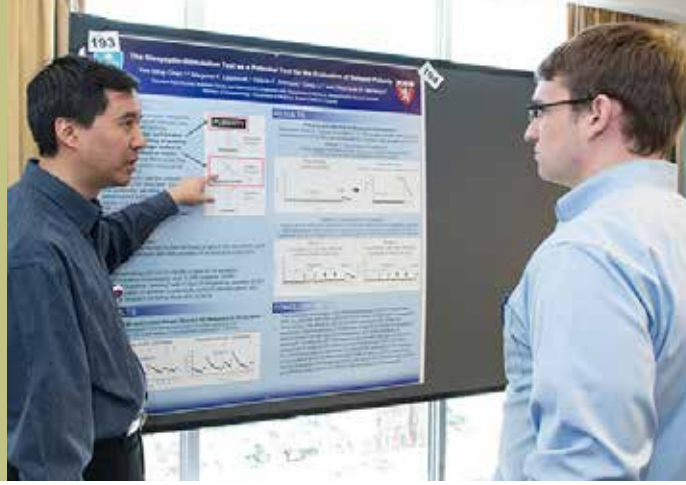
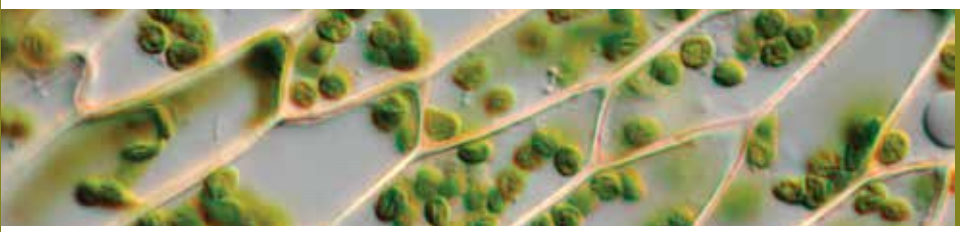
Executive Committee on RESEARCH | *Fostering Innovation at MGH*



67th Annual Meeting of the
MGH Scientific Advisory Committee

SAC 2014

Poster Session Abstracts



April 2, 2014
Wyndham Boston Beacon Hill
5 Blossom Street, 15th Floor

MGH 1811 RESEARCH Management | *Mainstay of MGH Innovation*

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SAC 2014

Agenda: Day One, Wednesday, April 2, 2014
Annual Celebration of Science at MGH

11:00 am–1:45 pm
Wyndham, 15th Floor

SAC 2014 Poster Session (light lunch available)

2:00–5:00 pm
Simches 3.110

Scientific Presentations

WELCOME
Peter L. Slavin, MD, President, Massachusetts General Hospital

OPENING COMMENTS AND INTRODUCTIONS
Robert E. Kingston, PhD, Chair, Executive Committee On Research (ECOR)

2014 MGH RESEARCH SCHOLARS
Robert E. Kingston, PhD

2:15–2:45 pm

2014 Martin Prize for Basic Research
Dedifferentiation of Committed Epithelial Cells into Stem Cells in Vivo
Jayaraj Rajagopal, MD

2:45–3:15 pm

2014 Martin Prize for Clinical Research
Ataxia, Dementia, and Hypogonadotropism Caused by Disordered Ubiquitination
Stephanie B. Seminara, MD

3:15–3:45 pm

2014 Goodman Award
The Role of IRA B Cells in Sepsis
Filip K. Swirski, PhD

3:45–4:00 pm

Break

4:00–5:00 pm

Keynote
INTRODUCTION
Peter L. Slavin, MD

Platelets and Extracellular Matrix—Under-appreciated Key Players in Metastasis
Richard O. Hynes, PhD, Daniel K. Ludwig Professor for Cancer Research
Investigator, Howard Hughes Medical Institute
Massachusetts Institute of Technology

5:00–7:00 pm
Russell Museum

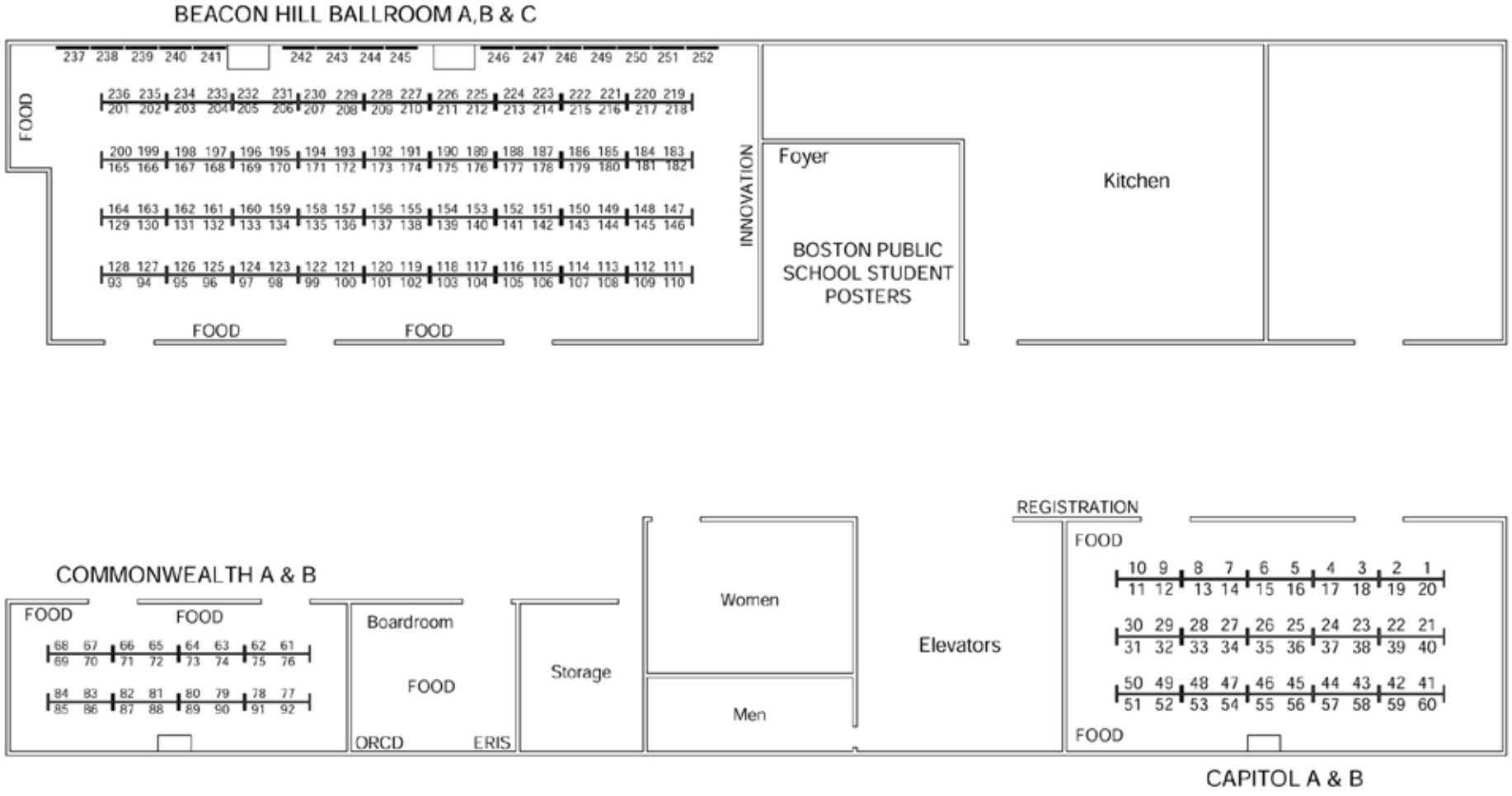
Reception (for invited guests)

SAC 2014

Agenda: Day Two, Thursday, April 3, 2014
Annual Celebration of Science at MGH

8:00–9:00 am *SERI 2nd Floor*	**BREAKFAST** SAC Members with small groups of MGH Faculty
9:00–9:15 am *Simches 3.110*	**WELCOME AND OPENING COMMENTS** **Peter L. Slavin, MD, President, Massachusetts General Hospital**
9:15–9:40 am	**ECOR REPORT 2013** **Robert E. Kingston, PhD, Chair, Executive Committee On Research (ECOR)**
9:40–10:00 am	**INTEGRATION OF MGH RESEARCH TO THE PARTNERS ENTERPRISE** **Anne Klibanski, MD, Partners Chief Academic Officer**
10:00–11:30 am	**DEPARTMENT REPORTS**
10:00–10:40 am	Neurology, **Merit E. Cudkowicz, MD, MSc**
10:40–10:50 am	Break
10:50–11:30 am	Surgery, **Keith D. Lillemoe, MD**
	The MGH Research Institute: Meeting the Challenges that Lie Ahead
11:30 am–12:00 pm	**OVERALL ORGANIZATION: THE RESEARCH INSTITUTE** **Harry W. Orf, PhD, Sr. Vice President for Research**
12:00–1:30 pm *SERI 2nd Floor* *Simches 3.120*	Lunch SAC Members with ECOR and Hospital Leadership ECOR Members & Speakers
1:30–1:40pm *Simches 3.110*	**CLINICAL RESEARCH REORGANIZATION**, **Jerrold F. Rosenbaum, MD**
1:40–2:00 pm	Discussion
2:00–2:10 pm	**TRANSLATIONAL RESEARCH CENTER**, **Mason W. Freeman, MD**
2:10–2:30 pm	Discussion
2:30–2:40 pm	**LIFE REGISTRY**, **Susan A. Slaugenhaupt, PhD**
2:40–3:00 pm	Discussion
3:00–3:15 pm	Break
3:15–3:45 pm *Simches 3.120*	**EXECUTIVE SESSION** (SAC members only)
3:45–4:15 pm *Simches 3.120*	**DEBRIEFING** (SAC members and MGH Leadership)

Session Floor Plan



Boston Public School Students

We would like to acknowledge the hard work of the students whose work is on display today. The school's participation is coordinated by the MGH Youth Programs Team in the MGH Center for Community Health Improvement (CCHI). Below is a list of the participating students; their MGH mentors are shown in parenthesis.

The James P. Timilty Middle School

- **Mari Cabreja, Grade 8**—Correlation of personality traits, age and gender with password security

  (Lori Rizzo, RN BSN MBA, Staff Specialist, Perioperative Services)

- **Naby Diallo, Grade 8**—Board & the brain

  (Bill Banchiere, Director, Environmental Service; Dee Dee Chen, Manager, Human Resources)

- **Loren Ferguson, Grade 8**—How does music effect concentration & memory?

  (Gabby Abrishamian-Garcia, Clinical Research Coordinator, Endocrine Unit)

- **Kaylin Florentino, Grade 8**—Do digital screens affect memory?

  (Pinar Avci, Research Fellow, Dermatology)

- **Lismeidy Francisco, Grade 7**—What is the effect of age on inkblots?

  (Cathy Zhang, Clinical Research Coordinator, Neurology)

- **Elijah Guillomaitre, Grade 7**—Robocars: Does the size of cars' wheels affect the reaction time when stopping on cement?

  (Boubacar Barry, Buyer, Materials Management)

- **Ana Ouais, Grade 7**—How much weight will be lost when certain types of food break down in gastric juice?

  (Amanda Meppelink, Research Technician, Plastic Surgery)

- **Mark Anthony Williams, Grade 8**—Can speed affect long jump?

