20 umol/L) significantly increased at 16 weeks (p=0.006), while copper (26.9±5.2 umol/L) and magnesium (0.85±0.13 mmol/L) levels significantly decreased over time (each < 0.04). Blood copper concentrations were significantly increased in TB subjects at baseline compared to controls (p<0.0001). Other measured minerals and trace elements did not differ between TB patients and controls. Dietary intake of most micronutrients significantly increased from baseline to 16 weeks in TB-infected subjects. All measured micronutrients met at least 100% of US Dietary Reference Intakes. Conclusion: Both dietary intake and blood levels of measured micronutrients significantly increased over the study period in this TB patient cohort. The decrease in blood copper concentrations despite increased dietary intake over time suggests the possibility of increased utilization of copper in TB-infected patients.

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Characteristics and Outcomes of Severe Sepsis Patients with HIV-1 and Chronic Alcohol Abuse
Rationale: Sepsis is one of the most common medical conditions in the ICU & is a leading cause of death in HIV-patients. Alcohol is one of the most commonly abused substances & chronic alcohol abuse (CAA) is associated with higher rates of organ dysfunction & decreased survival in sepsis patients. We aim to determine if CAA resulted in worse outcomes in HIV-septic patients. Methods: A prospective observational study of patients in adult surgical & medical ICUs at Grady Memorial Hospital who met the ACCP/SCCM definition of severe sepsis were enrolled between July 2006 & October 2012. CAA was defined as (AUDIT) > 8 for females & > 5 for males. We compared baseline demographics, severity of illness & hospitalization outcomes between HIV & non-HIV sepsis patients with & without CAA using t-tests & Chi square tests; p-values < 0.05 were considered significant. Results: Of 285 septic patients, 65 (23%) had CAA & 58 (20%) positive for HIV(mean CD4 count=114, SD=150). Primary source of infection-respiratory source in 67% of all patients, majority were African American. Those without CAA, HIV-positive patients were more likely to have a hospitalization of infection (p=0.11) but less likely to have diabetes (p < 0.05) & a history of sepsis compared to HIV-negative patients (p=0.05). HIV-negative patients also had a significantly lower mortality than HIV-positive patients (11% vs. 27%, p=0.01). Conclusion: This small study of HIV severe sepsis patients with & without CAA, CAA was not associated with higher mortality in HIV patients compared to non-HIV patients.

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HIV-associated non Hodgkin's Lymphoma in the late antiretroviral era in a southern Ryan-white–funded urban clinic

Silverton AL, Nguyen ML, Adamski M, Mosunjac M, Gunthel C

Background: Patients living with HIV/AIDS(PLWHA)are at a higher risk of developing Non-Hodgkin Lymphoma (NHL). Population–based studies have shown a dramatic decrease of the incidence of HIV-associated NHL (HA–NHL) in the late antiretroviral therapy (ART) era. We present our experience with HA–NHL.

Methods: This is a retrospective descriptive analysis of a cohort of PLWHA with NHL who received care at the Ponce de Leon Center in Atlanta from 1/1/2004 to 12/31/2010. Medical records and electronic charts were abstracted for demographic data, cancer stage, CD4 count, HIV viral load (HIVVL), ART, chemotherapy, and mortality.

Results: 35 patients with HA–NHL were identified. Among these patients, there were 7 Burkitt's, 20 DLBCL, 7 plasmablastic lymphoma and 1 Primary Effusion Lymphoma. 83% presented with advanced disease and 63% were not on ART at cancer diagnosis (cadx). Overall, patients completed 67% of planned chemotherapy cycles. Common causes for Cx termination were persistent myelosuppression (18.2%), social/patient preference (22.7%), and death (36.4%). 2-year overall survival was 40% for all NHL, 50% for DLBCL, and 14.3% for BL. Among the 285 septic patients, 65 (23%) had CAA & 58 (20%) positive for HIV(mean CD4 count=114, SD=150). Primary source of infection-respiratory source in 67% of all patients, majority were African American. Those without CAA, HIV-positive patients were more likely to have a hospitalization of infection (p=0.11) but less likely to have diabetes (p < 0.05) & a history of sepsis compared to HIV-negative patients (p=0.05). HIV-negative patients also had a significantly lower mortality than HIV-positive patients (11% vs. 27%, p=0.01). Conclusion: This small study of HIV severe sepsis patients with & without CAA, CAA was not associated with higher mortality in HIV patients compared to non-HIV patients.
The Malaria Host-Pathogen Interaction Center (MaHPIC): systems biology from the bench to non-human primates and humans
Galinski, MR, Moreno, A, Kissinger J and MaHPIC consortium (www.systemsbiology.emory.edu)

The Malaria Host-Pathogen Interaction Center (MaHPIC) is a large 5-year multidisciplinary systems biology research project consortium led from Emory University along with investigators from the CDC, UGA, GA Tech and around the world (www.systemsbiology.emory.edu). MaHPIC will generate unprecedented large datasets to understand malaria infections and disease processes. The project is designed to integrate clinical, hematological and parasitological information on malaria infections with biological data generated by innate and adaptive immune profiling, functional genomics, proteomics, lipidomics and metabolomics cores. The data will be analyzed with powerful methods of informatics, mathematical modeling and computational analysis and be made available to the scientific community through a relational database. It is hoped to aid the development of new anti-malarial drugs, vaccines and diagnostics. The central unifying hypothesis of the MaHPIC is that “Non-human primate host interactions with Plasmodium pathogens as model systems will provide insights into mechanisms as well as indicators for human malaria disease conditions”. The project will study malaria using several different non-human primate models as well as through collaborations with clinical scientists from malaria endemic countries across a variety of epidemiological settings, and each with distinctive research programs, interests and goals. The MaHPIC is supported by a research contract awarded by the National Institutes of Health, National Institute for Allergy and Infectious Diseases: contract # HHSN272201200031C.

Clinical Phenotypes and Disease Burden Of Discoid Lupus Erythematosus In A Sample Of Systemic Lupus Erythematosus Patients In The Southeastern United States
Cassidy LA, Bao G, Dunlop-Thomas CM, Lim SS, Drenkard C

Background/Purpose: Prior reports suggest that the rash of discoid lupus erythematosus (DLE) may be protective against severe disease in systemic lupus erythematosus (SLE); however, most studies have consisted of convenience samples of predominantly White patients. We examined the association of DLE with clinical manifestations and disease outcomes in a predominantly Black community-based cohort of SLE patients in the Southeastern United States.

Methods: Data was collected from the 2011-12 survey of Georgians Organized Against Lupus (GOAL). GOAL is a prospective cohort of validated SLE patients primarily derived from the Georgia Lupus Registry (GLR), a population-based registry of lupus patients in Atlanta, Georgia. GOAL collects self-reported data on health status, disease activity, and organ damage. We examined the association of DLE with clinical features and disease status, calculating the OR and 95% CI adjusted for demographic variables.

Results: Among 767 SLE patients, 196 (26%) had DLE. 168/597 (28%) Blacks versus 25/156 (16%) Whites (p=0.008), and 18/45 (40%) males versus 17/722 (25%) females (p=0.02) were affected. Mean educational attainment (years) was 13.7 (SD 2.8) and 14.4 (SD 2.9) in patients with and without DLE, respectively (p=0.004), and mean disease duration (years) was 15.5 (SD 10.4) and 12.6 (SD 8.1), respectively (p=0.0001).

Conclusion: DLE occurred in 26% of SLE patients, suggesting a stronger association of discoid rash with systemic manifestations than formerly believed. Despite differences in clinical phenotypes, disease outcomes were similar in patients with and without DLE. Our data suggest that environmental factors play a major role in outcomes of high-risk SLE patients.

A Novel Method for Diagnosis of Acute Influenza Viral Infection using Newly Generated Antibodies from Circulating Antibody Secreting Cells: Implications for H7N9 Influenza Virus.
Lee FEH, Halliley JL, Sanz I, Falsey AR, Walsh EE.

Surges of serum antibodies after immunization and infection are highly specific for the offending antigen, and recent studies demonstrate that viral infections induce transient increases in circulating antibody secreting cells (ASCs). High specificity of circulating ASC that lack bystander responses during acute infection highlight the diagnostic value of interrogating ASCs as an ideal one-time-point immune surrogate for serology during acute infection. We have developed a novel test to diagnose acute influenza infection using the circulating ASCs. Methods: Specificity of influenza-specific ASC during acute illness in N=148 patients with acute respiratory illness was tested using influenza-specific ASC Elispot assays for IgM, IgG, IgA in the blood. Twenty-six healthy asymptomatic adults were also enrolled as controls. Results: In the original pilot study of 23 influenza and 37 RSV confirmed adult respiratory infections, the test was positive with 80% sensitivity and 100% specificity. No influenza-ASC were detected in the 26 healthy subjects. A second validation cohort of 74 adult subjects admitted with respiratory infection had PCR by nasal swabs, blood RSV- and influenza- ASC assays performed during acute illness. PCR was positive in 16/27 patients and the influenza-specific ASC assay was positive in 20/27 patients. Interestingly, only 9 patients were positive by PCR and ASC assay. PCR positive patients often presented earlier whereas positive ASC assays occurred in those who presented later. Influenza type A & B as well as subtypes were discernable by the ASC assay. Conclusion: Influenza-specific IgM, IgG, & IgA ASC assays are effective in detecting acute influenza viral infections. PCR may
have advantages earlier in diagnosis but ASC assays are advantageous in patients who present later in their illness.

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Plasma cells in acute SLE flares are characterized by a highly diversified repertoire accentuated by clonal expansions of VH4-34 antibodies


Systemic Lupus Erythematosus (SLE) is an autoimmune disease in which faulty B cell tolerance promotes the generation of multiple autoantibodies. Typically, flares in SLE activity are accompanied by a substantial expansion of circulating antibody-secreting plasmablasts (PB). Similar expansions of PB observed in healthy subjects post-immunization contain large clonal expansions of antigen-specific cells derived from pre-existing memory cells. To determine if SLE PB expansions follow the same model, and to understand the diversity, origin and antigenic specificity of SLE PB expansions, we used immunoglobulin heavy-chain deep-sequencing to analyze sorted cells from patients experiencing acute flares. We found that PB in SLE acute flares are highly diversified with lower VH mutation rates than memory cells. However, within this diverse pool significant clonal expansions accounting for greater than 0.5% of the entire repertoire were regularly detected. Strikingly, VH4-34 sequences were among the largest clones in all SLE samples. We also found substantial fractions of PB in SLE to be highly evolutionarily related to a pool of activated naive precursors. This was in stark contrast to results obtained in PB samples from healthy, vaccinated subjects, which contained large, clonal expansions, high VH mutation rates and were largely related to switched memory cells. These cells also had an absence of autoreactive VH4-34 clonal expansions, signifying intact B cell tolerance. Combined, our data support a model of generalized naive and memory activation underlying the activation phase of human SLE. This polyclonal activation is accentuated, and possibly promotes, antigen-selected clonal expansions dominated by VH4-34-encoded autoreactive 9G4 antibodies.