  (Imran Ghare, Research Assistant, Molecular Biology)

Posters of Distinction

BASIC

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Generation of airway stem cell and functional epithelium with high efficient from human iPSC
Hongmei Mou, PhD
Instructor, Center for Regenerative Medicine

POSTER 70
Gliadin is a chemoattractant factor for neutrophils and induces migration via engagement of the formyl peptide receptor, FPR1
Karen Lammers, PhD
Assistant Immunologist, Mass General Hospital *for* Children

POSTER 123
Blockade of PIGF/NRP1 Signaling Inhibits the Growth and Spread of Pediatric Medulloblastoma
Ana Batista, PhD
Research Fellow, Radiation Oncology

POSTER 33
A small molecule ablates primordial germ cells through a novel mechanism of 3'UTR-mediated translational repression
Youngnam Jin, PhD
Research Fellow, Medicine

POSTER 95
Gene transfer of Human APOE isoforms differentially modulates the course of Alzheimer's disease in transgenic mouse models
Eloise Hudry, PhD
Instructor, Neurology

POSTER 140
β-Aminoisobutyric Acid Induces Browning of White Fat and Hepatic β-oxidation and is Inversely Correlated with Cardiometabolic Risk Factors
John O'Sullivan, MBChB, MS, PhD
Research Fellow, Heart Center

POSTER 98
A novel ontogenetic phospho-switch of GABA activity during brain development
Kristopher Kahle, MD, PhD
Resident, Neurosurgery

CLINICAL

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The role of predictive algorithm selection on the accuracy of MRI-based prediction of tissue outcome after acute ischemic stroke
Mark Bouts, PhD
Research Fellow, Radiology

POSTER 241
PET/MRI evidence of neuroinflammation in migraine
Nouchine Hadjikhani, MD, PhD
Associate Professor, Radiology

POSTER 174
Prophylactic Mastectomies: Implications of Occult Histology and Lifetime Cost of Surveillance vs. Surgery
David Mattos, BA
Graduate Student, Surgery

POSTER 245
Weight Loss Surgery Regulates Glucose Metabolism via Intestinal Metabolic Reprograming
Nima Saeidi, PhD
Research Fellow, Surgery

POSTER 223
A magneto-DNA nanoparticle system for target specific bacterial identification
Hyun Jung Chung, PhD
Research Fellow, Center for Systems Biology

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BASIC–OTHER

Poster Number 1

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Platelet TSC1 is an Important Mediator of Arterial Thrombosis

Investigators: Yong-Joo Ahn, MD; Sang Bok Kim, PhD; Eo Jin Kim, MD, PhD; Ji Hyeon Rho, MD; Min Ju Kim, MD; Maya H. Kim, BS; Hyung-Hwan Kim, PhD

Background: Arterial thrombosis is responsible for peripheral vascular disease, myocardial infarction, and stroke. Mammalian target of rapamycin (mTOR) inhibition promotes arterial thrombosis. To determine the contributions of platelet specific Tuberous sclerosis 1 (TSC1) deficiency to arterial thrombosis, we generated PF4Cre-TSC1flox/+ mice. Because the effect of mTOR activation on thrombus formation in vivo has not yet been studied, we hypothesize that mTORC1 signaling pathway in platelets may play an important role in arterial thrombosis.

Methods and Results: To determine the role of platelet TSC1 in arterial thrombosis, we generated a mouse strain with platelet-specific TSC1 deficiency (PF4Cre-TSC1flox/+) using conditional TSC1flox/flox mice and platelet-specific PF4-Cre mice. PF4Cre-TSC1flox/+ mice did not show any obvious phenotypic abnormalities. Arterial thrombus formation was investigated using in vivo ferric chloride induced thrombosis model. Ferric Chloride (5% FeCl3) induced thrombotic occlusion. Compared to PF4-Cre mice, PF4Cre-TSC1flox/+ mice exhibited elongated thrombotic occlusion time by 21.7%. Platelet specific TSC1 deficiency promotes arterial thrombosis via activation of mTOR and S6, a downstream target of mTORC1.

Conclusion: These finding indicate that platelet specific TSC1 deficiency promotes arterial thrombosis in vivo. These results suggest that activation of platelet mTORC1 may have therapeutic benefits in arterial thrombosis.

Poster Number 2

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In vivo molecular imaging of thrombosis and thrombolysis using a fibrin-binding PET probe

Investigators: Francesco Blasi, PhD, PharmD; Ilknur Ay, MD, PhD; Tyson A. Rietz, BS; Nicholas J. Rotile, BA; Helen Day, MS; Bruno L. Oliveira, PhD; Richard J. Looby, BS; Peter Caravan, PhD

Fibrin is a major component of both arterial and venous thrombi and represents an ideal candidate for molecular imaging of thrombosis. Here, we describe imaging properties and target uptake of a new fibrin-specific PET probe for thrombus detection and therapy monitoring in two rat thrombosis models.

The fibrin-binding probe FBP7 was synthesized by conjugation of a known short cyclic peptide to a cross-bridged chelator, followed by labeling with copper-64. Adult male Wistar rats (n=22) underwent either carotid crush injury (mural thrombosis model) or embolic stroke (occlusive thrombosis model) followed by rtPA treatment (10 mg/kg, i.v.). FBP7 detected thrombus location in both animal models with a high PET target-to- background ratio that increased over time (>5-fold at 30-90 min, >15-fold at 240-285 min). In the carotid crush injury animals, biodistribution analysis confirmed high probe uptake in the thrombotic artery (~0.5 %ID/g; >5-fold greater than blood and other tissues of the head and thorax). Similar results were obtained from ex vivo autoradiography of the ipsilateral vs. contralateral carotid arteries. In embolic stroke animals, PET-CT imaging localized the clot in the internal carotid/middle cerebral artery segment of all rats. Time-dependent reduction of activity at the level of the thrombus was clearly detected in rtPA-treated rats but not in vehicle-injected animals. Brain autoradiography confirmed clot dissolution in rtPA-treated animals, but enduring high thrombus activity in control rats.

Here we show that FBP7 is suitable for molecular imaging of thrombosis and thrombolysis in vivo, and represents a very promising candidate for bench-to-bedside translation.

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Poster Number 3

A Poly(A)-Independent Mechanism of MicroRNP-Mediated Translation Activation

Investigators: Syed Irfan A. Bukhari, PhD; S. Spencer Truesdell, MEng; Shobha Vasudevan, PhD

MicroRNAs are well documented as translational repressors. However, under certain cellular condition such as G0 (quiescent state), microRNAs can mediate translation activation of specific mRNAs. These mRNAs are translationally up-regulated by an FXR1a-associated microRNP complex (microRNA-protein complex), in quiescent (G0) mammalian cells and immature Xenopus laevis oocytes. The mechanism of this translation activation by microRNAs during the G0 state remains largely unknown. Here we show that microRNA-mediated activation requires short or no poly(A) tails on target mRNAs in oocytes and mammalian THP1 G0 cells, which holds true for endogenous targets of microRNA-mediated activation. Polyadenylated mRNAs are repressed, possibly due to poly(A) binding protein (PABP)-mediated enhancement of microRNA-mediated downregulation. Overexpression of PAIP2, which removes PABP from poly(A) tails, rescues microRNA-mediated upregulation of polyadenylated mRNAs in oocytes. Similarly inhibition of the deadenylase, poly(A) ribonuclease, PARN, prevents upregulation of translation activation in oocytes. Importantly, we also observed that the interaction of FXR1-associated microRNP with p97/eIF4G2, a paralog of the translation factor eIF4G without PABP-interacting domains, is required for translation activation. This mechanism is required for maintenance of the immature state in oocytes, with implications for related physiological function in G0 mammalian cells. Taken together, these data reveal a specialized mechanism of microRNA-mediated activation where the FXR1a-associated microRNP targets specific shortened poly(A) mRNAs for p97/eIF4G2 -mediated translation

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Poster Number 4

Advances in Developing a Clinically Feasible Cell Source for Auricular Cartilage Engineering

Investigators: Michael J. Cronce, BS; Irina Pomerantseva, MD, PhD; Alan Tseng, BS; Anya Kimura, BS; Joseph P. Vacanti, MD; Mark A. Randolph, MS; Cathryn A. Sundback, ScD

De novo engineering of the human auricle holds promise for treating those suffering from traumatic ear injuries or congenital defects. Over the past two decades, engineering auricular cartilage has become an area of focus for the field of tissue engineering. While great strids have been made, a number of issues continue to delay clinical application of engineered auricular cartilage.

Of those issues, perhaps the most important is that of cell sourcing. Primary chondrocytes must be greatly expanded to obtain sufficient cell numbers to engineer the cartilage of an adult-sized ear. However, standard culture conditions used for cell expansion lead to chondrocyte de-differentiation, senescence, and a decrease in chondrogenic potential. Also of significant concern is long-term stability of engineered cartilage in an immunocompetent recipient.

Here, we demonstrate an effective, yet reductionist, approach of utilizing basic fibroblast growth factor (bFGF) supplementation for expanding primary chondrocytes. At the cellular level, this approach inhibits chondrocyte de-differentiation, while increasing proliferative capacity, proliferation index, and chondrogenic potential to generate neocartilage. Moreover, we demonstrate that utilizing bFGF in an immunocompetent animal generates high quality cartilage with enhanced long-term stability. Our simple, cost-effective approach enhances engineered cartilage quality and neocartilage stability, while decreasing the length of time and number of cells required to generate a sufficient chondrocyte population. Our findings represent significant advances to the field of ear engineering and provide a foundation for clinical trial.

BASIC–OTHER

Poster Number 5

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Event Driven Surgical Gesture Recognition, from Surgery Simulation to Quality Control

Investigators: Gianluca De Novi, PhD; Gregory Loan; Mark P. Ottensmeyer, PhD

While simulation-based training for surgery has lagged behind fields such as anesthesiology and obstetrics, it is growing in importance and is becoming part of the surgical credentialing process. The lag has been in part due to the difficulty of measuring the complex manual and cognitive skills involved in a given procedure. Naïve approaches that measure global task duration, and instrument motion compactness and smoothness are insufficient to capture elements of technique and whether standard sequences through a given procedure are followed. Measuring outcomes after performing standard tasks does not provide immediate, contextual guidance for improvement. Current automated measurement techniques are also incapable of providing intra-operative feedback to flag errors and provide expert-derived advice.

Our ongoing work in developing an ocular trauma simulator includes development of surgical gesture recognition methods, which use recursive pattern recognition algorithms for online analysis of sequences of events generated by measuring the motions and actions of tracked surgical instruments. By identifying progress through phases of a procedure, through the steps of each phase, and the actions within each step, we can apply and weight appropriate performance scoring metrics. Recognition of progress through a procedure also enables presentation of useful tips and corrective advice when deviations from the text-book technique are made. The technique could be broadly applied to non-ocular surgical simulators, and with the addition of tracking sensors for intra-operative measurement during real procedures, it would enable measurements useful for continuous learning and monitoring for safety and quality improvement.

Poster Number 6

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Extracellular RNA Contributes to Myocardial Ischemia-Reperfusion Injury

Investigators: Yan Feng, MD, PhD; Chan Chen, MD, PhD; Lin Zou, MD, PhD; Jiayan Cai, BS; Wei Chao, MD, PhD

Introduction: Recent studies suggest that extracellular RNA (exRNA) released from injured cells may induce inflammation and participate in tissue injury. However, the role of exRNA in myocardial ischemia-reperfusion (I/R) injury is unknown.

Methods: RNA in media or sera was extracted and stained with a RNA-selective fluorescent dye followed by gel electrophoresis. microRNA (miRNA) and cytokine mRNA were quantified by qRT-PCR and MIP-2 measured by ELISA. Myocardial I/R was created by coronary artery ligation for 45 min. Myocardial leukocyte recruitment was detected by FACS, myocardial apoptosis examined by caspase 3 activity, and myocardial infarct measured by TTC staining.

Results: Gel electrophoresis demonstrated that necrotic macrophages or hypoxic cardiomyocytes released RNA into the culture media. qRT-PCR studies indicated that hypoxic cardiomyocytes in vitro or ischemic heart in vivo released miR-208a, miR499, miR-1, and miR-133 into the culture media or to the blood, respectively. Moreover, necrotic macrophages induced multiple cytokine (e.g. IL-1β, MIP-2, IL-6 and KC) mRNA production in cardiomyocytes. RNase, but not DNase, pretreatment of the necrotic cells markedly attenuated the cytokines transcript production. Meanwhile, both necrotic macrophages and cardiomyocytes induced a robust and dose-dependent MIP-2 production in cardiomyocytes, which was significantly inhibited by RNase. Finally, compared to saline-injected mice, mice treated with RNase had significantly reduced myocardial cytokine responses, leukocytes infiltration, caspase 3 activity, and, most importantly, reduced infarct size following I/R.

Conclusions: These studies demonstrate that exRNA mediates necrotic cell-induced inflammation in cardiomyocytes in vitro and may contribute to myocardial I/R injury in vivo.

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Poster Number 7

Improving Quality and Safety for Diverse Populations: An Innovative Interprofessional Curriculum

Investigators: Karey S. Kenst, MPH; Gail B. Gall, PhD, APRN, BC; Joseph R. Betancourt, MD, MPH; Alexander R. Green, MD, MPH

Patients with limited English proficiency (LEP) are more likely to suffer adverse events than English-speaking patients, and these events tend to have more serious consequences. Health professions students do not typically receive training on the principles of patient safety and caring for patients with LEP. The Disparities Solutions Center at MGH and the MGH Institute of Health Professions designed an interprofessional curriculum for medical and nursing students, Providing Safe and Effective Care for Patients with Limited English Proficiency. The curriculum is built on an e-learning platform with associated classroom sessions and online assignments. Objectives include educating students on disparities among LEP patients, developing skills to work effectively with interpreters, and exploring how systems can be improved to ensure quality and safety.

The curriculum was pilot tested with 16 HMS and MGH IHP nursing students and an advisory board of HMS and MGH IHP faculty. Survey results show average faculty and student ratings of 4.5 and 4.3 out of 5.0, respectively, for the overall learning experience. Additionally:

- 100% of students and faculty agreed/strongly agreed that the course will help students provide safe care for patients with LEP;
- 93% of students and 100% of faculty agreed/strongly agreed that it was helpful to learn in an interprofessional environment; and
- 87% of students and 100% of faculty agreed/strongly agreed that the course should be a required component of medical and nursing school curricula.

This curriculum provides both the structure and flexibility to be adopted by academic institutions.

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Poster Number 8

Meniscus Injuries After the Kinematics of the Knees with ACL Deficiency

Investigators: Ali Hosseini, PhD; Jing-Sheng Li, MS; Thomas J. Gill IV, MD; Guoan Li, PhD

Objective: Most research has investigated the knee biomechanics with an isolated anterior cruciate ligament (ACL) injury; however ACL injuries combined with meniscus tear is very common. We investigated the effect of meniscus tear on the kinematics ACL injured knee.
Methods: 5 isolated ACL injuries (Group I), 8 combined ACL and medial meniscus injuries (Group II), 8 combined ACL and lateral meniscus injuries (Group III) were recruited. Both knees were scanned during stair climbing using a dual fluoroscopic imaging to measure the knee kinematics during stair climbing. Anteroposterior and mediolateral translations and axial tibial rotation were compared between the injured and intact contralateral knees.

Results: injured knees in groups I and III showed more than 2 mm increased anterior tibial translation close to full knee extension. In group II, no difference was observed. Near full extension, in groups I and III, injured knees had less that 1 mm increased medial tibial translation, and 1.8 mm increase in lateral tibial in group II. In regard to axial tibial rotation, group I showed an increased external tibial rotation (~ 5°), group II had little variation, and a group III had increased internal tibial rotation (~ 2°).

Conclusions: The results demonstrate that a combined ACL/meniscus injury could alter the kinematics of the ACL injured knees in a different way than knees with isolated ACL tears. Future studies should focus on specific treatment of patients with combined ACL and meniscus injuries in order to protect the joint from abnormal kinematics and subsequent post-operative cartilage degeneration.

BASIC–OTHER

Poster Number 9

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Using Dictyostelium as a model system for studying the function of Batten disease proteins

Investigators: Robert J. Huber, PhD; Susan L. Cotman, PhD; Michael A. Myre, PhD

Batten disease refers to a group of mostly childhood neurodegenerative disorders also known as the neuronal ceroid lipofuscinoses (NCL). Juvenile NCL is the most common subtype and results from recessive, loss-of-function mutations in the CLN3 gene. The social amoeba Dictyostelium discoideum is a genetically tractable model system that has been used successfully as a biomedical model to study a variety of human diseases, including neurodegenerative disorders. In order to elucidate the earliest pathogenic events caused by mutant CLN3, we created a loss-of-function model by deleting the cln3 gene in Dictyostelium (Cln3, protein) and assessing phenotypes during both growth and development. cln3- cells proliferated ~20% faster than parental AX3 cells and this phenotype could be rescued by overexpressing GFP-Cln3 in mutant cells. During growth, GFP-Cln3 predominantly localized to the contractile vacuole system and to a lesser extent to compartments of the endocytic pathway. Although cln3- cells were able to complete development, they formed ~30% more aggregation territories compared to parental cells. Development of cln3- cells was precocious, resulting in the accelerated formation of mid- and late developmental structures, specifically tipped mounds, slugs, and fruiting bodies. Cln3 deficiency also increased slug migration. Expression of GFP-Cln3 or GFP-CLN3 in cln3- cells restored the timing of slug and fruiting body formation and suppressed the enhanced slug migration. The accelerated formation and enhanced migration of slugs was also rescued through calcium chelation. Taken together, our results indicate that Cln3 functions as a negative regulator of growth and development in Dictyostelium.

Poster Number 10

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Assessing Invariance of the Revised Professional Practice Environment Scale: A Multi-International Site Study

Investigators: Ives Erickson, Jeanette, DNP, RN; Ditomassi, M., DNP, RN; Jones, D., PhD; Guarino, A.J., PhD

This study evaluated the measurement equivalence/invariance (ME/I) of the The Revised Professional Practice Environment (RPPE) Scale across five international sites. The RPPE is a 39 item instrument that assesses staff perception on eight components of the professional clinical practice environment in the acute care setting. Items are scored on a four point Likert-type scale with anchor from strongly disagree (1) to strongly agree (4). The eight components of the RPPE are (a) leadership and autonomy over practice, (b) staff relationships with physicians, (c) control over practice, (d) communication about patients, (e) teamwork, (f) handling disagreement and conflict, (g) internal work motivation, and (h) cultural sensitivity. The RPPE produces scores for each of the eight components that weave a tapestry of staff perceptions of the environment in which they practice. It was hypothesized that invariance would occur for all eight scales across the five international sites.

To assess ME/I, a multi-model confirmatory factor analysis (CFA) was conducted using Amos version 20. To determine any significant measurement differences, two models were proposed, (1) an unconstrained model (which assumes that the groups are yielding different values of the parameters) and (2) a constrained model (which assumes that the groups are yielding equivalent values of the parameters) when the model is applied to the data. Results confirmed that the eight scales of the RPPE were in fact invariant as hypothesized. These findings offer chief nurses across international sites a comprehensive mechanism to assess the professional practice environment in the aggregate.

Poster Number 11

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Non-Thermal, Irreversible Electroporation: A Novel Tool for Scarless Skin Regeneration

Investigators: Saiqa Khan, MD; Alexander Golberg, PhD; G. Felix Broelsch, MD; Stefan Bohr, MD; Martin C. Mihm, MD; Hassan Albadawi, MD; Michael T. Watkins, MD; Martin L. Yarmush, MD, PhD; William G. Austen, Jr., MD

Background: Hypertrophic scarring dramatically affects quality of life. Methodology to induce scarless adult wound healing is required to comprehend tissue regeneration. IRE is a non-thermal method of ablation, which destabilizes cell membranes while preserving the extracellular matrix (ECM) and microvasculature. IRE induces temporary vasoconstriction, which reduces the immune response allowing for rapid tissue regeneration with decreased scarring.

Methods: We investigated rat skin regeneration following IRE. Twenty-three rats were treated. Third-degree burns served as positive controls, and untreated skin served as negative controls. To minimize thermal effects, specific IRE parameters were used. An expert dermatopathologist analyzed the histopathology.

Results: Local blood flow was evaluated with Laser Doppler Imaging, which revealed preservation of blood flow in the IRE-ablated tissue, in contrast to the burned area, which suffered decreased blood flow. Specimens were harvested after 24 hours, 1 week, 3 weeks, and 2 months. 24 hours after IRE, massive inflammation was observed along with mast cell degranulation, and damaged muscle fibers and hair follicles. However, complete regeneration of epidermis, sebaceous glands, hair follicles, and panniculus carnosus is demonstrated two months after ablation. There was no evidence of scar tissue formation as collagen maintained its normal architecture. Collagen architecture was completely lost in the burned skin along with lack of regeneration of sebaceous glands and hair follicles. We hypothesize that the regeneration observed after IRE is secondary to preservation of the vasculature and ECM.

Conclusion: IRE may become a key tool in regenerative medicine, as it allows for rapid scarless tissue regeneration following ablation.

Poster Number 12

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Smooth Muscle Cell Specific TSC1 Mediate Arterial Thrombosis and Arterial Injury

Investigators: Eo Jin Kim, MD, PhD; Yong-Joo Ahn, MD; Sang Bok Kim, PhD; Ji Hyeon Rho, MD; Min Ju Kim, MD; Maya H. Kim, BS; Hyung-Hwan Kim, PhD

Background: Arterial thrombosis and arterial injury are main leading causes of cardiovascular associated death after myocardial infarction and ischemic stroke.

Methods and Results: To understand the role and mechanism of smooth muscle cell specific TSC1 in arterial thrombosis and arterial injury, we generated smooth muscle cell specific TSC1 conditional knockout mice (TSC1SM22-/-) using a conditional allele of TSC1 and a cre recombinase allele regulated by the smooth muscle protein 22 promoter. Arterial injury was induced using a left common carotid artery ligation model in heterozygous mice (TSC1SM22+/-). Arterial thrombosis and the intima and media ratios were measured at 14 days after arterial ligation. The neointimal formation was significantly increased in TSC1SM22+/- mice (intimal thickness/medial thickness ratio; 1.14 ± 0.14, $p<0.001$) compared with the control mice (0.13 ± 0.03). Two weeks after arterial injury, arterial thrombus area was increased in TSC1SM22+/- mice (thrombus area/luminal area ratio; 72.1 ± 4.4, $p<0.001$) compared with control mice (0.1 ± 0.0). Reduction of TSC1 and mammalian target of rapamycin complex 1 (mTORC1) activation including mTOR and S6 signaling pathway were observed in artery lysates of TSC1SM22+/- mice.

Conclusion: These findings suggest that arterial thrombus formation and neointimal formation using TSC1-mTORC1 mechanism might be a useful target for therapeutic intervention in arterial thrombosis and arterial injury.

BASIC–OTHER

Poster Number 13

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Platelet Rich Plasma: An alternative to Fetal Bovine Serum in cell culture

Investigators: Katherine M. Kulig, BA; Xiang-Hong Liu, PhD; Joseph P. Vacanti, MD; Irina Pomerantseva, MD, PhD; Scott Goldman, MS; Cathryn A. Sundback, ScD; Gino Bradica, PhD; Craig M. Neville, PhD

Fetal Bovine Serum (FBS) in the culture of cells destined for regenerative medicine and cell therapies can potentially limit clinical use. FBS has been used for years to supplement medium with essential components such as growth factors and cytokines to maintain expansion of cultured cells. However, bovine derived-clinical products are of a general concern to the Food and Drug Administration (FDA) because of the possibility of transmissible pathogens.

We are investigating the use of Platelet Rich Plasma (PRP) as a substitute for FBS in cell culture medium. Autologous PRP is FDA approved for clinical use, targeting orthopedic injuries. Platelets are circulating cell fragments that are a natural source of growth factors. We have developed a robust and reproducible protocol for concentrating platelets (PRP). We compared the sustainability of several cell types (adipose derived-mesenchymal stem cells, human umbilical vein endothelial cells, human foreskin fibroblasts, and NIH 3T3 fibroblasts) through several passages in medium supplemented with FBS, PRP, or Platelet Poor Plasma (PPP). PRP was able to support cell survival and expansion as well as FBS. Additional studies are underway to evaluate functionality of the cultured cells in a variety of tissue engineering models.