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Inotrope Use and Outcomes in Hospitalized Heart Failure: Impact of Etiology, Systolic Blood Pressure, and Cardiac Output – Insights from ESCAPE

Kalogeropoulos AP, Marti CN, Papadimitriou L, Nasir S, Al-Anbari R, Georgiopoulou VV, Butler J

Introduction: Despite limited evidence and mostly negative signals, inotropes are widely used in hospitalized heart failure (HHF). We investigated whether HF etiology, systolic blood pressure (SBP), or cardiac index (CI) modify the effect of inotropes on outcomes in patients with HHF.

Methods: In the ESCAPE trial, we examined the association of in-hospital inotrope use (dobutamine, milrinone, or dopamine) with 1) 180-day major events (death, left ventricular assist device, or cardiac transplantation) and 2) study days alive and out of hospital, in relation to HF etiology, admission SBP, and, in a subset of patients, CI.

Results: 107/215 (49.8%) ischemic and 87/216 (40.3%) nonischemic patients received inotropes. Events were more frequent among patients who received inotropes, both with ischemic (42.1% vs. 20.4%; P<0.001) and nonischemic (33.3% vs. 16.3%; P=0.003) HF. Risk with inotropes declined over time in ischemic (adjusted HR at 30 days: 4.63; 95% CI: 2.01 to 10.6; P<0.001, HR at 90 days: 2.19; 95% CI: 1.23-3.90; P=0.008) but not in nonischemic (HR 2.13; 95% CI: 1.16-3.94; P=0.015) patients, Figure. 1. Admission SBP did not modify risk with inotropes; adjusted HR was 3.38 (95% CI: 1.10-10.3; P=0.033), 2.56 (95% CI: 1.49-4.42; P=0.001), and 1.80 (95% CI: 0.80-4.09; P=0.16) in patients with SBP <90, 90-109, and ≥110 mmHg, respectively, Figure. 2. CI (N=196) did not modify risk with inotropes. Inotrope use was associated with fewer study days alive and out of hospital in all subgroups.

Conclusion: In advanced HHF, inotrope use is associated with unfavorable outcomes regardless of etiology, SBP, or CI.

Figure 1

Figure 2
Combined mononuclear phagocyte system depletion and blockade permits functional adenoviral hepatic insulin gene transfer in pigs

**Thule PM, Jia DW, Campbell AG, Paveglio SA, Olson DE**

Assessing pigs provides a better indication of human responses to gene therapy than rodent studies. However, gene transfer into pig liver is difficult, and has required partial hepatectomy with ex vivo transfection, hepatic circulatory isolation, or segmental hepatic catheterization. In pigs, adenovirus induces circulatory collapse through activation of the pulmonary and hepatic mononuclear phagocyte system (MPS). We performed experiments in 12 pigs to determine if MPS depletion and/or blockade enhances adenoviral (Ad5) hepatic gene transfer. Hanford barrow swine (10-20kg) were administered increasing mesenteric vein doses of Ad5 with or without MPS depletion by gadolinium chloride (GdCl3) or liposome encapsulated clodronate (Lipo-Cl). Most Lipo-Cl pigs also received MPS blockade with poly-inosinic acid (pi). MPS depletion with GdCl3 reduced pulmonary MPS uptake of particulate copper and Ad5, and increased vector uptake in liver and persistence in blood. However, GdCl3 failed to prevent Ad induced circulatory collapse. In contrast, Lipo-Cl pretreatment alone prevented Ad5 induced circulatory collapse (3.5x1011Vp/kg). A combination of screening to exclude Ad5 serum neutralizing factors (SNF), Lipo-Cl treatment, and MPS blockade permitted survival, and diffuse pan-hepatic transduction (0.5-5%) in 5 pigs, as determine by green fluorescent protein microscopy. In a STZ-diabetic pig, administration of a metabolically responsive, liver specific insulin transgene produced hepatic transduction sufficient to induce weight gain and lower blood sugars from >300mg/dl to <100mg/dl without exogenous insulin. We conclude that in pigs, Lipo-Cl MPS depletion combined with blockade produces functionally significant hepatic gene transfer without inducing circulatory collapse.

The Impact of Perioperative Hyperglycemia in Patients With and Without Diabetes Undergoing Coronary Artery Bypass Surgery: A Prospective Multicenter Observational Study

**Cardona S, Farrokh F, Adeel A, Pasquel FJ, Smiley D, Jacobs S, Peng L, Umpierrez GE.**

This Emory prospective study compared the prevalence and severity of hyperglycemia, the need for continuous insulin infusion (CII), and perioperative complications in 200 CABG surgery patients with (n= 106) and without diabetes (n= 94). There were no differences in the number of surgical grafts, duration of surgery, APACHE score, or need for vasopressors (all p=NS). During the perioperative period, 100% of DM and 93% of non-DM patients developed hyperglycemia (>140 mg/dL), with 100% of DM and 80% of non-DM treated with CII (p<0.001). The mean insulin dose and duration of CII in the ICU were higher in DM (129 ±138 units and 30±25 hours) compared to non-DM patients (42±46 units and 15±14), p<0.001. After CII, 100% of patients with DM and 19% of non-DM required transition to subcutaneous (SC) insulin. Patients with DM had higher rates of ICU complications compared to non-DM (23% vs. 13%, p=0.07), but the greatest number of complications were among non-DM with hyperglycemia (BG > 140 mg/dl, p<0.003) compared to DM with hyperglycemia (p=0.63). There were no differences in length of ICU stay or in mortality between patients with and without DM.

Conclusion: Perioperative hyperglycemia and need for CII is common in CABG patients with and without diabetes. Patients with diabetes and non-DM with glucose > 140 mg/dl have higher number of complications compared to normoglycemia. An ongoing randomized trial at Emory will determine the importance of perioperative glycemic control on clinical outcomes in patients undergoing CABG surgery.

Indeterminate Common Bile Duct Strictures with “Atypical Cells” on Cytologic Brushing: Long-term Outcomes

**Runge TM, Gamboa AM, Chawla S, Yarandi SS, Keilin S, Woods KE, Willingham FF, Cai Q**

Purpose: Common bile duct strictures may be the lone sign of pancreaticobiliary malignancy in patients with negative cross-sectional imaging. ERCP with cytopathology is a critical step in the workup of these strictures. The appropriate course of action when cytology indicates “atypical cells” is often not clear. Knowledge of the long-term outcomes of CBD strictures with this finding may assist in management of these patients.

Methods: Between 2000-2009, 109 cases of indeterminate CBD strictures were identified. Patients with a mass on imaging or history of liver transplant were excluded. Cases interpreted as having “atypical cells” on cytopathology were identified, and the long-term outcomes of these cases were reviewed. Final diagnoses were made by surgical pathology, subsequent biopsies, or subsequent radiographic findings. For statistical comparisons, Chi-Square, Mann-Whitney U Test, and multiple logistic regression analyses were performed.

Results: Of 109 patients in our database, 56/109 patients ultimately were diagnosed with malignant disease. 14 had brushings with “atypical cells present.” Ultimately 13/14 of these patients were diagnosed with malignancy. 9/14 were diagnosed by surgical specimens, 2/14 by image-guided biopsy, and two by imaging. Adjusted for other risk factors, the presence of atypical cells was the strongest predictor of a malignant diagnosis (OR 5.67, p <0.05).

Conclusion: At our institution, the finding of “atypical cells” on brush cytology was strongly associated with malignancy. Close evaluation of such patients is necessary, and while treatment decisions should be individualized, consideration should be
given to surgical management when atypical cells are found on cytology.

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Mechanical Strain-induced Osteopontin Expression in the Aorta

Caesar C, Lyle AN, Taylor WR

Recent studies suggest elevated mechanical strain in disease states, such as hypertension, leads to vascular inflammation. However the underlying molecular mechanisms modulating this process are still largely unknown. In this study, we use in-vivo and in-vitro approaches to determine if elevated mechanical strain increases osteopontin (OPN), a pro-inflammatory protein, in a hydrogen peroxide (H2O2) dependent manner. In two mouse models of hypertension, induced via angiotensin-II (Ang-II) and norepinephrine (NE), we found that blood pressures were increased 1.5-fold and 1.3-fold increase respectively. Aortic OPN mRNA expression was also increased 7-fold (p<0.05) in the Ang-II treated group and 2.5-fold (p<0.05) in the NE treated animals. In-vitro studies utilizing rat aortic smooth muscle cells that were cyclically strained showed ~3-fold (p<0.001) increase in OPN mRNA and ~5-fold (p<0.001) increase in secreted protein expression. H2O2 levels were also increased 4-fold (p<0.05) in cyclically strained cells. Finally, to determine if increased OPN is H2O2-dependent, cells were simultaneously stretched with PEG Catalase, a H2O2 scavenger, which blunted OPN expression by 65.14% ± 0.22 (p<0.05). These results suggest that elevated mechanical strain, as experienced by the vascular wall under hypertensive conditions, could lead to increases in inflammatory protein expression, such as osteopontin, via a H2O2-dependent pathway.

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Role of βPIX in redox-dependent lamellipodia formation during PDGF-induced migration in VSMC

Duran CG, Williams HC, Griendling KK, San Martin A

During atherosclerosis and postangioplasty restenosis, the release of growth factors, such as platelet derived growth factor (PDGF), results in the migration of vascular smooth muscle cells (VSMCs) into the intima. We have demonstrated that PDGF-induced ROS production via Nox1 is indispensable for VSMC migration. Cell migration is a coordinated process that starts with the extension of the lamellipodia, a surface-attached protrusion at the leading edge. The intracellular signaling cascades involved in lamellipodia formation are unclear. Because Nox1 activation requires the small GTPase Rac1, we investigated the role of the guanine exchange factor βPIX in PDGF-induced lamellipodia formation in VSMC. We found that treatment of rat aortic smooth muscle cells (RASMs) with PDGF induced the activation of Rac1 between 1 and 5 min with a peak at 2 min. Likewise, we were able to pulldown βPIX from PDGF-treated cell lysates using purified GST-Rac1, suggesting the participation of βPIX in PDGF-induced Rac1 activation. Lucigenin assays demonstrated that siRNA knockdown of βPIX completely abrogated PDGF-induced superoxide formation in VSMC (50% increase in control vs 6% increase for siβPIX). Using live cell imaging, we observed that treatment of cells with siβPIX abrogated PDGF induced lamellipodia protraction rates (1.4μm/min SEM ± 0.37 vs 2.6μm/min SEM ± 0.71 for siControl) and retraction rates (1.3μm/min SEM ± 1.4 vs 3.0μm/min SEM ± 2.4). In addition, knockdown of βPIX reduces protrusion distance (0.6μm SEM ± 0.16 vs 1.1μm SEM ± 0.2). Taken together, our work demonstrates a critical role of βPIX mediating PDGF-induced Rac1/Nox1 activation and lamellipodia formation, thus implicating this signaling pathway in the regulation of VSMC migration.

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Pioglitazone attenuates alcohol-induced alveolar macrophage oxidative stress and dysfunction by up-regulating Nox4-associated microRNAs

Yelligar SM, Harris FL, Brown LA, and Hart CM

Rationale: Alcohol abuse increases risk of respiratory infections through enhanced oxidative stress and impaired alveolar macrophage (AM) phagocytic function. PPARγ ligands have been shown to reduce lung oxidative stress through down-regulation of NADPH oxidase 4 (Nox4). We hypothesized that treatment with pioglitazone (PIO), a PPARγ ligand, would attenuate alcohol-induced AM dysfunction by up-regulating Nox4-related microRNAs and subsequently decreasing AM oxidative stress.

Methods: AMs were obtained from the bronchoalveolar lavage (BAL) fluid of C57BL/6J mice fed ± ethanol (20% w/v) in the drinking water for 12 wks and treated ± 10 mg/kg/day PIO during week 12. In parallel, MH-S cells, a mouse AM cell line, were treated ± 0.08% ethanol for 3d ± 10 μM PIO for 1d. Levels of miRs (miR-25, -32, -92a, -92b, -363, and -367), which bind to the 3'UTR of Nox4 to increase Nox4 levels, were assessed by qRT-PCR. Nox4 mRNA and protein expression were measured by qRT-PCR and western blot. Oxidative stress was measured with DCFH-DA and Amplex Red assays. AM function was evaluated by phagocytosis assay (S. aureus internalization).

Results: In vivo and in vitro, ethanol: 1) decreased AM miR-92a, -92b, and -363 levels, 2) increased Nox4 mRNA and protein expression, 3) enhanced oxidative stress, and 4) impaired phagocytic capacity. PIO treatment reversed these ethanol-induced AM derangements.

Conclusions: Alcohol-induced AM oxidative stress and dysfunction were attenuated by PIO treatment. Our studies suggest PIO as a clinically relevant intervention that will ameliorate alcohol-induced AM dysfunction by up-regulating Nox4-associated miRs despite continued alcohol ingestion.
Lipid droplets (LD) are far more complex than just sites for neutral lipid storage. Such highly dynamic and mobile organelles derive from the endoplasmic reticulum (ER) and are present in most cell types and organisms, displaying a complex proteomic profile that reflects possible functions in signaling, metabolism, protein folding/maturation and intracellular traffic. Whether LDs integrate redox signaling in non-adipocytes is unknown. We have detected Nox4 in LD fraction with a ~2-fold increase after oleic acid incubation. In order to verify if LD could be involved in ROS-dependent processes, the redox state of LD was measured. Using the fluorescent redox-sensing probe C11-bodipy 581/591, we detected a fluorescence pattern consistent with a small basal level of lipid peroxidation in VSMC LD, which was increased upon incubation with oleic acid (60μM for 20 h). However, neither the known Nox4 regulator Poldip2 nor Nox4 subunit p22phox was associated with LD. Moreover, LD fraction does not present diaphorase or NADPH oxidase activity, measured by the electron acceptor INT and DHE oxidation, respectively. Thus, Nox4 appears to be inactive in LD. However, ROS scavengers (PEG-SOD + PEG-catalase) and flavoenzyme inhibition (DPI) promote LD translocation to the cytoplasm periphery when compared to basal condition. Interestingly, LD number increases with GKT (Nox4 inhibitor) or in Nox4 knockout MEFs, correlating with a previously described anti-adipogenic effect of Nox4. Consolidating the above results, we suggest that the vascular pool of Nox4 present in LD is not active and that cytoplasmic ROS (possibly derived from Nox4) interfere with LD traffic/translocation via microtubules.

**Conclusions:** Alcohol exposure decreases the expression of Trx1, which is associated with decreased Nrf2-ARE nuclear binding and decreased expression of a key enzyme in glutathione synthesis. These findings may help explain how alcohol induces oxidative stress and TGF-β1, resulting in many pathophysiological features of the ‘alcoholic lung’. Further experiments are necessary to more clearly delineate the balance between Nrf2 and Trx1.

**Progression of Pulmonary Artery Systolic Pressures by Echocardiography Among Ambulatory Patients without Pulmonary Hypertension at Baseline**

**Abstract Book**

**Methods:** We evaluated 106 ambulatory HF patients enrolled in a prospective cohort study with (1) available echocardiographic PASP at baseline; (2) baseline PASP <45 mmHg and (3) at least one follow-up PASP estimate ≥6 months later. We used mixed-effect models with random intercept and slope for PASP to estimate mean slope of PASP and its individual variation.

**Results:** Average number of serial echo studies was 3 (range, 2-6) over a median of 3.1 years (interquartile range, 2.0-4.1). Mean age of patients was 56±12; 52% were white; 44% were black; 58% were male; 41% had ischemic HF; left ventricular ejection fraction (LVEF) was 41±16%; and baseline PASP was 33±7 mmHg. Average slope of PASP progression was 1.0 mmHg/year (P=0.01); however, there was significant individual variation, with 95% of values lying between -2.9 to 3.9 mmHg/year. Higher baseline PASP was associated with accelerated PH progression (Figure 1). Patients with more-than-moderate mitral regurgitation had a trend towards more rapid PH progression (1.4 vs. 0.4 mmHg/year; P=0.055 for interaction). However, age, LVEF, functional class, and creatinine, among others, were not associated with progression of PH.
Conclusion: In ambulatory HF patients with no PH at baseline, the progression of PH is highly variable with higher PASP at baseline being associated with accelerated progression. Standard HF prognostic factors explain only a small fraction of the variance in PH progression.

Figure 1.

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Glucose Challenge Test Screening for Diabetes and Dysglycemia in a High-risk Primary Care Population


Diabetes prevention and care are limited by lack of screening. Screening with a 50g oral glucose challenge test (GCT), with measurement of glucose 1 hour later, regardless of meal status - similar to screening for gestational diabetes - has been shown to be accurate, convenient, and cost-effective in volunteers. We hypothesized that the GCT would also be useful in high risk patients. In a VA clinic, subjects with BMI >25 or age >45 had measurement of A1c, plasma and capillary random glucose (RPG and RCG), and plasma and capillary GCT (GCTpl and GCTcap). At a second visit, they had a diagnostic 75g OGTT. 1440 subjects had mean age 57 years and BMI 30.4, 94% were men, and 74% were black. By OGTT criteria, diabetes was present in 10% and high-risk prediabetes (IGT and/or IFG with glucose 110-125 mg/dl) in 22%. The GCTpl provided areas under receiver-operating characteristic curves (AUC) of 0.84, 0.76, and 0.70 for detection of diabetes, dysglycemia, and high-risk prediabetes, respectively. GCTcap performed similarly, with AUCs of 0.82, 0.74, and 0.69 (all p=ns vs. GCTpl). In 958 patients with complete screening data, GCTpl and GCTcap were significantly more accurate than A1c, RCG, and RPG for detection of diabetes or dysglycemia (all p<0.05). Conclusions: Primary care patients with BMI >25 or age >45 have a high prevalence of previously unrecognized diabetes. GCT screening, followed, if abnormal, by an OGTT, would be convenient and would be more accurate than random glucose or A1c for identifying these problems.

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The Role of the Receptor for Advanced Glycation Endproducts (RAGE) in Collateral Vessel Formation


Diabetes is a major health problem in the United States associated with the many complications. Specifically, cardiovascular disease is the leading cause of death in diabetic patients and over 60% of all non-traumatic lower limb amputations occur in diabetics. One potential mechanism for the complications is that chronic hyperglycemia can lead to the irreversible, nonenzymatic glycosylation of proteins to form advanced glycation endproducts (AGEs). The receptor for advance glycation endproducts (RAGE), is thought to be one of mechanisms through which AGEs elicit changes. The impaired ability of diabetics to form collateral vessels has already been well established, and work in our lab has shown that RAGE-/- mice have improved reperfusion over wildtype (WT) even in diabetic models. The goal of this project was to determine signals that regulate the expression of RAGE. Our hypothesis was that RAGE is upregulated in response to hypoxia and hydrogen peroxide. Using a mouse hind limb ischemia (HLI) model we found that RAGE was upregulated in the ischemic leg and that the more hypoxic gastrocnemius muscle had increased RAGE expression compared to the adductor region. RAGE expression following HLI was decreased in mice treated with peg-catalase indicating hydrogen peroxide signaling upregulates RAGE. Future work will determine which cells are involved in RAGE expression to begin to help us understand RAGE expression in a collateral growth model.