Poster Number 14

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Dynamic Biodistribution of Extracellular Vesicles in Vivo Using a Multimodal Imaging Reporter

Investigators: Charles P. Lai, PhD; Osama Mardini, BPharm; Maria Ericsson, BSc; Shilpa Prabhakar, MSc; Casey Maguire, PhD; John W. Chen, MD, PhD; Bakhos A. Tannous, PhD; Xandra O. Breakefield, PhD

Extracellular vesicles (EVs) are nano-sized vesicles released by normal and diseased cells as a novel form of intercellular communication, and can serve as an effective therapeutic vehicle for genes and drugs. Yet, much remains unknown about the in vivo properties of EVs such as tissue distribution, and blood levels and urine clearance - important parameters that will define their therapeutic effectiveness and potential toxicity. Here we combined Gaussia luciferase and metabolic biotinylation to create a sensitive EV reporter (EV-GlucB) for multimodal imaging in vivo, as well as monitoring of EV levels in the organs and biofluids ex vivo after administeration of EVs. Bioluminescence and fluorescence-mediated tomography imaging on mice displayed a predominant localization of intravenously administered EVs in the spleen followed by the liver. Monitoring EV signal in the organs, blood and urine further revealed that the EVs first undergo a rapid distribution phase followed by a longer elimination phase via hepatic and renal routes within six hours, which are both faster than previously reported using dye-labeled EVs. Moreover, we demonstrate systemically injected EVs can be delivered to tumor sites within an hour following injection. Altogether, we show the EVs are dynamically processed in vivo with accurate spatiotemporal resolution, and target a number of normal organs as well as tumors with implications for disease pathology and therapeutic design.

Poster Number 15

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In vivo kinematic features of the knee after cruciate-retaining TKA during weight-bearing flexion

Investigators: Chunbao Li, MS; Ali Hosseini, PhD; Jing-Sheng Li, MS; Tsung-Yuan Tsai, PhD; Yang-Min Kwon, MD; Harry E. Rubash, MD; Li Guoan, PhD

Although total knee arthroplasty (TKA) has been widely used to restore function of end-stage osteoarthritis (OA) knee, the intrinsic kinematics of TKA knee has always been debated. While the surgical transepicondylar axis (TEA) of the femur is widely used to help determine femoral component rotation, the description of knee kinematics using TEA may represent the femoral condylar translations. The objective of this study was to quantitatively investigate the in vivo contact biomechanics and posterior femoral translation of posterior cruciate ligament-retaining (CR) TKA patients when performing a single leg weight bearing flexion.

Eleven TKA patients with advanced osteoarthritis were included. 3D computer model and the femoral TEA axis were created on native knee. Then dual fluoroscopic analyses were performed to invest tibiofemoral articular contact points and femoral component translations during flexion.

Here we show that the tibiofemoral contact locations moved in a small range. However, when measured using the TEA, both the medial and lateral femoral condyles showed anterior translation at early flexion, but consistent posterior translation till the maximal flexion.

These data indicated that the femoral condyle motions could be very different if described using different methods. Although substantial posterior femoral condyle translations were measured in this group of patients, the polyethylene liner articulated with the femoral component almost at the same locations in the medial and lateral compartment. This kinematic feature may be not optimal to the longevity of the polyethylene component. Future development of TKA surgery should improve the articular contact kinematics of the knee.

Poster Number 16

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Bone morphogenetic protein signaling contributes to the vascular calcification but not smooth muscle de-differentiation associated with matrix Gla protein deficiency

Investigators: Rajeev Malhotra, MD, MS; Trejeeve Martyn, MD; Hannah R. Shakartzi, BS; Timothy E. Thayer, BS; Megan F. Burke, BA; Caitlin O'Rourke; Pingcheng Li, BS; Matthias Derwall, MD; Ester Spagnolli, MD; Starsha A. Kolodziej, BS; Claire Mayeur, MD; Emmanuel S. Buys, PhD; Paul B. Yu, MD, PhD; Kenneth D. Bloch, MD

Rationale: Matrix Gla protein (MGP) is a mineral-binding protein that can inhibit bone morphogenetic protein (BMP) signaling in vitro. MGP deficiency is associated with osteogenic transdifferentiation of vascular smooth muscle cells (SMCs) and calcification of the medial arterial layer.

Objective: We tested the hypothesis that MGP prevents vascular calcification by inhibiting BMP signaling.

Methods and Results: MGP-/- mice were treated with vehicle or one of two inhibitors of BMP signaling, LDN-193189 or ALK3-Fc, beginning one day after birth. Aortic calcification was assessed in 28-day-old mice by measuring the uptake of a fluorescent bisphosphonate probe and by staining tissue sections with Alizarin red. Aortic calcification was reduced by 80% in MGP-/- mice treated with either LDN-193189 or Alk3-Fc compared to vehicle-treated mice ($P<0.001$ for both). LDN-193189-treated MGP-/- mice survived longer than did vehicle-treated MGP-/- mice. Levels of phosphorylated Smad1/5 and Id1 mRNA (downstream targets of BMP signaling) did not differ in the aortas from MGP-/- and wild-type mice. LDN-193189 treatment reduced aortic Id1 mRNA levels by 70-80% in MGP-/- mice. Markers of SMC differentiation were reduced in MGP-/- aortas before the development of vascular calcification, and this de-differentiation was not prevented by inhibiting BMP signaling.

Conclusions: Pharmacologic inhibition of BMP signaling reduces vascular calcification and improves survival in MGP-/- mice. MGP deficiency does not appear to increase aortic BMP signaling in mice and induces SMC de-differentiation via a mechanism independent of BMP signaling. These findings suggest that BMP signaling represents an important therapeutic target in the treatment of vascular calcification.

Poster Number 17

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FAI Negates the Acetabular Labral Seal during Pivoting Maneuvers, but not Normal Gait

Investigators: Maureen Dwyer, PhD; Hugh Jones, BS; Richard Field, PhD; Philip Noble, PhD; Joseph McCarthy, MD

The acetabular labrum enhances the stability of the hip joint by forming an articulating seal which regulates the exchange of synovial fluid between the central and peripheral compartments of the hip. Experimental disruption of the acetabular labrum compromises its sealing function and alters cartilage lubrication. However, whether pathological changes to the labrum secondary to femoro-acetabular impingement have a similar impact on labral function is unknown. This study was performed to determine the effect of natural labral damage secondary to abnormal femoral morphology on the labral seal. We showed that the acetabular seal, quantified by intra-articular pressure, was affected by the presence of labral damage secondary to impingement. Visual observation following testing showed that each specimen with cam femoro-acetabular impingement morphology exhibited secondary damage of the labrum and the adjacent chondral surface, while specimens of normal morphology were undamaged. The specimens with labral damage exhibited reduced peak central compartment pressure during simulated pivoting motions when compared to intact specimens. Conversely, no differences in peak pressure were detected between specimens with and without labral damage during simulated gait and stooping. As degeneration is progressive with repetitive impingement, loss of the labral seal starts to be seen during pivoting and may progress from there, but at this time point (50 years), the seal remains intact during gait and stooping.

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Poster Number 18

Fluorogenic turn-on probes for bioorthogonal chemistry

Investigators: Labros G. Meimetis, PhD; Jonathan C. T. Carlson, MD, PhD; Ralph Weissleder, MD, PhD

Many diagnostic and imaging applications are based on fluorescent compounds that change their optical properties upon reaction. A recent development has been the discovery of extraordinary fluorescence turn-on properties (several 1,000 fold) when certain fluorochromes are conjugated to tetrazines and subsequently reacted with trans-Cyclooctene (TCO). While the initial discovery was fortuitous, more recent understanding of the underlying through-bond energy transfer (TBET) mechanism has allowed the development of alternative fluorochrome tetrazines.

We synthesized over 20 fluorochrome-tetrazine conjugates and evaluated their optical properties and reaction kinetics with respect to TCO-conjugates. We identified lead compounds with > 3,000 fold turn-on ratios for BODIPY and coumarin fluorochromes. Importantly, these ratios hold true under physiologic conditions, i.e. in serum and can thus be used for cellular and molecular imaging.

We show that this powerful paradigm for the first time allows imaging of receptors and targets without the need to wash cells (in vitro) or without the need to wait for physiologic clearance (in vivo). The described generic chemistry platform is a powerful one enabling new applications

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Poster Number 19

Generation of airway stem cell and functional epithelium with high efficient from human iPSC

Investigators: Hongmei Mou, PhD; Anthony Arvanites, PhD; Lance Davidow, PhD; Karen Kotkow, PhD; Tata Purushothama Rao, PhD; Vladimir Vinarsky, MD; Kevin Lam, PhD; Lee Rubin, PhD; Jayaraj Rajagopal, MD

We have recently published a step-wise method for the generation of airway progenitors from human iPSC. However, the major obstacle preventing the actual development of human airway disease models using patient-specific iPSC is the inability to produce with near purity airway stem cells and then to subsequently differentiate into functional airway epithelial cells in vitro. In this work, we have optimized our differentiation protocol to produce human airway progenitors from human iPSCs by using an unbiased chemical screen. By performing additive/synergistic experiments using our lead hits, we have generated a robust, economically efficient and generally applicable protocol for a highly enriched population of NKX2.1+ lung progenitors. The derived lung progenitors can be sorted to purity and matured into airway basal stem cells and further differentiated into physiologically active ciliated cells in vitro. In addition, we successfully generated the first “humanized” mouse model by replacing the native murine airway epithelium with human airway epithelium by engrafting human airway stem cell populations. Thus, we have developed a new technology that finally enables us to study the airway stem cell biology in vitro and in vivo and to realize the personalized medicine in the future.

Poster Number 20

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Renal epithelial cell processing of nanoparticles in vivo

Investigators: Anilkumar V. Nair, PhD; Edmund J. Keliher, PhD; Dennis Brown, PhD; Ralph Weissleder, MD, PhD

Nanotechnology approaches are actively being pursued in drug delivery, novel diagnostics, implantable devices, and consumer products. Yet there is considerable lack of knowledge how these materials interact with host cells or excreting renal cells. These concerns are of particular importance in FDA-approved nanoparticles in the sub 100 nm range, and newer particles in the sub 10 nm range currently under development. The extremely active endocytic machinery of proximal tubules (PT) avidly internalizes filtered proteins, and we hypothesized that this may also be the case for renally filtered nanoparticles. To test this hypothesis, we IV-injected mice with either 6 or 13 nm nanoparticles made by cross-linking 10 KD dextran molecules or 5 nm poly(amido amine) dendrimers (PAMAM). The effects of these nanoparticles on major PT proteins were studied under two conditions: 1) 24 h after a single injection, 2) three injections with 48 h between each treatment. The expression of aquaporin-1, megalin, clathrin, actin, cystatin, and kidney injury marker-1 (KIM-1) was investigated in fixed kidney slices. Nanoparticle injection induced no detectable KIM-1 expression. All proteins except cystatin showed reduced expression after nanoparticle injection, PAMAM having the greatest effect. Cystatin expression increased in treated tissues, indicating reduced intracellular degradative processing. Injecting fluorophore-tagged albumin after nanoparticle application confirmed this observation. As expected, chronic repeated nanoparticle infusion led to more pronounced effect than single injection. These nanoparticle-induced changes in protein expression are predicted to result in defective PT function, highlighting the importance of investigating the consequences of nanoparticle-tissue interactions.