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S-adenosylmethionine improves the oxidative stress-induced lung epithelial barrier dysfunction in HIV-1 transgenic rats

Fan X, Raynor R, Joshi PC, Koval M and Guidot DM

Despite antiretroviral therapy, lung disease is a major cause of death in people living with HIV. HIV- related viral proteins can directly induce epithelial dysfunction in the lung via mechanisms involving oxidative stresses such as glutathione (GSH) depletion. Because S-adenosylmethionine (SAMe) is a glutathione precursor, we tested the hypothesis that supplementation of SAMe would antagonize oxidative stress-induced impairment of alveolar epithelial function in HIV-1 transgenic (Tg) rats. Monolayers derived from primary alveolar epithelial cells (AECs) isolated from HIV-1 Tg rats had markedly decreased intracellular GSH levels, abnormal tight junction protein expression and assembly within the cell membranes, and increased permeability compared monolayers derived wild type littermates. In contrast, treatment with SAMe for 5 days in the culture medium restored intracellular GSH levels and improved the localization of tight junction proteins in the plasma
membrane of HIV-1 Tg monolayers. More importantly, SAMe attenuated the HIV-1 viral protein-induced epithelial monolayer dysfunction as evidenced by both an increase in the transepithelial electrical resistance (TER) and a decrease in FITC-dextran paracellular flux. Further, AEC monolayers derived from HIV-1 Tg rats whose diets had been supplemented with SAMe for 8 weeks had significantly improved function compared to AEC monolayers from untreated HIV-1 Tg rats. Our findings raise the possibility that augmenting antioxidant defenses within the alveolar space could improve the lung health of HIV-infected individuals.

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Safety and Efficacy of Sitagliptin Therapy for the Inpatient Management of General Medicine and Surgery Patients with Type 2 Diabetes: A Pilot, Randomized Controlled Study

Pasquel, F; Gianchandani, R; Smiley, D; Jacobs, S; Wesorick, D; Newton, C; Farokhi, F; Peng, L; Reyes, D; Pradhan, S; Umpierrez, G

No previous studies have reported on the use of dipeptidyl peptidase-4 (DPP-4) therapy for the inpatient management of hyperglycemia in patients with type 2 diabetes (T2D). Accordingly, we conducted a pilot, open-label, randomized clinical trial to determine the safety and efficacy of sitagliptin (Januvia™) alone or in combination with basal insulin in the management of general medicine and surgery patients with T2D. A total of 90 patients with known history of T2D, with an admission BG between 140 mg/dl and 400 mg/dl and treated with diet, oral antidiabetic agents or with low total daily dose insulin (≤0.4 unit/kg/day) were randomized to receive sitagliptin alone or in combination with basal insulin (glargine) or with basal bolus regimen (glargine and lispro). If needed, all groups received supplemental doses of lispro before meals. Major study outcomes included differences in daily blood glucose (BG), frequency of treatment failures (defined as ≥3 consecutive BG≥240 mg/dl or a mean daily BG >240 mg/dl), and frequency of hypoglycemia (BG <70 mg/dl).

Glycemic control improved similarly in all treatment groups. There were no differences in the mean daily BG after the 1st day of treatment (p=0.23), number of readings within a BG target of 70-140 mg/dl (p=0.53), number of BG readings >200 mg/dl (p=0.23), and number of treatment failures (p>0.99). The total daily insulin dose and number of insulin injections were significantly less in the sitagliptin groups compared to the basal bolus group (both <0.001). There were no differences in the number of adverse events, length of hospital stay, or in the number of hypoglycemic events between groups (p=0.86).

In summary, these preliminary results indicate that treatment with sitagliptin alone or in combination with basal insulin is safe and effective for the management of hyperglycemia in general medicine and surgery patients with T2D.

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Urea Transport in the Collecting Duct, Regulated by an Endothelin/Nitric Oxide Signaling Pathway, is Required for Sodium Reabsorption

Rogers RT, Redd SK, Hong SM, Blount MA

The kidney modifies blood pressure by controlling fluid homeostasis. Nitric oxide (NO) production in the inner medullary collecting duct (IMCD) regulates sodium and water reabsorption to stabilize blood volume by regulating transporter function. Ablation of the IMCD urea transporters, UT-A1 and UT-A3, induces polyuria in UT-A1/A3 null (KO) mice. Disruption of water reabsorption in these mice leads us to speculate that NO production is altered and, in fact, we found that urinary nitrate/nitrite levels were increased 3-fold in UT-A1/A3 KO mice. Using qRT-PCR, we observed that NOS2 and NOS3 mRNA expression levels in the IMCD of UT-A1/A3 KO mice where similar to WT; however, NOS1 expression was increased 40-fold. Because IMCD endothelin (ET-1)/NO signaling pathway modulates pressure-natriuresis, we investigated this pathway in the UT-A1/A3 KO mice. We found that serum aldosterone levels and mineralocorticoid receptor protein expression, regulators of ET-1 concentration, were increased and endothelin type-B (ETB) receptor protein expression, the target of ET-1, was also increased in UT-A1/A3 KO mice compared to WT. Urinary sodium excretion in UT-A1/A3 KO was increased 57% and blood pressure was slightly lower in these mice compared to WT in accordance with the natriuretic and antihypertensive effects of ET-1 and ETB expression. Renal handling of sodium and water has always been linked processes in regulation of blood volume. The present studies suggest that urea handling in the IMCD, acting through endothelin-simulation of NOS1, also regulates sodium reabsorption.

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TGF-β mediates Focal Adhesion Maturation by a smad/Nox4-dependent mechanism that involves regulation of Hsp27 and Hic5


Focal adhesions (FAs) link the cytoskeleton to the extracellular matrix, and as such play a critical role in growth, contractility and vascular resistance in vascular smooth muscle cells (VSMCs). The maturation of FAs is regulated by Nox4, an H2O2-producing enzyme present in FAs. However, its downstream effectors are unknown. Here we examine the role of FA resident protein Hic5, a potential binding partner to heat shock protein (Hsp) 27, in Nox4-mediated FA maturation. We found that TGF-β promotes the formation of Hic5-containing mature FAs, increases both Hic5 (20834±520 vs. 11391±1024;p<0.01) and Hsp27 (98.2±0.1 vs. 25.4±3.9;p<0.001) expression and induces their co-localization.
in stress fibers and cytosol (5.5±0.2 vs. 1;p<0.01). These effects were blocked in siNox4 treated cells (34.5±10.9 vs. 98.2±1.1;p<0.001). Next, we evaluated the role of the TGF-β canonical downstream effectors, smads. Interestingly, siSmad blocked Nox4 expression and siNox4 impeded smad activation suggesting that these proteins participate in a forward feedback loop that is initiated by TGF-β. Similar to siNox4 cells, Hsp27 and Hic5 protein upregulation was blocked in smad4 deficient cells (27228±1688 vs. 10863±1604;p<0.0001 and 2.8±0.5 vs. 1.5±0.1;p<0.01 respectively). Additionally, Hic5 downregulation prevented TGF-β-mediated FA maturation, while the effects of Hsp27 were partial, identifying Hic5 as the more downstream effector. In Hsp27 deficient cells, Hic5 fails to localize to FAs even though Hic5 expression is unaffected. Taken together, our results support the idea that smad/Nox4 mediate TGF-β-induced FA maturation by upregulation of Hic5 and Hsp27. Furthermore, the interaction of Hic5 and Hsp27 facilitates Hic5 localization to FAs.

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**Metformin Attenuates Atherosclerosis and Vascular Aging Even in the Absence of Diabetes**

**Forouzandeh F, Salazar G, Patrushev N, Xiong S, Hilenski L, Alexander RW**

Cardiovascular disease due to atherosclerosis can be further exacerbated by other age-related changes in blood vessels such as arterial stiffness mediated by angiotensin II (Ang II). Thus, strategies that can target atherosclerosis and vascular aging concomitantly are of special interest. Metformin was shown to attenuate all-cause mortality and myocardial infarction compared with other standard diabetes medications, suggesting that metformin has protective effects in the cardiovascular system perhaps beyond its glucose control activities. However, whether the vasculoprotective effects of metformin persist in non-diabetic models have not been evaluated convincingly before. Thus, using the ApoE-/- mouse model, we examined whether metformin can attenuate the progression of atherosclerosis and/or vascular aging in response to Ang II treatment or high fat diet. In the Ang II-treatment model, we found that both Ang II-induced hypertension and vascular aging were almost completely abolished by metformin. Moreover, metformin treated animals had significantly less atherosclerotic plaques compared with control groups. Similar vasculoprotective effects were observed when metformin was given to the mice fed high fat. However, the vasculoprotective findings in both models were not accompanied by significant differences in blood glucose levels. Based on our recent ongoing studies, these effects of metformin could be explained, at least in part, by its role in modulation of the mTOR pathway that plays a major role in response to nutrition status.

This study further elucidates whether metformin can be used as a primary or secondary preventive therapy for patients at risk of suffering cardiovascular complications even in the absence of diabetes.

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**Pancreatic Insufficiency Secondary to Tobacco Exposure, Preliminary Results from the Prospective Controlled Cohort Study**


Introduction: Tobacco exposure is an established risk factor for pancreatic cancer and chronic pancreatitis, and has been associated with histologic changes in the pancreatic parenchyma. It is not known if tobacco exposure is an independent risk factor for pancreatic insufficiency in the absence of pancreatic disease.

Design: Prospective, controlled cohort study.

Methods: A priori sample size calculation based on a 15% difference in prevalence indicated a target enrollment of 100 patients in each arm. Data collection included a focused history, questionnaires, and validated inventories of alcohol and tobacco consumption [smokers (≥ 20 pack years); controls (≤ 5 pack-years and ≥7 years of abstinence)]. Pancreatic insufficiency was assessed using the fecal elastase-1 (FE-1) level.

Results: 7,854 patients were approached, 226 were interviewed, and 200 met inclusion criteria and were enrolled. The prevalence rate of pancreatic insufficiency in smokers [18% (18/100)] was significantly greater than in controls [6% (6/100)] (p=0.009). The mean FE-1 concentration in smokers (368.0 ug/g) was also significantly less than in controls (411.4 ug/g) (p=0.027). In multivariate logistic regression, the risk of pancreatic insufficiency in smokers was significantly increased (p=0.026, OR=3.39 [1.16-9.98]) when controlled for alcohol use, age, BMI, diabetes, ace-inhibitor usage, and dyslipidemia.

Conclusion: In addition to protean manifestations in other organ systems, this study establishes tobacco exposure as an independent risk factor for pancreatic insufficiency. While alcohol has historically been considered the primary exposure for pancreatic disease, this study suggests that smoking may have a greater impact on pancreatic function prior to the onset of clinical symptoms.

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**Encapsulation of Mesenchymal Stem Cells to Improve Stem Cell Therapy Following Myocardial Infarction in Rats**

Introduction: Cell-based therapy has emerged as a strategy to prevent decline in systolic function following myocardial infarction. One key barrier limiting the success of cell therapy is poor retention of viable stem cells around the myocardium. Our hypothesis is that encapsulation of MSCs in alginate will improve cell retention and cardiac function following myocardial infarction in rats.

Methods: Rat mesenchymal stem cells (MSCs) were encapsulated in 1% alginate and applied in a 4% polyethylene glycol-maleimide (PEG-MAL) hydrogel to the epicardial surface of rat hearts immediately after LAD ligation. We delivered one million MSCs by one of three methods: alginate-encapsulated MSCs in PEG hydrogel, non-encapsulated MSCs in PEG hydrogel, or direct injection of MSCs in the border zone of the infarcted myocardium. Cardiac function was assessed by echocardiography.

Results: While there was a trend towards improvement in all three treatment groups, only the direct injection group had improvement that reached statistical significance. Future analysis will compare the three delivery methods in their ability to decrease scar size, decrease apoptosis, and improve neovascularization in the infarcted rat heart.

Conclusion: Our finding that only direct myocardial injection of MSCs improved fractional shortening is inconsistent with our previous data in nude rats where encapsulation of MSCs in alginate was the superior delivery method for preserving systolic function. It is possible that the fully intact immune response to the hydrogel in Sprague-Dawley rats limited the efficacy of this delivery strategy. Future experiments will characterize the immune response to these biomaterials in this model.

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Impaired airway epithelial barrier function in Cystic Fibrosis-related Diabetes
Koval M, Molina SA, Hunt WR, Hanson JM and McCarty NA.

Cystic Fibrosis (CF)-Related Diabetes (CFRD) impacts ~50% of adult CF patients. CFRD is associated with abnormally high airway glucose and an accelerated decline in pulmonary function, indicating a failure in the airway epithelial barrier to regulate glucose permeability. Thus, we examined tight junctions of airway cells expressing wild type CF Transmembrane Conductance Regulator (CFTR) to cells expressing mutant ΔF508-CFTR. Increasing basolateral glucose from 100 to 450 mg/dL compromised tight junctions of ΔF508-CFTR cells by ~25% vs. control cells which were significantly less affected. Impaired barrier function was associated with decreased expression of claudin-4 and displacement of junctional ZO-1. Using a dual Luciferase-based ER stress reporter assay, cells expressing ΔF508-CFTR exhibited higher levels of ER stress than control cells. ER stress was further induced upon exposure to high glucose medium. Thus, ER stress amplified by expression of ΔF508-CFTR impairs the airway glucose barrier and may predispose the lungs of CF patients to the detrimental consequences of systemic diabetes.

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Depressive Symptoms, Oxidative Stress, Inflammation, and Vascular Dysfunction
Held EP, Al Mheid I, Obideen M, Morris A, Uphoff I, Cunningham L, Brigham K, Martin GS, Jones DP, Vaccarino V, and Quyyumi AA

Background: Psychosocial factors influence the incidence of cardiovascular disease (CVD) risk, and concomitant affective disorders forecast dismal outcomes in patients with CVD. We investigated the relationship between depressive symptoms and subclinical CVD, with the hypothesis that depressive symptoms will be associated with cardiometabolic abnormalities, arterial stiffness, oxidative stress burden, and increased levels of inflammatory markers. Methods: 1220 Atlanta residents (49±10yrs, 36% African American) compiled from both the Emory Predictive Health Initiative study (n=660) and the Morehouse and Emory Team up to Eliminate Health Disparities (META-Health) study (n=560) completed the Beck Depression Inventory-II (BDI) survey. Anthropomorphic measurements, lipid profile, C-reactive protein (CRP) levels and tumor necrosis factor alpha (TNFa) levels were obtained. Arterial stiffness was assessed by tonometry-derived pulse wave analysis to calculate the augmentation index (AIX) and the subendocardial viability ratio (SEVR). Oxidative stress was measured as total plasma glutathione (GSH).

Results: Mean BDI score was 7.0±7.1, and higher scores were associated with higher resting heart rate(r=0.1,p<0.001), systolic blood pressure(r=0.1,p<0.01), triglyceride levels(r=0.1,p<0.01), and lower HDL(r=-0.1,p<0.01). The BDI score correlated with AIX(r=0.1,p<0.036), CRP(r=0.1,p<0.001), SEVR(r=-0.09,p<0.01) and GSH levels(r=-0.09,p<0.01). Multivariable adjustment for the Framingham Risk Score, age, race, BMI, and study subset confirmed an independent association between higher BDI scores and higher AIX(R=0.43,B=0.16,p=0.004), lower SEVR (R=0.29,B=-3.36,p=0.05), lower GSH(R=0.276,B=-0.04,p=0.031), higher CRP levels(R=0.47,B=0.24,p=0.001) and higher TNFa levels(R=0.4,B=0.24,p=0.048).

Conclusion: Adults with depressive symptoms exhibit worsened cardiometabolic profiles and subclinical vascular disease, evidenced by the increased oxidant stress, levels of inflammatory markers and arterial stiffness in those with higher BDI scores, irrespective of traditional risk factor burden.
A Cost Analysis Study of Basal Bolus and Sliding Scale Insulin Regimens in General Surgery Patients with Type 2 Diabetes

The Rabbit-2 surgery trial reported better glycemic control and lower number of hospital complications with use of a basal bolus (BB) regimen compared to sliding scale regular insulin (SSI) in general surgery patients with type 2 diabetes. The financial impact of such interventions; however, is unknown. This post-hoc cost analysis determined differences in hospitalization costs and resource utilization using cost-charge ratios from Centers for Medicare and Medicaid Services, among 180 of 211 patients in the Rabbit 2 Surgery trial treated with BB (n=88) and SSI (n=92) regimens. Patients treated with BB regimen had improved glycemic control (p < 0.001) and lower perioperative complications compared to SSI (24% vs. 7%, p=0.002). Total hospital costs for the SSI group were higher at $26,841±15,928 compared to $22,998±12,040 for the BB group (p=0.09), with a log-transformed cost savings of $2,105 (95% CI: -$3,303 - $7,000), p=0.53. Compared to BB regimen, treatment with SSI resulted in higher resource utilization for pharmacy, radiology, laboratory, ICU and consultation services (p=0.25) and in greater resource utilization costs ($7,207±13,322 vs. $5,467±5,724, p=0.50). Among patients with ICU utilization, SSI-treated patients had higher post-surgery ICU use (2.7±3.5 vs 1.1±0.3 days, p=0.025) and higher total costs ($48.3±24.7K vs $31±7.0 K, p=0.02) compared to BB group. In conclusion, treatment with BB regimen is associated with improved glycemic control, lower number of complications, lower resource utilization and hospitalization costs compared to SSI treatment in general surgery patients with type 2 diabetes.

Anxiety Symptoms, Major Clinical Events, and Healthcare Resource Utilization in Outpatients with Heart Failure in The Atlanta Cardiomyopathy Consortium (TACC) Study

Introduction: Anxiety and depression are common comorbidities in heart failure (HF). Reports on the role of anxiety on risk for hospitalization have not been conclusive yet.

Methods: We evaluated anxiety by the Generalized Anxiety Disorder (GAD)-7 tool at baseline and its association with major clinical events (death, heart transplant, and ventricular assist device) and healthcare resource utilization in 323 outpatients enrolled in a prospective HF cohort study. Anxiety symptoms were stratified as minimal (0-4), mild (5-9), or moderate/severe (10-21). We used Cox models for major clinical events and Poisson models for healthcare resource utilization.

Results: Mean age was 57±12 years; 64% of patients were male; 50% were white and 47% black; 42% had ischemic HF. Mean left ventricular ejection fraction was 39±15%. At baseline, 203 patients (63%) had minimal anxiety; 66 (20%) had mild anxiety; and 54 (17%) had moderate or severe anxiety. GAD-7 score was not associated with major clinical events or rate of HF-related admissions. Patients with moderate-to-severe vs. those with minimal or mild anxiety had 18.5% higher rates of all-cause hospitalizations (P=0.049) and 36.1% higher rates of cardiovascular hospitalizations (P=0.031) (Table 1). Patients with mild (vs. those with minimal anxiety) spent 11.5% more days in the hospital (P=0.005) and 33.0% more days in hospital for HF (P<0.001), Table 1.

Conclusion: Anxiety is not associated with major clinical events or HF admissions in stable outpatients with HF. However, patients with moderate-to-severe anxiety have higher rates of all-cause and cardiovascular admissions. Mild anxiety was associated with more in-hospital days.

POSTER WITHDRAWN

One-Year Mortality Among Patients With Heart Failure and Preserved Ejection Fraction at Presentation: Implications for Clinical Trials

Introduction: Most data on outcomes in patients with heart failure with preserved ejection fraction (HFP EF) come from hospitalized heart failure (HF) databases. Hospitalization for HF is associated with substantially elevated mortality rates in the following months. Data on outpatients with HFP EF are scarce.

Methods: We evaluated the medical records of 390 outpatients without congenital heart disease or mechanical circulatory support who received care in Q1 2012 associated with ICD-9 codes 402.X1, 404.X1, 404.X3, and 428.XX. We recorded (1) documentation of HF; (2) latest and previous ejection fraction (EF) reports; and (3) specific cardiomyopathies, and classified HF into HFP EF (EF >40% without previous EF≤40%); HF with recovered EF ([HFrEF]; EF >40% without previous EF≤40%); and HF with reduced EF ([HFrE EF]; EF ≤40%).

Results: Mean age was 63±15 years; 54.6% were male; 48.0% white and 44.6% black. Overall, 76 (19.5%) of patients had HFP EF; 66 (16.9%) had HFrE EF; and 235 (60.3%) had HFrE EF; documentation was incomplete for 13 patients (3.3%). Unadjusted 1-year mortality was 5.3% (95% CI: 2.0% to 13.4%) for patients with HFrE EF; 3.0% (95% CI: 0.8% to 11.6%) for
patients with HFrEF; and 8.5% (95% CI: 5.6% to 12.9%) for patients with HFrEF (P=0.19 for log-rank).

Conclusion: Outpatients with HF presenting with EF >40% (either HFP EF or HFrEF) have a relative low 1-year mortality, which tends to be lower compared to HFrEF. Patients with HFrEF tend to have the lowest mortality. Although further confirmation in larger patient samples is necessary, these findings have implications for the design of clinical trials.