Poster Number 21

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Cell fate of basal cells in the adult mouse epididymis

Investigators: Jeremy W. Roy, PhD; Vlad Vinarsky, MD; Tata Purushothama, PhD; Jayaraj Rajagopal, MD; Sylvie Breton, PhD

The epididymis is a male reproductive organ whose function is critical for the maturation and storage of spermatozoa. It is composed of a single tubule lined with a pseudo-stratified epithelium composed of principal, narrow/clear and basal cells. In many organs basal cells function as adult stem cells capable of differentiating into other cell types. In the epididymis, we observed a subpopulation of clear cell “like” cells that also express cytokeratin 5 (KRT5), a marker of basal cells (BCs), and in animals in which Tdtomato is under the control of KRT5 we observed a subpopulation of clear cells that are Tdtomato+. These results indicated the possibility that clear cells originate from basal cells. To study the cell fate of basal cells in the epididymis, we generated mice in which KRT5 promoter drives the expression of Tamoxifen-activated Cre recombinase (CreER) in membrane Tdtomato/membrane EGFP LoxP(mT/mGLoxP) reporter mice (KRT5CreER/mTmGLoxP). KRT5CreER/mTmGLoxP mice were treated with Tamoxifen (2 mg) or corn oil for 5 days, and epididymis were collected 3 and 14 days post induction. Three days post induction we observed mG+/KRT5+ BCs. Fourteen days post induction, in addition to mG+/KRT5+ BCs, we observed a significant number of mG+ clear cells (ATP6v1B1+, KRT5-), but no mG+ principal cells (AQP9+). In conclusion, our results demonstrate that under homeostatic conditions epididymal basal cells function as adult progenitor cells capable of differentiating into clear cells. Additional long-term and injury-repair studies will elucidate whether basal cells or clear cells can differentiate into principal cells.

Poster Number 22

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The Underutilization of the Internet in Obtaining Health Information

Investigators: Catherine Sutherland, CIP; A. J. Guarino, PhD

Purpose: The primary objective of this study was to assess patient utilization of the Internet in obtaining health related information.

Participants: The sample was comprised of N = 93 patients undergoing infusion at a large metropolitan hospital in New England. The demographic make-up was mostly female (82%), Caucasian (91%), and well educated (79% reported a bachelor's degree or higher).

Instrumentation: The Information Needs Survey (INS)(Sorensen, 2010) is comprised of eleven items scored on a four-point Likert-type scale with higher ratings indicating greater levels. The INS is designed to assess the following, (a) sources of health related information on the Internet, (b) awareness of the hospital Internet information source, and (c) usage of emailing their health provider.

Results: Results of the binomial test indicated a statistically significant ($p < .001$) smaller proportion of patients (i.e., only 15%) utilized Internet health information sources. The chi-square goodness-of-fit test indicated that statistically significantly more patients were unaware of the hospital Internet information source (79.4%, $p < .001$) and significantly less ever logged on to a site (19.1%, $p < .01$). Lastly, only 40% of the respondents emailed their health providers.

Discussion: Results indicated that patients were either unaware of Internet health information and/or do not elect to use this medium; a surprising finding considering the education level of this sample. Further studies are needed to investigate the barriers preventing patients from utilizing this invaluable resource.

Poster Number 23

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Clinically relevant long-term ventilator-induced lung injury in mice

Investigators: Margit Veronika Szabari, MD; Luiz Fernando dos Reis Falcão, MD, PhD; Guido Musch, MD

Mice models offer unique opportunity to study the molecular mechanisms of ventilator-induced lung injury (VILI). However, most injurious ventilation protocols in mice do not reflect the clinical scenario of VILI because they are performed with exceedingly large tidal volumes applied for a short time. We investigated the effect of protective and injurious mechanical ventilation in mice over time scales and tidal volume ranges closer to those encountered in the clinical setting.

Mice were ventilated with protective (Vt=6-8 ml/kg, RR=180/min, n=10) or injurious (Vt=15-20 ml/kg, RR=52-80/min, n=12) ventilation with FiO2=50%. Ventilation was continued for 16 hours or until the mouse death. We measured lung tissue elastance (H) with broadband frequency oscillation technique and inspiratory capacity (IC) at 30 cmH2O. At the end of the experiment, lung tissue was collected to determine pulmonary oedema with wet/dry ratio.

16-hour mortality was significantly higher in the injurious ventilation group ($p=0.004$) Over the course of the study, IC decreased significantly in the injurious group (1.04 vs. 0.94 ml, $p<0.001$) whereas the decrease was less in the protective group (1.04 vs. 1.00 ml, n.s.). H increased significantly in both groups ($p<0.001$), but this change was again more marked in the injurious (Δ=27%) than the protective (Δ=19%) group. We did not find difference between the two groups in the wet/dry ratio.

We developed a long-term mouse model of protective mechanical ventilation using clinically relevant ventilatory parameters. Survival was significantly better, and deterioration of respiratory mechanics was less, in this model than in one with higher tidal volume.

BASIC–OTHER

Poster Number 24

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Dedifferentiation of committed epithelial cells into stem cells in vivo

Investigators: Purushothama Rao Tata, PhD; Hongmei Mou, PhD; Ana Pardo-Saganta, PhD; Rui Zhao, PhD; Mythili Prabhu, BS; Brandon M. Law, BS; Vladimir Vinarsky, MD; Josalyn L. Cho, MD; Sylvie Breton, PhD; Amar Sahay, PhD; Benjamin D. Medoff, MD; Jayaraj Rajagopal, MD

Cellular plasticity contributes to the regenerative capacity of plants, invertebrates, teleost fishes, and amphibians. Recently, the study of plasticity in higher vertebrate regeneration has gained momentum. In vertebrates, differentiated cells are known to revert into replicating progenitors to generate more cells of a given lineage, but these cells do not persist as stable stem cells. We now present evidence that fully differentiated luminal epithelial cells can revert into stable stem cells in vivo. Following diphtheria toxin-induced ablation of airway stem cells, we observed a surprising increase in the proliferation of secretory cells. In vivo lineage tracing demonstrated that the luminal secretory cells had dedifferentiated into basal stem cells. Dedifferentiated secretory cells were morphologically indistinguishable from stem cells and functioned normally to repair infectious and toxin-induced airway damage. Indeed, single secretory cells clonally dedifferentiated into functional multipotent stem cells when cultured without basal stem cells, but direct contact with a single basal cell was sufficient to prevent dedifferentiation. In analogy to classic descriptions of amphibian nuclear reprogramming, the propensity of the committed cell to dedifferentiate was inversely correlated with its state of maturity. The ability of a differentiated epithelial cell to stably acquire a functional stem cell fate in vivo suggests that the dedifferentiation of committed cell types into stem cells may contribute more generally to the regenerative capacity of higher vertebrates. Furthermore, the ability of differentiated cells to convert into stem cells has implications for the origins and behavior of cancer.

Poster Number 25

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Enhancing Synthetic Bone Scaffolds with Dentin Matrix Protein I

Investigators: Veronica Toro; Andrew Grottkau; Yonggang Pong, MD; Brian Grottkau, MD; Craig Neville, PhD

The current advances in orthopedic surgery have increased the demand for more efficient synthetic bone scaffolds that may substitute for autologous live grafts. The biggest detriment of synthetic bone scaffolds that are currently being produced is that they are osteoconductive. They serve as a scaffold for new bone growth but they do not promote bone growth (the growth is perpetuated by the native bone itself). This is a problem when it comes to the replacement of large deficits, because the bone will not be able to regenerate completely in an appropriate amount of time. Our goal is to develop osteoinductive synthetic bone scaffolds.

Dentin Matrix Phosphoprotein I is an abundant extracellular matrix protein that plays a critical role on both the mineralization of bone and dentin, and the differentiation of odonto- and osteoblasts. It is usually highly phosphorylated and depending on the level of phosphorylation, may either promote the formation calcium phosphate crystals (which make up bone) or inhibit mineralization. Incorporating DMPI into bone scaffolds may make them osteoinductive. We have cloned the human DMPI gene and expressed it in E. coli to produce the large amounts of protein necessary to coat synthetic bone scaffolds. We have co-expressed the genes that encode Casein Kinase II in order to produce phosphorylated DMPI. We are currently evaluating the impact of DMPI on bone formation.

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Poster Number 26

An optical platform for single cell transplantation in live animals

Investigators: Raphaël Turcotte, MSc; Clemens Alt, PhD; Judith Runnels, PhD; Juwell W. Wu, PhD; Walid Zaher, MD; Luke J Mortensen, PhD; Wen-Shuo Kuo, PhD; Lev Silberstein, MD, PhD; Daniel Côté, PhD; Andrew L. Kung, MD, PhD; Charles P Lin,PhD

The success of bone marrow transplantation depends on the ability of hematopoietic stem cells (HSC) to home to specific bone marrow microenvironments. The interactions between HSC and their microenvironment are essential in regulating the reconstitution of all blood cells after chemotherapy or radiotherapy. A single HSC injected into the blood stream has the potential to regenerate the entire blood cell population. Finding the site where the single HSC initially home is nevertheless not possible, and that microenvironment can thus not be characterized. A system having the ability to directly transplant single cells in spatially defined locations would enable a better description of the relationship between HSC and their microenvironments, and how it impacts reconstitution.

We have developed an all-optical system allowing the delivery of single cells in the calvarium bone marrow of live mice. Laser ablation is used to drill a microchannel to reach a specific location in the bone marrow. A single HSC is then brought in proximity of the channel with a micropipette, and released into an optical tweezer. The trapped cell can be positioned in the desired location through the microchannel. All manipulations are performed under real-time optical microscopy guidance. The platform was successfully used to deliver HSC, but also mesenchymal stem cells and leukemic (Nalm6) cells. Here, we also demonstrate the ability of delivered HSC to proliferate and reconstitute blood cells.

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Poster Number 27

Yap Is Required for the Maintenance of Airway Basal Stem Cells and Proper Epithelial Architecture

Investigators: Rui Zhao, PhD; Timothy R. Fallon, BS; Srinivas Vinod Saladi, PhD; Ana Pardo-Saganta, PhD; Vladimir Vinarsky, MD; Hongmei Mou, PhD; Naveen Nunna, BS; Fernando Camargo, PhD; Leif W. Ellisen, MD, PhD; Jayaraj Rajagopal, MD

Our understanding of how stem cells are regulated to maintain appropriate tissue size and architecture is incomplete. We show for the first time that Yap is required for the maintenance of an adult mammalian stem cell. Without Yap, airway basal stem cells are lost through differentiation, resulting in the simplification of a pseudostratified epithelium into a columnar one. Conversely, Yap overexpression causes increased stem cell proliferation and blocks terminal differentiation, resulting in epithelial stratification. Furthermore, Yap overexpression in secretory cells causes them to lose markers of terminal differentiation and acquire stem cell markers. Conversely, Yap knockdown prevents the dedifferentiation of secretory cells into stem cells. We demonstrate that Yap functionally and genetically interact with p63, and that p63 loss phenocopies Yap loss. Thus, Yap plays essential roles in adult epithelial stem cell maintenance, the regulation of stem cell identity, and the control of epithelial architecture.

CELL SIGNALING

Poster Number 28

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Calcium mediated signaling leading to podocyte cell death causes terminal kidney disease

Investigators: Frank Dubois; Philip Castonguay; Sookyung Kim; Constantine Tarabanis; Dequan Tian, PhD; Anna Greka, MD,PhD

Podocyte loss is the critical step in various forms of kidney disease. Clinical experience with ACE inhibitors (ACEi) and angiotensin receptor blockers (ARBs) has proven significant reno-protective benefits beyond blood pressure control. Podocyte specific overexpression of the angiotensin receptor type 1 (AT1R) in rats leads to podocyte loss and kidney failure. Our laboratory has shown that AT1R activation causes calcium influx through TRPC5 and TRPC6 channels in podocytes. Recently we established that TRPC5 deletion or pharmacological blockade is protective in acute models of glomerular disease. Here we show the effects of targeting TRPC5-mediated calcium influx downstream of a constitutively upregulated AT1R in immortalized mouse podocytes, as an in vitro model to study mechanistically the steps leading to podocyte death in the AT1R transgenic rat. In addition, we elucidate the effects of Angiotensin II / AT1R signaling on important podocyte signaling molecules, including TRPC channels, at the transcriptional level. Finally, we explore in vitro and in vivo the effects of excess calcium influx through AT1R/TRPC signaling on mitochondrial and ER stress, leading to cell death.

We conclude that targeting excess calcium influx downstream of the AT1R may be a novel mechanism to protect podocytes and prevent progression to kidney failure.