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Providing Antibiotic Cost Data Reduces High-Cost Antibiotic Prescribing
Newman KL, Varkey J, Mohan AV

Background: Physicians frequently prescribe antibiotics to inpatients without any knowledge of medication cost. It is not well understood whether providing cost data would change prescribing behavior. The study goal was to evaluate the effect of providing cost data alongside culture and antibiotic susceptibility results in a tertiary care hospital.

Methods: We conducted a quasi-experimental, pre-post analysis of all patients diagnosed with bacteremia or UTI who received positive culture results that were susceptible to antibiotics from more than one cost category at two teaching hospitals during a 12-month baseline period and subsequent 12-month intervention period during which we added cost category data for each antibiotic ($, $$, $$$, or$$$$) to culture and susceptibility testing data. Our main outcome measure was change in the average cost per patient of antibiotics prescribed after the receipt of susceptibility testing results.

Results: There was a significant decrease in the average cost category of antibiotics per patient after the intervention (pre-intervention=1.9 $ vs. post-intervention=1.8 $, p=0.003). After adjusting for age, sex, and length of stay prior to sensitivity test results, the odds ratio (OR) of a patient’s average antibiotic cost being $ or $$ vs. $$$ or$$$$ after the intervention was 1.77 (95% confidence interval (CI) 1.06, 2.97).

Conclusion and Relevance: Providing physicians with cost data alongside culture and antibiotic susceptibility results was associated with a significant decrease in prescription of high cost antibiotics. This intervention is easy to implement, low cost, and may shift providers towards less expensive medications when equally acceptable options are available.

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Hospital Discharge Rates due to Pulmonary Embolism in the State of Georgia
Mohamed Kelli H, Tahhan A, Kelli M, Winawer N, Lattouf OM

Background: Pulmonary embolism (PE) is a potentially fatal disorder. As diagnostic methods have developed over time, it is unclear if these advances have contributed to early detection of PE. The aim of this study is to analyze the hospital discharge rates due to PE in Georgia. Methods:

Data from the Georgia Department of Public Health database were utilized based on ICD -9 and ICD-10 codes for PE to identify hospital discharge rates from 1999-2010. The statistical difference of gender and race were compared and graphs were obtained to show the rate progression in the time period. Results:

The overall hospital discharge rates due to PE has approximately doubled from 23.9 per 100,000 in 1999 to 51.4 per 100,000 in 2010 over the study period (P=0.00). A similar raising trend was observed in gender, with higher rates in females (P=0.02). Hospital discharge rates were also consistently higher in African-Americans with widening gap in difference over time but it was not statistically significant (P=0.27).

Conclusions: Hospital discharge rates due to PE in Georgia have steadily increased over time. Females have consistently higher rates compared to males. Although there was no statistically significant difference in race comparison, the gap difference widened over the study period. We theorize that the overall increase in hospital discharge rates is likely due to the improvement and application of diagnostic modalities that contribute to the early detection of PE during admission. Further studies should address whether improved detection of PE reduces overall mortality and hospital cost.

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The Patients’ Experiences in Emergency Research-ProTECT Phase (PEER-ProTECT)
Dickert NW, Mah, VA, Baren JM, Biros MH, Fleischman R, Govindarajan P, Jones EB, Pancioli A, Wright DW, Pentz RD

Background- Research on emergent conditions often requires exception from informed consent (EFIC). Concerns exist regarding patients’ views of enrollment in EFIC studies, and it is unclear whether community views reflect enrollees’ views.

Methods- The Patients’ Experiences in Emergency Research-ProTECT (PEER-ProTECT) study was nested within the Progesterone for the Treatment of Traumatic Brain Injury (ProTECT III) Trial, a placebo-controlled trial of progesterone in traumatic brain injury. ProTECT III patients or surrogates (if patient died or remained impaired) were enrolled from 12 sites. Structured, interactive interviews ascertained views of acceptance of EFIC enrollment in ProTECT III. Closed-ended responses were analyzed using descriptive and bivariate statistics. Text data were analyzed thematically.

Results- 31 patients and 54 surrogates were interviewed. 71
(84%) respondents were glad they (or family member) were included in ProTECT III. 66 (78%) found EFIC inclusion acceptable; 4 (5%) found it unacceptable, and 15 (18%) were neutral or uncertain. Female subjects were more likely to agree with EFIC enrollment (87% vs. 67%, p=0.025). Black (n=11) subjects were less likely to agree with EFIC enrollment than white (n=59) or Hispanic (n=6) subjects (55% vs. 83% and 100% respectively, p=0.029). Positive responses mostly related to potential direct medical benefits (88%). Subjects generally accepted placebo use (79%) and randomization (91%).

Conclusions- Most enrollees and surrogates in this placebo-controlled EFIC trial had positive views of enrollment; approval rates were higher than in other studies and in community consultation reports from ProTECT III. Male and black subjects' lower acceptance, however, demonstrate heterogeneity within this population.

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Gene Expression Profiling Analysis Of Human B-Cell Subsets in Health and Systemic Lupus Erythematosus
Wei C, Ramos E, Allaire N, Zak S, Kalled S, Ranger A, Sanz I

SLE is an autoimmune disease, which exhibits multiple B cell abnormalities including expanded populations of plasmablasts and DN memory cells as well as a contracted unswitched memory subset. The transcriptional profiles that underlie these homeostatic changes are poorly understood. To remedy this knowledge gap and generate insight into the disease pathogenesis, we carried out transcriptome analysis of five B cell subsets sorted from 12 healthy controls (HC) and 13 SLE patients with low disease activity (SLEDAI<6). Overall, there are fewer differentially expressed genes (DEGs) among the three memory B cell subsets than between naive and each of the three memory subsets in both healthy subjects and SLE patients, suggesting that the gene expression program is quite similar among all the memory B cells in HC and SLE. The transcriptome of multiple B cell subsets was remarkably similar between HC and SLE patients with low disease activity suggesting that most differences in active disease may be due to extrinsic differences. Most DEGs differentials between the two cohorts were identified in the DN subset. Our results are consistent with the important role of IL-4 and IL-21 as growth factors for naïve cells and the known activity of IL-6 in memory differentiation into plasma cells. Of great interest is the upregulation of TACI, AICDA and FcRL4 in DN B cells, a population expanded in SLE. The implications for the potential germinal center origin and activation status of these cells, reported to represent exhausted cells in HIV infection, will be discussed.

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Ovariectomy Induces Short-Term Hemopoietic Stem Cell Expansion through T Cells
Li J-Y, Adams J, Laura MC, Weitzmann MN, and Pacifici R.

Estrogen deficiency expands bone marrow (BM) hemopoietic stem and progenitor cells (HSPCs) and mature blood lineages but neither the involved mechanism nor the affected HSPC populations are known. Since T cells contribute to ovariectomy (ovx) induced bone loss, we investigated the role of T cells in the HSPC expansion induced by ovx. Ovx increased the number of HSPCs (Lin-Sca-1+cd45+Kit+ cells) by ~2 fold and host survival after BM transplantation by ~4 fold in WT mice and T cell reconstituted-T cell deficient mice. By contrast ovx had no effects on HSPCs expansion and survival after BM transplantation in T cells deficient mice. Analysis of SLAM receptor expression on HSPCs and competitive repopulation assays demonstrated that ovx specifically expands short-term HSCs (ST-HSCs) without exhausting long-term HSCs (LT-HSCs) in T cell replete mice but not in T cell null mice. Mechanistic studies revealed that ovx expands ST-HSCs and improves host survival after BM transplantation through a dual role of the T cell costimulatory molecule CD40L. This surface receptor is required for ovx to stimulate T cell production of Wnt10b, a Wnt ligand that activates Wnt signaling in HSPCs and stromal cells (SCs). Attesting to the relevance of CD40L and Wnt10b, ovx expanded ST-HSCs and survival after BM transplant in CD40L null mice and in animals lacking global or T cell expression of Wnt10b. In summary, T cell expressed CD40L and the resulting increased production of Wnt10b play a pivotal role in the mechanism by which ovx regulates hemopoiesis.

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“Administrative” Versus Clinically Adjudicated Heart Failure With Preserved Ejection Fraction Among Outpatients: A Preclinical Trial Registry

Introduction: Administrative databases report that heart failure with preserved ejection fraction (HFpEF) represents 40%-60% of heart failure (HF) cases but limited prospective data suggest a lower proportion. Also, enrollment in recent HFpEF trials has been slower than anticipated, suggesting that the proportion of HF patients fulfilling all clinical criteria for HFpEF may be considerably lower in practice.

Methods: We evaluated the medical records of 390 outpatients who received care in Q1 2012 associated with ICD-9 codes 402.X1, 404.X1, 404.X3, and 428.XX excluding those with congenital heart disease or mechanical circulatory support. We recorded (1) HF documentation; (2) latest (index) and previous ejection fraction (EF) reports; and (3) specific cardiomyopathies. True HFpEF was defined as HF with index EF >40% without previous EF ≤40%.

Results: Mean age was 63±15 years; 54.6% were male; 48.0% white and 44.6% black. Overall, 152 patients (39.0%) had index...
EF >40%; among these, 66 (43.4%) had a previous documentation of EF ≤40% and therefore had recovered EF, 10 (6.6%) had no documentation of HF, and 76 (50.0%) had true HFpEF. Among cases with HFpEF, 16 had specific cardiomyopathies (amyloid, other restrictive, hypertrophic, or primary right-sided disease) for a total of 60 HFpEF cases potentially eligible for HF therapies. The proportion of these cases was 15.4% (95% CI: 11.8% to 19.0%) in the entire sample and 39.4% (95% CI: 31.6% to 47.3%) among patients with EF >40% at index visit.

Conclusion: Prevalence of true HFpEF among HF outpatients is considerably lower than that reported from administrative data.