Poster Number 29

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Toll-like receptors up-regulate complement factor B via NF-kB pathway in human kidney tubular cells

Investigators: Dan Li, MD; Lin Zou, MD, PhD; Yan Feng, MD, PhD; Yu Gong, MD, PhD; Wei Chao, MD, PhD

Introduction: Toll-like receptors and complements are two important parts of the host innate immunity. Complement factor B (cfB) is a necessary component of the alternative pathway (AP) of complement activation. Our previous studies have shown that mice deficient of cfB were partially protected from acute kidney injury (AKI) and had improved survival during bacterial sepsis. However, how cfB synthesis is regulated in the kidney in sepsis is less clear.

Methods: Human proximal tubular cells (HK-2 cells) were stimulated with different concentrations of lipopolysaccharides (LPS, a TLR 4 ligand) and polyinosinic-polycytidylic acid (Poly(I:C), a TLR3 ligand) for various periods of time. Ten uM of BAY 11-7082, a specific NF-kB inhibitor, was added 30 min prior to HK-2 cells treatment with 100 ug/ml of LPS or 25 ug/ml of poly IC for 24 h. HK-2 cells were also stimulated with 2 ug/ml of lipoteichoic acid (LTA ,a TLR 2 ligand), 1 ug/ml of Pam3cys(a TLR2 ligand) or 0.25 uM CpG oligodeoxynucleotides (CpG, a TLR 9 ligand) for 48 h. cfB expression was determined by Western blot.

Results: Poly I:C and LPS induce time- and dose-dependent increase in cfB expression in HK-2 cell. These effects were significantly inhibited by BAY 11-7082. LTA and Pam3cys, but not CPG, also induced cfB expression in HK-2 cell.

Conclusions: These data demonstrate that activation of TLR2, TLR3 and TLR4, but not TLR9, induce a robust cfB expression in human proximal tubular cells in vitro and this effect appears to be mediated via NF-kB signaling.

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Poster Number 30

PRP19 Transforms into a Sensor of RPA-ssDNA after DNA Damage and Drives ATR Activation via a Ubiquitin-Mediated Circuitry

Investigators: Alexandre Maréchal, PhD; Ju-Mei Li, PhD; Jennifer Ji, Ching-Shyi Wu, PhD.; Shizhou Liu, PhD; Jianping Jin, PhD; Lee Zou, PhD

The DNA damage response protects cells against genotoxic lesions that can produce deleterious mutations and lead to cancer onset. RPA-coated single-stranded DNA (ssDNA) is a key platform which turns on the DNA damage response during replicative stress. RPA-ssDNA acts as a mobilizer of signaling proteins such as the ATR-ATRIP kinase complex and can also recruit a number of genome maintenance proteins to sites of damage to activate cell-cycle checkpoints, repair lesions and promote replication fork stability and restart. To discover new players in the response to replicative stress, we performed a proteomic screen to identify proteins capable of recognizing RPA-ssDNA. Using this approach, we identified the Prp19 E3 ubiquitin ligase as a sensor of DNA damage. Prp19 is involved in pre-mRNA splicing and also participates in the DNA damage response but its role in the maintenance of genome stability remains elusive. Here we show that PRP19 binds RPA directly and localizes to DNA damage sites via RPA, promoting RPA ubiquitylation in a DNA damage-induced manner. PRP19 facilitates the accumulation of ATRIP, the regulatory partner of the ATR kinase, at DNA damage sites. Depletion of PRP19 compromised the phosphorylation of ATR substrates, the recovery of stalled replication forks, and the progression of replication forks on damaged DNA. Importantly, PRP19 mutants that cannot bind RPA or function as an E3 ligase failed to support the ATR response, revealing that PRP19 drives ATR activation by acting as an RPA-ssDNA-sensing ubiquitin ligase in response to damage.

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Poster Number 31

Ex-vivo calcium imaging in freshly isolated glomerulai probes podocyte calcium dynamics

Investigators: Dequan Tian, PhD; Sookyung Kim BS; Anna Greka, MD, PhD

Background: Many cell signaling pathways are triggered by calcium influx through ion channels. The calcium permeable ion channels TRPC5 play a crucial rule in the regulation of cytoskeletal remodeling and cell migration in podocytes, cells critical to the structure and function of the kidney filter.

Hypothesis: In order to explore the precise mechanisms by which TRPC5 and TRPC6 may trigger podocyte damage, we asked whether we can measure podocyte calcium dynamics in situ in intact glomeruli.

Methods: We freshly isolate mouse glomeruli. Ex-vivo glomeruli from TRPC5 knockout and littermate control mice were seeded on glass cover slips and loaded with the calcium indicator Fura-2 AM. Calcium measurements are done using an inverted microscope equipped with a fluorescence camera and analyzed using MetaMorph imaging software.

Conclusion: Ex-vivo glomerular calcium imaging is a powerful approach to address in-vivo signaling transduction questions. We show attenuated calcium dynamics in TRPC5 knockout mice compared to littermate controls. We further show that a novel TRPC5-specific inhibitor abrogates disease-associated calcium signaling. These data support the use of this novel calcium imaging technique for the analysis of calcium dynamics and the validation of compounds targeted to the protection of the kidney filter.

DEVELOPMENT

Poster Number 32

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Mesenchymal Cell Fate is Influenced by Elastin Signaling During Lung Alveolarization

Investigators: Alwiya M. Ahmed, BS; Cassandra M. Kelleher, MD

Introduction: Understanding the role of elastin signaling in alveolarization is likely to provide mechanistic information about newborn lung diseases, such as bronchopulmonary dysplasia and diaphragmatic hernia, and insights into novel lung development-protective therapies. To determine how elastin signaling regulates alveolar development we tested how elastin-signaling blockade regulates MAPK signaling in rat fetal lung fibroblasts (RFL-6) and postnatal mouse pup lung explants undergoing alveolarization.

Methods: Monoclonal anti-elastin antibody (clone BA4) was used to inhibit elastin signaling. RFL-6 cells were cultured with or without 10ng/ml BA4 for eight days. Agarose-inflated lungs obtained from postnatal day 4 mice were sectioned, imbedded in collagen matrix, and similarly incubated with or without BA4, but for four days. Immunohistochemistry was used to evaluate cell proliferation and differentiation. Quantitative polymerase chain reaction and western bloting were performed to determine mRNA and protein expression, respectively.

Results: RFL-6 cells and lung sections grown in the presence of BA4 showed decreased proliferation and elastin gene transcription, protein expression and matrix deposition. Moreover, BA4-treated RFL-6 cells and lung sections had nearly absent filamentous-actin (F-actin) immunoreactivity. Additionally, BA4-treatment decreased ERK1/2 phosphorylation, which is known to be activated by elastin signaling. No differences were observed in these parameters in RFL6 cells or lung sections treated with G3A1, an isotype control antibody.

Conclusion: We report for the first time that elastin signaling plays an important role in regulating parenchymal cell proliferation and differentiation during alveolarization. The elastin-signaling pathway may represent a novel therapeutic target in the treatment of newborn lung disease.

Poster Number 33

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A small molecule ablates primordial germ cells through a novel mechanism of 3'UTR-mediated translational repression

Investigators: Y Jin, PhD; P. Schlueter, PhD; N. Jurisch-Yaksi, PhD; R. Peterson, PhD

Germ cells are essential for transmitting genetic information to offspring. Abnormal regulation of germ cells can lead to infertility and testicular/ovarian cancers. However, the underlying mechanisms by which germ cells are specified and maintained remain elusive. Our aim was to discover novel factors involved in these processes. We performed a small molecule screen with zebrafish embryos expressing EGFP in primordial germ cells (PGCs). One small molecule named primordazine was found to ablate PGCs with other cell types being unaffected. We discovered that primordazine acts via the 3' untranslated region (UTR) of nanos and deadend, essential genes for PGCs. The presence of these 3'UTRs is sufficient to allow primordazine to repress translation. Fluorescence in situ hybridization revealed that primordazine treatment results in formation of abnormal mRNA granules of nanos and deadend in PGCs. Introduction of mRNA of nanos, deadend, or both into embryos partially rescued the loss of PGCs, indicating that nanos and deadend are central players for primordazine-induced PGC loss. To gain further insight into how primordazine represses translation via the 3'UTR, nanos's 3'UTR was used as bait for proteomic approaches. We identified ksrp and tia1l as RNA binding proteins. Knockdown of each gene phenocopies primordazine-induced PGC loss. Embryos with homozygous .knockout of ksrp or tia1l show significantly reduced translation of luciferase mRNA with these 3'UTRs, compared to wild type embryos. These results reveal a novel mechanism of RNA regulation as well as germ cell development and provide insights into potential therapeutic strategies for infertility or germ cell cancers.

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Poster Number 34

Upregulation of eIF5B-mediated general translation controls cell cycle arrest and specific developmental stages

Investigators: Sooncheol Lee, PhD; Samuel S. Truesdell, MS; Syed I. A. Bukhari, PhD; Ju Huck Lee, PhD; Olivier LeTonqueze, PhD; Shobha Vasudevan, PhD

Proliferation arrest and distinct developmental stages inhibit general translation yet maintain ongoing translation. Their alternative translation mechanisms remain uncharacterized. We investigated the translation mechanism in three cell states considered to have reduced canonical translation: immature Xenopus laevis oocytes, mouse embryonic stem cells (ESCs) and the transition state of proliferating mammalian cells to quiescence (G0) upon growth factor deprivation. In these conditions, we find that canonical general translation is blocked by phosphorylation and thereby, inactivation of eukaryotic initiation factor 2 (eIF2), the conventional translation factor that recruits tRNA-Meti for general translation. Our data reveal transient increase of eIF5B, the eukaryotic orthologue of the bacterial initiator tRNA recruitment factor, IF2, in these conditions. EIF5B associates with tRNA-Meti upon serum-starvation of THP1 mammalian cells and functions as an alternative mechanism to promote translation in these eIF2-compromised conditions. EIF5B had been found to enable eIF2-alternative translation of specific viral and stress-related mRNAs by promoting tRNA-Meti recruitment, supporting our findings. Importantly, we find that eIF5B is an antagonist of G0 and G0-like states, as eIF5B overexpression promotes maturation of G0-like immature oocytes and causes cell death, the alternative to G0 upon serum-starvation of THP1 cells. Consistently, eIF5B depletion blocks maturation of G0-like, immature oocytes and hastens early G0 arrest upon serum-starvation of THP1 cells. These data reveal a novel, non-canonical general translation mechanism that regulates critical cell and developmental stages.

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Poster Number 35

Imaging The Murine Calcified Aortic Valve Using Micro Optical Coherence Tomography (μOCT)

Investigators: Ken Chu, PhD; Joseph Gardecki, PhD; Guillermo Tearney, MD, PhD

Background and Objectives: Severe aortic valve stenosis is progressive disorder, and it is associated with a difficult and morbid clinical course without surgical intervention. Many researchers have been coping with the process of aortic valve calcification from the molecular aspects. Various imaging modalities have been developed for specific purposes as well. Micro Optical Coherence Tomography (μOCT) has been enabled 1-μm resolution imaging. We applied this technique to valve microcalcifications ex vivo. The objective of this study was to determine the μOCT in visualizing key pathological features associated with early initiation and progression of CAVD.

Method: 8 murine valves (2 wild type mice, 3 ApoE-deficient mice, 3 double knockout mice) were dissected from the root of aorta and placed in a sample chamber which were submerged phosphate-buffered salines (PBS) to prevent tissue dehydration and quickly transported from Brigham and Women's Hospital to Massachusetts General Hospital. We identified region of interest by gross visual and conventional optical frequency domain imaging.

Result: μOCT system obtained images at a rate of 40 frames per second with a resolution of 1μm×1μm×1μm (x,y,z) in tissue. We could image detailed cellular system with enough resolution. Here we show representative pathological features (2D images) visualized by μOCT and 3D reconstructive image. μOCT imaging has potential to become a new imaging tool in the field of microscopic analysis.

DEVELOPMENT

Poster Number 36

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Early Mesenchymal Progenitors in Developing Bones Express Type II Collagen Gene

Investigators: Noriaki Ono, DDS, PhD; Wanida Ono, DDS, PhD; Henry M. Kronenberg, MD

During skeletal development, matrix-producing osteoblasts, hematopoiesis-supporting stromal cells and adipocytes are continually generated as bones become bigger. In endochondral bones, chondrocytes in growth plates continue to proliferate postnatally, providing engines for bone lengthening. The current concept holds that growth plate chondrocytes eventually undergo apoptosis through hypertrophy, and mesenchymal cells of various origins including perichondrium migrate into and proliferate in the marrow space. However, the source of these mesenchymal cells is elusive, without identification of an explicit stem cell population fueling continual bone growth. Here we show that self-renewing mesenchymal progenitors in developing bones express a transgene driven by the type II collagen (Col2) promoter active in chondrocytes and their precursors. Using cre-mediated lineage marking, we found that Col2+ descendants contributed to a majority of perichondrial cells, osteoblasts, stromal cells and mesenchymal progenitor cells in bone marrow. Pulse-chase experiments using a tamoxifen-inducible creER system revealed that Col2+ cells progressively contributed to multiple cell types including adipocytes, while continuing to replicate for the duration of mice's lifespan for 18 months. In contrast, marked osteoblast precursors expressing osterix (Osx) were mostly transient, gradually disappearing from the perichondrium and the growing part of bones as the chase extended. These results indicate that Col2+ cells include self-renewing multipotent mesenchymal progenitors earlier than Osx+ osteoblast precursors in vivo, suggesting that skeletal stem cell populations may be found in chondrocytes or their precursors.

Poster Number 37

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A Mouse Model with Generation of Full-Thickness Skin by Tissue-Culture-Expanded Human Cells

Investigators: Xunwei Wu, PhD

The goal of regenerative medicine is to reconstruct fully functional organs from tissue-culture-expanded humans cells. Here we report a mouse model with the production of human skin (hRSK) using cultured cells. The hRSK contains an intact epidermis, dermis, mature cycling hair follicles, sebaceous glands, eccrine glands and most notably, a subcutis. It shows normal human skin morphogenesis, retains human characteristics for over 6 months and differentiates normally. The hRSK establishes its own stem cell niches and can heal after injury. Enhancing β-catenin expression of adult epidermal cells used in the construct increased the number of hair follicles formed. The hRSK promises to be valuable as a model for studying problems unique to human skin, such as cell lineage questions, genetic diseases, new drug development and scarless wound healing.

Poster Number 38

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Water Relaxation Parameters and the State of Coagulation of a Protein for Vascular Repair

Investigators: Ming Zhao, MS; Jerome L. Ackerman, PhD

Coagulation of a protein biomaterial by MR-induced RF heating is a novel means to effect repair of vascular defects such as aneurysms or arteriovenous malformations. These defects are conventionally repaired by surgical clipping or by filling/occluding with endovascularly delivered wire coils, and in some cases with particles or a polymeric material. Our novel method, MR Coagulation, is intended to achieve a comparable result by coagulating a thermosetting material (such as a protein solution) delivered endovascularly by catheter and coagulated by RF-induced heating of an intracatheter resonant wire antenna in the MRI scanner. Human serum albumin (HSA) is the most abundant protein in the blood. Because of its natural biocompatibility and coagulability when heated with lasers or other energy sources, it has been used surgically to seal blood vessels and to stem diffuse bleeding. In image guided MR coagulation, we need to determine the biomaterial's rheological state to establish when coagulation is complete using its MR characteristics. Egg white, a 20 wt% protein solution, can be used as an inexpensive HSA substitute for investigating heat coagulation behavior and MR relaxation properties. In this study, the MR relaxation properties of egg white were studied as a function of temperature and degree of coagulation to determine which relaxation time constants would be most appropriate for future imaging studies to ascertain the coagulation state in vivo. We find that the spin-spin relaxation time and rotating frame spin-lattice relaxation time both indicate clearly the state of coagulation of the protein.

Poster Number 39

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Unbiased screen for novel barriers of somatic cell reprogramming

Investigators: Marti Borkent, MSc; Brad Lackford, PhD; Nimet Maherali, PhD; Stephen Elledge, PhD; Guang Hu, PhD; Konrad Hochedlinger, PhD

The discovery that differentiated adult cells can be reprogrammed towards pluripotency by overexpression of a handful of transcription factors has great potential in regenerative medicine and disease modeling. However, reprogramming remains inefficient and the underlying mechanisms are poorly understood. Barriers of the reprogramming process likely exist to protect against malignant transformation by preventing cellular transformation.Thus, we set out to perform an unbiased screen for genes whose down-regulation facilitates reprogramming. Specifically, we are using a pooled genome-wide shRNA library in combination with a 'reprogrammable mouse' model, which allows for homogeneous expression of all four required reprogramming factors. In this approach, shRNAs were introduced into reprogrammable embryonic fibroblasts (MEFs) and subsequently retrieved by PCR from derived induced pluripotent stem cells (iPSCs) to generate an enriched shRNA library. In order to eliminate false positive hits, this cycle was repeated twice and enriched shRNAs where then identified by deep sequencing. We have already discovered a number of genes whose shRNAs are consistently enriched in the iPSCs. Furthermore, we have confirmed for some that their knockdown significantly increases reprogramming efficiency, suggesting these genes constitute roadblocks of the reprogramming process. Some of the candidates have already been described in cancer biology, while others have not yet been linked to reprogramming and constitute a novel cellular pathway in changing cell fate. By investigating the biology behind these 'unusual suspects' in cellular plasticity, we hope to improve our understanding of reprogramming into iPSCs as well as better understand how cells protect themselves from transformation into cancerous cells.

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Poster Number 40

Cell-based studies using accurate genetic models of juvenile NCL implicate CLN3 function at the intersection of calcium homeostasis and autophagy pathway regulation

Investigators: Uma Chandrachud, PhD; Mathew Walker, MS; Alexandra Simas, BA; Sasja Heetveld, MS; Anton Petcherski, MS; Wen-Ning Zhao, PhD; Stephen Haggarty, PhD; Emyr Lloyd-Evans, PhD; Susan L. Cotman, PhD

Loss-of-function CLN3 mutations cause the childhood neurodegenerative disorder juvenile neuronal ceroid lipofuscinosis (JNCL), which is characterized by the autophagosomal-lysosomal accumulation of subunit c of the mitochondrial ATP synthase. Using a genetically accurate murine neuronal precursor cell model, which displays an abnormal accumulation of autophagosomes marked by GFPLC3, we previously identified a significant effect of thapsigargin on further increasing levels of GFPLC3-marked autophagsomes, more-so than in wildtype cells. Thapsigargin blocks the SERCA endoplasmic reticulum (ER) pump leading to elevated cytosolic calcium, and has previously been demonstrated to inhibit autophagosome-lysosome fusion. We have therefore now investigated 1) whether the observed sensitivity of our murine JNCL neuronal cells to thapsigargin treatment was calcium mediated, and 2) whether thapsigargin acted at a late step in the autophagy pathway in these cells. Indeed, calcium chelation reversed the effect on GFPLC3 autophagosome accumulation, and mutant cells displayed abnormalities in calcium homeostasis, including in ER and lysosomal stores. Consistent with a deficiency in autophagosomal-lysosomal pathway flux, we further observed elevated p62/SQSTM1 aggregates in mutant cells, which was worsened by thapsigargin treatment. Moreover, treatment with bafilomycin to block autophagosome-lysosome fusion sensitized wildtype cells to thapsigargin, recapitulating the effect of Cln3 mutation alone. Finally, JNCL neuronal progenitor cells, derived from human patient induced pluripotent stem cells, similarly displayed autophagosomal accumulation and sensitivity to thapsigargin treatment. These studies importantly validate the use of our genetically accurate murine cell model for study of JNCL pathogenesis, and highlight a possible role for CLN3 in regulating calcium-mediated autophagy pathway flux.

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Poster Number 41

Common variants associated with plasma triglycerides and risk for coronary artery disease

Investigators: Ron Do, PhD; Cristen J. Willer, PhD; Global Lipids Genetics Consortium; Goncalo R. Abecasis, PhD; Mark J. Daly, PhD; Benjamin M. Neale, PhD; Sekar Kathiresan, MD

Plasma triglycerides are transported in specific triglyceride-rich lipoproteins; in observational epidemiologic studies, increased triglyceride levels correlate with higher risk for coronary artery disease (CAD). However, it is unclear whether this association reflects causal processes. Genetic information can help assess causality, and can be useful in dissecting the influences of correlated measures such as triglycerides, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). We used 185 common polymorphisms recently mapped for plasma lipid traits ($P<5x10-8$ for each) to examine the role of triglycerides on risk for CAD. First, we highlight loci associated with both LDL-C and triglycerides, and show that the direction and magnitude of both are factors in determining risk for CAD. Second, we consider loci with a strong magnitude of association with triglycerides but a minimal one with LDL-C, and show that these loci are also associated with CAD. Finally, in a model accounting for effects on LDL-C and/or HDL-C, a polymorphism's strength of effect on triglycerides is correlated with the magnitude of its effect on CAD risk. These results suggest that triglyceride-rich lipoproteins may causally influence risk for CAD and that novel therapeutic approaches targeted to triglyceride-rich lipoproteins might be expected to reduce risk of CAD.

GENETICS / GENOMICS

Poster Number 42

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Improving Genome Targeting Specificity Using Modified CRISPR/RNA-guided Nucleases in Human Cells

Investigators: Yanfang Fu, PhD; Jeffry D. Sander, PhD; Deepak Reyon, PhD; Vincent M. Cascio, BS; J. Keith Joung, MD, PhD

CRISPR RNA-guided nucleases (RGNs) have recently emerged as a simple and highly efficient method for targeted genome engineering in a wide range of organisms. However, we and others have previously shown that high frequency indel mutations can be induced at off-target sites harboring up to five mismatched nucleotides and that these effects can be site dependent and challenging to predict. Improving the specificity of CRISPR RGNs is of utmost importance if these reagents are to be used for therapeutic applications. Here we report a modified CRISPR RGN architecture that substantially improves the specificities of these reagents. We demonstrate that our modified RGNs can reduce mutagenesis rates by up to 5000-fold or more at previously defined off-target sites in human cells, without sacrificing on-target gene modification activity. Furthermore, to evaluate the general specificity of our modified RGNs, we tested a large number of additional candidate off-target sites, covering nearly all sites in human genome harboring one or two mismatches and found only very low level mutation frequencies at a small number of these sites. Furthermore, we show that our modified gRNAs can be combined with a previously reported dual nickase method to enhance the specificities of both strategies. Our modified RGNs provide an important advance that will help to further advance the use of CRISPR RGNs as potential therapeutics for genetic diseases.

Poster Number 43

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Human embryonic stem cells exhibit a dynamic, conserved and functional m6A epitranscriptome

Investigators: Benoit Molinie, PhD; Jinkai Wang, PhD; Kaveh Daneshvar, PhD; Chan Zhou, PhD; Alan Mullen,MD, PhD; Yi Xing, PhD; Cosmas Giallourakis, MD

The N6-methyladenosine (m6A) RNA modification pathway is linked to developmental decisions in lower eukaryotes. However, little is known concerning the dynamic extent, conservation and potential function(s) of RNA modifications in human development. Herein, we report the genome-wide analyses of m6A modifications in human embryonic stem cells (hESCs) differentiated towards endoderm. m6A sites are observed on thousands of transcripts including those encoding master regulators of stem cell identity and differentiation. Moreover, we identified a conserved topological enrichment of m6A sites at the 3¹ end of genes among single exon and multi-exon mRNAs that extends to lncRNAs. Comparative genomic analyses of mouse and human ESCs reveals a striking level of conservation of methylated genes and their respective sites of modification. Moreover, endoderm differentiation is distinguished by dynamic regulation of m6A peak intensities. Importantly, we demonstrate that hESCs are reliant on the m6A methyltransferase component METTL3 for normal endoderm differentiation. Thus, we reveal a novel layer of stem cell regulation at the epistrancriptomic level.