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A Compelling Need for Bed Exit Prediction Tools: Voices from the Front Lines of a Skilled Nursing Facility

Background: About half of all skilled nursing residents fall each year, with older, ambulatory, and cognitively impaired residents especially at risk. Most falls in skilled nursing occur in out-of-view places such as bedrooms and bathrooms. Existing tab or pressure monitors alert staff of bed and chair exits at a point in time when little can be done to prevent falls. Methods: This small case study of a top-rated skilled nursing facility in Atlanta, Georgia, used in-depth interviews with administrators and focus groups with licensed nursing staff and certified nursing assistants to explore their perceptions regarding the potential role of new bed exit prediction technology for predicting future bed exits within the context of current fall management approaches, work arrangements, and existing tools and technology. Transcripts of recorded interviews were analyzed thematically. Results: Staff expressed dread and lack of control with regard to resident falls and “huge down time” when falls occur. They described a culture that collectively monitors fall-risk residents and collectively responds when falls occur. However, typical staffing ratios, limited communication technology, and lack of location information from bed exit alarms lead to poorly coordinated fall response efforts. Study participants expressed high interest in predictive technologies as well as concern about technology maintenance, noise disruption, infringement on resident and staff privacy, and threats to collective fall management culture. Conclusion: There is an urgent need for bed exit prediction technology. However, technological development of this kind needs to take account of staff culture and concerns.

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Gender Differences in the Prognostic Value of Angina-Related Health Status in Patients with Suspected Coronary Artery Disease

Background: Angina-related health status measured by the Seattle Angina Questionnaire (SAQ) has been shown to predict outcomes in patients with coronary artery disease (CAD), but few data are available for women. We sought to investigate gender differences in the prognostic role of angina health status domains towards long-term outcomes.

Methods: 3100 patients (age 62 ± 11, 65% male) undergoing coronary angiography were followed for mean 3.5 years for all-cause death and/or myocardial infarction (MI). Three domains of SAQ were assessed: health-related quality of life (HRQOL), physical limitation (PL), and angina frequency (AF), on a scale of 0 to 100. Severity of CAD was measured using the Gensini score and depressive symptoms with the Patient Health Questionnaire (PHQ)-9.

Results: Composite death/MI occurred in 545 patients (18%) during follow-up. Women reported worse HRQOL (63±28 vs. 68±27, P <0.001) but had significantly lower Gensini scores (23±43 vs. 49±65, P<0.001). HRQOL was independently associated with severity of CAD in both genders (p<0.001) after adjustment for conventional risk factors and depressive symptoms. In women, after adjusting for age, traditional risk factors, depressive symptoms, ejection fraction, and Gensini score, HRQL was an independent predictor of death/MI (HR=0.969 per 5-point score increment, P=0.03). In contrast, in men, after adjusting for the same factors, HRQL was not an independent predictor of death/MI (HR=1.001, p=0.95). Results for the other SAQ domains were similar.

Conclusion: Despite being associated with severity of CAD in both genders, HRQOL and other angina-related health status domains are significant predictors of outcomes in women only.

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Therapeutic ERCP in Children as Compared to Indication-Matched Adult Controls, A Four-Year Experience
Zaid Alnoah, MD, Saurabh Chawla, MD, Alvin Freeman, MD, Kevin Woods, MD, MPH, Steven Keilin, MD Qiang Cai, MD, PhD, Cary Sauer, MD, MSc, Field Willingham, MD, MPH

Background: In many large institutions, endoscopic retrograde cholangiopancreatography (ERCP) in children is performed by adult gastroenterologists; however, there is little data regarding the safety and efficacy of ERCP in the pediatric population.

Purpose: To compare the technical success, clinical success, complications and outcomes of therapeutic ERCP in pediatric patients versus indication-matched adult controls.
Methods: Case-controlled, retrospective review of therapeutic ERCP in a pediatric population compared with an adult control group matched 1:2 on indication and ASGE grade at an adult tertiary care center.

Results: 34 children (aged 5-17, mean 12.8) and 68 matched adults (aged 19-91, mean 55.4) underwent 63 and 154 procedures, respectively. All procedures were performed with adult duodenoscopes using standard devices. The groups were equivalent by indication and ASGE grade. Main indications for therapeutic ERCP in children were choledocholithiasis (26%), biliary strictures (23%), and pancreatic indications (23%). Technical and clinical success in children was similar to adult controls (technical success 93% vs 95%, p=0.7; clinical success 76% vs 85%, p=0.2). There was no significant difference with regards to technical details (fluoroscopic time, total time, cannulation device) or complications. The most common complication was post-ERCP pancreatitis or worsening of pre-existing pancreatitis, which occurred in 2/34 (5%) children and 3/68 (4%) adults. Significantly more pediatric procedures were performed under general anesthesia as compared to adult controls (p value < 0.001).

Conclusion: Therapeutic ERCP performed by subspecialized adult gastroenterologists is safe and efficacious in pediatric populations. Technical and clinical outcomes were equivalent in cohorts of pediatric and indication-matched adult controls.

80 Circulating progenitor cell levels are higher in patients with ST-segment elevation myocardial infarction compared to other acute coronary syndrome presentations. Patel SP, Ghasemzadeh N, Patel RS, Li Q, Eapen D, Khayata M, Waller EK, Quyyumi AA

Background: Circulating progenitor cells (CPCs) are mobilized in response to myocardial injury and reflect reparative/regenerative potential. CPC levels have been shown to correlate with cardiovascular outcomes; however, data about the role of CPCs in acute coronary syndrome (ACS) is limited. We hypothesized that the degree of mobilization of CPCs differs based upon the phenotypic ACS presentation.

Methods: We recruited 90 ACS patients (mean age 65±15 yrs, 73% male) presenting for cardiac catheterization. Nine patients presented with ST-segment elevation myocardial infarction (STEMI), 69 with non-ST-segment elevation myocardial infarction (NSTEMI), and 12 with unstable angina (UA). Blood samples were obtained at the time of cardiac catheterization for enumeration of CPCs as CD45 dim cells using flow cytometry.

Results: No differences in CD34+ or CD34+/CD133+ levels were observed between the different phenotypes of ACS. Analysis of more specific CPCs revealed that CD34+/VEGF2R+ and CD34+/CXCR4+/VEGF2R+ cells were increased in patients presenting with STEMI when compared to patients with NSTEMI (0.37±0.38 vs. 0.22±0.21 cells/μL, p=0.05; 0.37±0.38 vs. 0.21±0.21 cells/μL, p=0.04) or UA (0.37±0.38 vs. 0.09±0.07 cells/μL, p=0.004; 0.37±0.38 vs. 0.08±0.07 cells/μL, p=0.004). CD34+/VEGF2R+ and CD34+/CXCR4+/VEGF2R+ levels correlated with peak troponin levels following ACS (r=0.28, p=0.012 and r=0.29, p=0.009, respectively). These differences persisted after analysis of covariance to account for age, gender, diabetes, smoking status, and statin use.

Conclusions: These results suggest that CPC counts vary based on phenotypic ACS presentations. Increased severity of myocardial injury appears to result in increased CPC recruitment. Further investigation is needed to validate these findings.


Introduction: Since 1975, the incidence of esophageal adenocarcinoma (EAC) has increased at a faster rate than any other cancer. Standard treatment for esophageal cancer is esophagectomy with lymph node dissection, which is associated with significant morbidity (20-50%) and mortality (2-5%). Smaller series suggest that endoscopic therapy may be safe and effective for early EAC (stage Tis, T1a, T1b); however this has not been examined in a national population based sample.

Methods: Data from 2004-2010 on early esophageal adenocarcinoma was extracted from the Surveillance, Epidemiology and End Results (SEER) database. Mortality data were measured in months from diagnosis. Mean survival was compared according to treatment allocation. Survival analysis was performed for patients managed with endoscopy versus surgery using Kaplan-Meier curves.

Results: 1755 cases of early EAC were identified. 428 underwent endoscopic therapy and 1327 underwent surgical therapy. The mean survival time for patients receiving endoscopic therapy was 58 months compared with 61.6 months for surgery. Kaplan Meier analysis was plotted by treatment using Kaplan-Meier curves.

Conclusions: Mortality outcomes were compared between endoscopic and surgical therapy for early stage esophageal adenocarcinoma. Endoscopic therapy was associated with a similar survival rate compared to surgical therapy.
Increased Circulating Progenitor Cells in Women with Microvascular Coronary Dysfunction: Results from the NHLBI-Sponsored Women’s Ischemia Syndrome Evaluation - Coronary Vascular Dysfunction Study (WISE-CVD)


Background: Women with ischemia but no obstructive CAD often have microvascular coronary dysfunction (MCD). Ischemia stimulates a reparative response resulting in mobilization of progenitor cells (PCs), but PCs in subjects with vascular disease may not have the capacity to proliferate. Preliminary studies show that PC colonies in culture are reduced in MCD. We hypothesized that women with ischemia from MCD will mobilize PCs into the circulation.

Methods: In 160 women with ischemia but no obstructive CAD enrolled in the WISE study, we measured intracoronary adenosine-mediated coronary flow reserve (CFR) (n=160), acetylcholine-mediated microvascular endothelial function (n=48), and nitroglycerin-mediated endothelium-independent function (n=49). Circulating PC counts were measured using flow cytometry for expression of CD34, CD133, and CXCR4 epitopes on CD45med mononuclear cells.

Results: Subjects were 53±11 years, 38% were hypertensive, 10% diabetic, 28% hyperlipidemic, and 7% smokers. Lower CFR with adenosine correlated with higher circulating levels of CD34+ (r = -0.19, p=0.015) and CD34+/CD133+ (r = -0.18, p=0.020). Lower vasodilation with acetylcholine correlated with higher CD34+/CD133+ cells (r = -0.29, p=0.044). In multivariate analyses, after adjusting for the aforementioned covariates, lower CFR remained significantly associated with elevated CD34+ (p=0.020), CD34+/CD133+ (p=0.029) and CD34+/CXCR4+ (p=0.046) cells. Moreover, lower acetylcholine responses remained independently associated with higher CD34+ (p=0.048), CD34+/CD133+ (p=0.031), and CD34+/CXCR4+ (p=0.018) cells. PC levels did not correlate with nitroglycerin responses.

Conclusion: In women with MCD, reduced microvascular endothelial function and impaired CFR are associated with mobilization of PCs, suggesting that vascular dysfunction and/or ischemia stimulate PC mobilization.

Therapeutic neovascularization and long-term fate of purified endothelial cells derived from human pluripotent stem cells via a clinically-compatible system

Lee S, Kim S, Byun J, Han J, Park I, Kim W, Kwon P, and Yoon Y

Current endothelial cell (EC) differentiation systems from human pluripotent stem cells (hPSCs) have limited clinical use due to low efficiency and purity, and undefined components. Moreover, the long-term fate of hPSC-derived ECs implanted in cardiovascular animal models is unknown. We developed a fully defined clinically-compatible system to differentiate hPSCs into ECs by stepwise treatment with a GSK3β inhibitor, angiogenic factors, and a Notch ligand, and sorting with CDH5 (VE-cadherin). This protocol generated similar yields of ECs in five hPSC lines. The resultant hPSC-CDH5+ cells showed committed EC characteristics and pro-angiogenic activities, and enhanced recovery when implanted into hindlimb ischemia. Histologically, transplanted cells contributed to neovascularization through angiogenesis at an early phase, and a vessel-guiding role and vasculogenesis at a later phase. Notably, the proportion of perivascularly-localized and vessel-incorporated cells was gradually increased over 10 months of follow-up. These hPSC-CDH5+ cells and this insight into their long-term in vivo behavior will be useful for cell therapy and drug discovery.

Complication from Biopsy of the Ampulla of Vater


Background: Biopsy of the ampulla of vater is indicated for evaluation of ampullary adenomas, tumors, and carcinomas. More recently biopsy of normal appearing papillas with immunohistological staining for IgG4 has been used for
autoimmune pancreatitis. While generally assumed to be safe, there are reports of severe adverse events following biopsy, and the complication rate has not been systematically evaluated.

Objective: The primary aim of the study is to systematically evaluate the rate and type of complications following biopsy of the ampulla of vater.

Design: Observational, retrospective cohort study

Methods: Data was abstracted from a large endoscopy database for patients undergoing ampullary biopsy from December 1, 2008 to May 30, 2013. The data included patient demographics, findings at endoscopy, rate of complications, and final pathologic diagnosis.

Results: 561 procedures were reviewed, and 159 were included in the analysis, including 54 ERCP, 45 EUS and 60 EGD. Complications occurred in 9 cases (5.7%), including pancreatitis 7 (4.4%), bleeding 2 (1.3%), and infection 2 (1.3%). The presence of a large ampulla by appearance or adenoma on histology were not associated with a decrease in the complication rate (p-values of 0.783 and 0.926 respectively).

Conclusions: Biopsy of ampulla of vater is safe and effective in the diagnosis of ampullary mass lesions. On systematic evaluation, complication rate was 5.7%. The complication rate was similar for large and small ampullas.

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Multidisciplinary Group Education Sessions Teaching Patient Self-Management Skills Improves Outcomes For Low-Income, Low-Literacy Patients in an Urban Setting

Schmid S, Fost M, Flucker SA, Miller L, Bussey-Jones J, Levitch D, Quairoli K, Brown M.

Statement of Problem: In an urban outpatient setting serving large numbers of low-income, low-literacy patients, do multidisciplinary group sessions for obesity improve patient (1) perceptions of overall health, (2) perceptions of healthcare delivery, and (3) outcomes in weight loss?

Methods: Enrollees with obesity and at least one other chronic condition attended group sessions teaching self-management skills for making healthy behavioral choices related to diet and exercise. A team of healthcare providers (physician, pharmacist, nutritionist, and nurse) led sessions twice per month. Patients were invited to attend five sessions in total. We treated three groups of patients, with each group conducted in successive quarters over the year.

Measures of Success: Patients completed pre and post intervention surveys: Short Form 36 (SF 36) Health Survey assessing perceptions of overall health, and the Patient Assessment of Chronic Illness Care (PACIC) Survey assessing patient activation, goal setting, and problem-solving in line with the chronic care model. We measured pre- and post-survey scores using matched pairs t-tests.

Results to Date: Fourteen patients completed pre- and post-surveys. Significant differences in overall scores for both the PACIC [t= -2.42, p=0.04, CI (-2.056, -0.048)] and SF-36 [t=3.52, p=0.01, CI (2.509, 14.004) were observed. There was no significant difference in overall mean arterial pressure pre- and post-intervention. Weight loss did reach a statistically significant change [t= -2.48, p=0.02, CI (-9.542, -0.752)], mean of the differences = -5.147, although this did not meet clinical significance (defined as >5% weight loss).

Key Lessons for Dissemination: In our urban outpatient clinic serving low-income, low-literacy patients, group classes teaching self-management skills related to diet and exercise improved obese patients’ perceptions of overall health, as well as their satisfaction with healthcare delivery in line with PCMH principles and the chronic care model. A larger sample size is needed for definitive analysis and should be followed over time.

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Understanding the Stimulatory Pathways Responsible for Naïve B Cell Activation in Systemic Lupus Erythematosus

Blalock EL, Scharer C, Jenks S, Boss J, and Sanz I.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by multiple B cell abnormalities, including naïve B cell activation. Because the mechanisms of this activation remain unclear, we investigated activation marker expression on activated naïve (aN) and resting naïve (rN) B cells of SLE patients and the stimulatory pathways responsible for their expression. Multidimensional flow analysis was utilized to determine B cell activation marker levels on SLE aN and rN B cells versus healthy controls (HC). PBMCs from SLE and HC patients were stimulated and compared via flow analysis. Genome-wide DNA methylation status of SLE and HC B cells was determined using MeDIP-Seq. Compared to HC, SLE aN B cells exhibited low CD21, CD24, and CD83 levels, while CD86 levels were increased. Following 16h of stimulation, SLE aN B cells exhibited increased CD21, CD83, and CD86 expression and decreased CD24 levels. Expression of CD21 decreased at 48h, followed by CD83 at 4d post-stimulation. Global DNA methylation analysis of SLE aN B cells revealed several genes, including interferon-regulated genes, were hypomethylated when compared to HC. Interestingly, the CD83 gene was hypermethylated in SLE aN B cells; a result consistent with low CD83 levels observed via flow and RNASeq analysis of several SLE patients. The stimulation of PBMCs through distinct pathways reveals that activation markers are temporarily expressed on SLE aN B cells, only to decrease with prolonged stimulation. Hypermethylation of CD83 may explain several lupus B cell characteristics including: decreased marginal zone...
maturation, decreased IL-10 production, and increased Ig secretion.

Score-based versus Clinical Evaluation of Heart Failure Severity Among Patients Listed for Heart Transplantation

Introduction: Identifying patients with heart failure (HF) who will need advanced therapies, including heart transplant (HTx), is challenging. Although prediction schemes are available, clinical assessment is most often used for this purpose. The association between clinically assigned HF severity compared to formal risk prediction schemes has not been described.

Methods: We retrospectively evaluated 320 adults (≥18 years old) listed for HTx from 1997 to 2011. Listing decision was based on cumulative patient data discussion among HTx team members without use of risk prediction scores. HF severity was assigned to patients based on United Network for Organ Sharing listing status 1A, 1B, or 2 and was compared to average Seattle Heart Failure Score (SHFS).

Results: Overall 33 patients were listed as status 1A (10.3%), 136 (42.5%) as 1B, and 151 (47.2%) as 2. Age was 51.5±11.3 years; 62 (19.4%) were women; 102 (31.9%) black; 209 (65.3%) white; ejection fraction was 15.1±7.8%; and 137 (42.8%) patients had ischemic HF. Treatment included angiotensin system inhibitors in 248 patients (77.5%), beta-blockers in 252 (78.8%), and aldosterone antagonists in 163 (50.9%), whereas 261 (81.6%) had a biventricular pacemaker and/or defibrillator. The median (interquartile range) SHFS was 1.85 (1.32, 2.04), 1.36 (0.73, 2.04), and 0.64 (0.07, 1.18) in patients listed as status 1A, 1B, and 2, respectively, P<0.001 (Figure 1), corresponding to projected 1-year mortality of 25%, 19%, and 10% (Figure 2).

Conclusion: Clinically evaluated listing for HTx correlates well with formal risk scores, as well as with clinically meaningful differences in projected prognosis.

Cholangiocarcinoma is notoriously difficult to diagnose, and is surgically resectable in approximately 30% of cases. Orthotopic Liver Transplantation (OLT) is potentially curative for an otherwise fatal diagnosis; however, the topic remains controversial and is suitable only for patients with unresectable peri-hilar cholangiocarcinoma without lymph node metastasis (LNM). The detection of LNM is critical for consideration for transplant listing.

Data regarding patients with confirmed cholangiocarcinoma was abstracted from the Surveillance, Epidemiology and End Results (SEER) national population database. Data through 2010 was analyzed to calculate the incidence and location of cholangiocarcinoma LNM, and data was stratified using multiple tumor and patient factors.

The incidence of LNM increases with increasing tumor grade (Table 1). Tumor size greater than 2cm and age less than 60 years are correlated with greater incidence of LNM (Tables 1 and 2). Prior neoadjuvant chemotherapy was associated with decreased incidence of LNM (36.1% vs 44.0% without neoadjuvant therapy).

High-grade tumors and tumor size greater than 2cm were associated with increased incidence of nodal metastasis in patients with cholangiocarcinoma. Neoadjuvant chemotherapy was associated with decreased incidence of LNM. Patient race, sex and tumor location were less predictive.

Further analysis from a national population-based sample may be able to aid in predicting liver transplant candidacy.

Comparison of Circuit Survival Between Two Different Continuous Renal Replacement Therapy Machines

Background: Continuous renal replacement therapy (CRRT) is an important tool in the care of critically ill patients. The purpose of this study was to transition to a model of ICU nurse based CRRT delivery and, subsequently, evaluate the effectiveness of CRRT delivery with ICU nurse delivery of CRRT while comparing two FDA-approved CRRT devices with particular attention to continuity of therapy and circuit patency. This article presents the data comparing these two different CRRT machines.

Materials and Methods: A group of ICU nurses were selected to undergo expanded training in CRRT operation and empowered to deliver all aspects of CRRT. The ICU nurses then provided all aspects of CRRT on two FDA-approved CRRT devices over six months. Each device was used exclusively in the
designated ICU for a two-week run-in period followed by three months data collection period. The primary end point for the study was the differences in average number of filter exchanges per day during the CRRT event.

Results: A total of 45 unique patients were included who underwent 64 separate CRRT treatment periods. Four CRRT events were excluded (see text for details). Twenty-eight CRRT events occurred in the NxStage System One arm and 32 events in the Gambro Prismaflex arm. Average filter exchanges per day for the NxStage System One machine was 0.443 (±0.60) and for Gambro Prismaflex machine was 0.553 (±0.65) (P = 0.09).

Conclusions: There was no demonstrable difference in circuit patency as defined by the rate of filter exchanges per day of CRRT therapy.
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Oral presentations are located in the front of the abstract book in the order in which they will occur at the event.