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Poster Number 44

The Partners HealthCare Biobank: Phenotyping of the first 10,000 consented subjects

Investigators: Alison G. Hoffnagle, BS; Nicole Allen, MPH; Lynn Bry, MD, PhD; Vivian S. Gainer, MS; Kent B. Lewandrowski, MD; Susan A. Slaugenhaupt, PhD; Patrick Sluss, PhD; Jordan W. Smoller, MD, ScD; Scott T. Weiss, MD; Elizabeth W. Karlson, MD

Background: The Partners HealthCare Biobank is a research study that aims to define genetic, lifestyle and environmental factors associated with human diseases. The Biobank aims to collect consented samples linked to comprehensive health information, family history, lifestyle and environmental data from 100,000 Partners Healthcare patients from Brigham and Women's Hospital (BWH), Massachusetts General Hospital (MGH), and Spaulding Rehabilitation Hospital (SRH). Currently over 10,000 patients have been consented to this initiative, and provided DNA, plasma, and serum samples through direct outreach at over 20 outpatient clinics collaborating with over 100 participating physicians.

Methods: We conducted a Research Patient Data Repository (RPDR) analysis of demographic data, and billing data according to phenotype groupings from published PheWAS studies based on ICD9 codes.

Results: The Partners Biobank is comprised of 57.6% females, 42.4% males. Race/ethnicity distribution includes White (83.3%), Black (6.2%), Hispanic (4.6%), Asian (2.2%) and other (3.7%) individuals. Common phenotypes in the 10,165 consented subjects include heart disease (61%), joint pain (61%), hypertension (57%), gastroesophageal reflux disease (32%), osteoarthritis (30%), obesity (23%), stroke (22%), major depression (21%), diabetes (21%), and kidney disease (18%).

Conclusion: The Partners HealthCare Biobank provides large numbers of consented samples from subjects with common diseases for clinical and translational research. This initiative is a powerful opportunity for collaboration by patients, physicians, and researchers who together can make strides in understanding the causes of disease and in finding new treatments and cures.

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Poster Number 45

Fine mapping of inflammatory bowel disease risk loci using immunochip

Investigators: Hailiang Huang, PhD; Luke Jostins, PhD; Kyle Kai-How Farh, MD, PhD; Brendan Bulik-Sullivan; The International IBD Genetics Consortium; Jeffrey C. Barret, PhD; Mark J. Daly, PhD

Inflammatory bowel disease (IBD) is an autoimmune disorder that affects 2.5 million people of European ancestry. There are two distinct but etiologically related forms of IBD, Crohn's disease (CD) and ulcerative colitis (UC). The latest study in IBD genetics reported 163 genomic loci that are associated with IBD. Comprehensively assessing variants within these risk loci gives insights into the genetic mechanisms of IBD. Here we propose a Bayesian based fine mapping approach that can define the number of independent association signals in each locus, the disease form of each signal and a minimal set of variants that we are 95% confident to contain the causal variants.

We apply this approach to 66,849 European samples from the International IBD Genetics Consortium's immunochip project (18,603 CD, 14,307 UC and 33,938 healthy controls). Genotypes of these samples have been stringently QC'ed and imputed to the 1000 genome reference panel. We reduced 19 associations to a single variant and 43 associations to ≤5 variants. Known causal variants in NOD2 (fs1007insC, R702W and G908R) and IL23R (V362I and G149R) were mapped as the only variant in the credible set. Small credible sets (≤5 variants) are enriched for coding variants (10-fold, p-value=1E–11) and potential splice variants (3.6-fold, 1E–4), suggesting high confident causal variants. 11 credible sets have overlap with the best cis-eQTL signals and we have observed a significant enrichment (p-value=1E–10) of the H3K4me1 marker in the credible variants.

Poster Number 46

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Identifying somatic mutation hotspots across protein family alignments

Investigators: Marcin Imielinski MD, PhD; Charles Du; Matthew Meyerson MD, PhD

In cancer, somatic driver mutations often target "hotspots" of paralogous residues across evolutionarily related members of a single protein family. These hotspots usually confer a dominant oncogenic function, such as constitutive catalytic activity or binding. These hotspots generally represent the most "druggable" genetic alterations. Examples of protein families targeted in such a manner include the Ras and receptor Tyrosine Kinase family. Protein family hotspots are not directly assessed by standard computational approaches for statistically nominating genes under positive somatic selection, such as MutSig, InVex, or MuSic. These methods only assess single genes for statistical enrichment of total mutation burden or the presence of mutation hotspots. As a result, such approaches are only powered to detect protein family hotspots in cases where each (or at least one) protein family member is very frequently mutated (e.g. KRAS, NRAS, HRAS). They are not powered to detect hotspots in highly functionally redundant families, of which each member may be only mutated in <<1% of a tumor type. To directly identify such protein-family hotspots, we have developed a computational framework, MutPfam. This framework superimposes mutation data onto Pfam (http://pfam.sanger.ac.uk/) (protein family and subfamily multiple sequence alignments and identifies statistically significant hotspots. We demonstate our method on protein families involved in the catalysis of small-molecular catabolism using the TCGA Pan-Cancer dataset (https://tcga-data.nci.nih.gov/) comprising somatic mutation calls for 4608 whole-exome sequenced patients and 21 tumor types. As we demonstrate, our framework enables the de novo discovery of somatic mutation hotspots across protein families.

Poster Number 47

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Excessive daytime sleepiness and obesity: evidence for a common genetic basis points to pleiotropy

Investigators: Jacqueline Lane, PhD; Andrew Bjonnes, MS; Frank Scheer, PhD; Susan Redline, PhD; Richa Saxena, PhD

Nearly half of US adults report suffering from excessive daytime sleepiness (EDS) at least a few days a month, leading to cognitive, psychological and metabolic issues, such as obesity. We therefore asked if there is a common genetic basis for both mass index (BMI) and EDS. We evaluated the relationship between EDS and genetic variants identified by genetic studies for BMI. We used data from the CARe study with genotype and phenotype data (self-report dichotomous trait) (n=11,737). Analysis was adjusted for age, gender, and ancestry. A fixed effects, inverse-variance meta-analysis was performed. Risk scores were calculated using 14 SNPs previously associated with BMI. We find increased odds of EDS in overweight (OR 1.020, p=0.041), obese (OR 1.083, p<0.0001), and morbidly obese (OR 1.195, p<0.0001) individuals of European ancestry versus normal BMI (N=11,737). We find a significant association between EDS and genetic variants in BDNF and FTO. In total 11/14 SNPs demonstrate decreased risk of EDS with BMI risk alleles, even after adjustment for BMI. A weighted BMI risk score is significantly associated with EDS (Risk Coef -1.0875, p=1.56e-05) and is independent of BMI. Our association results capture an effect beyond BMI. Paradoxically, BMI raising alleles individually or aggregated are associated with a decreased odds of EDS, indicating divergent physiological roles for underlying pathways. This suggests individuals with a predicted genetic risk of BMI might be at a lower risk for developing EDS. Mechanistic understanding of BMI risk variants may provide parallel insights into excessive daytime sleepiness.

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Poster Number 48

Mutations in ZFPM2 (FOG2) and its Downstream Genes in 275 Congenital Diaphragmatic Hernia Exomes

Investigators: Mauro Longoni, MD; Meaghan K. Russell, MPH, PhD; Frances A. High, MD, PhD; Kasper Lage, PhD; Julie Wells, PhD; Carol J. Bult, PhD; Kate G. Ackerman, MD; Charles Lee, PhD; David Andrews, MD; Barbara R. Pober, MD; Patricia K. Donahoe, MD

Congenital Diaphragmatic Hernia (CDH) is a common, genetically heterogeneous birth defect. In the majority of CDH patients, causative mutations are not known. ZFPM2 (also known as FOG2) gene defects cause CDH and/or conotruncal defects in humans and animal models. We estimated the prevalence of damaging ZFPM2 mutations in a cohort of 275 sporadic CDH patient exomes to be 2.5%. ZFPM2 mutations were mostly missense, but included one stop gain and one frameshift, and were frequently inherited from an unaffected parent, consistent with the hypothesis that environmental factors or genetic modifiers are necessary to cause a clinically recognizable diaphragmatic defect. Genetic analysis of a multigenerational family identified an intragenic ZFPM2 deletion with an estimated penetrance of only 30%, which has important implications in genetic counseling. Similarly, a low penetrance ZFPM2 frameshift mutation was observed in a second family with isolated CDH. In order to understand the mechanism of ZFPM2-related CDH, we generated microarray expression profiles from microdissected Zfpm2 knock-out embryonic diaphrams at E11.5, when the diaphragmatic defect is thought to arise. Unbiased enrichment analysis showed that the 123 upregulated and 441 downregulated genes were in the retinoic acid, muscle, and tendon development pathways. Nine Real Time qPCR validated genes downstream of Zfpm2 had rare, heterozygous, likely pathogenic variants in 29/275 (10%) CDH exomes. Our data confirm that the ZFPM2-related pathway is central in diaphragm development and suggest that developmental expression studies are a useful tool for the interpretation of exome sequencing data when studying birth defects.

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Poster Number 49

Identification of LMX1B as a novel oncogene in human ovarian cancer

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Ovarian cancers are thought to result from the accumulation of multiple genetic aberrations that transform ovarian and/or fallopian tube surface epithelial cells, allowing for their abnormal growth, proliferation and metastasis. We carried out genome-wide copy-number analysis using array comparative genomic hybridization on a panel of mouse ovarian cancer (OVCA) cell lines previously established in our laboratory. We identified a recurrent focal amplification on mouse chromosomal region 2qB, which contains the LIM homeodomain containing transcription factor 1B (Lmx1b) gene. LMX1B is not expressed in normal human ovary, but is expressed in many human OVCA cell lines and primary tumors. High expression of LMX1B correlates with poor outcome. To clarify the role of LMX1B in ovarian carcinogenesis, we transduced LMX1B into a panel of mouse and human OVCA cell lines and demonstrated that LMX1B strongly promotes migration of cancer cells in culture and accelerates xenograft growth in nude mice. Conversely, knockdown of LMX1B in a human cell line with endogenous high expression of LMX1B inhibits cell migration in vitro and tumor growth in vivo. Microarray analysis of cells overexpressing LMX1B identified NF-κB pathway as a potential mediator of tumor progression and subsequent treatment of NF-κB inhibitor decreased the migratory capacity of these cells. Thus, our data demonstrate that LMX1B functions as an oncogene in OVCA pathogenesis.

Poster Number 50

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Whole Exome Sequencing in Cohort of Very Early Onset Inflammatory Bowel Disease

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Background: Many of the genetic loci implicated in adult-onset inflammatory bowel disease (IBD) have also been shown to play a role in pediatric-onset IBD. However, rare loss-of-function mutations in children with very early-onset IBD (VEO-IBD) have recently been described. We hypothesized that patients with VEO-IBD or extreme gastrointestinal symptoms likely harbor rare, causative genetic variants.

Methods: Patients with IBD with onset <6years old (and their parents) were recruited along with patients with extreme gastrointestinal symptoms. Salivary or whole blood DNA was collected. Exome capture was performed by Agilent Whole Exome SureSelect kit. Sequencing was performed on Illumina HiSeq. Sequences were processed by Picard pipeline and aligned with GRCh37,h19. Variants were called using GATK toolkit and filtered for minor allele frequencies <1% in controls. Functional significance was assessed by SIFT/Polyphen2. RESULTS: 70 VEO-IBD patients were sequenced (30 full parent-proband trios). A novel, frameshift mutation in RIPK2 (Chr8,90784949T>TA) was identified in a patient with aggressive, ileocecal Crohn's disease (CD) whose father also possessed the variant and aggressive CD. A novel,homozygous, in-frame insertion variant in LXRB(Chr19, 50881822A>ACAA) was identified in affected siblings. We identified a homozygous, in-frame deletion in SMPD1 (involved in Niemann-Pick disease) in an infant with severe, secretory diarrhea and hepatomegaly and compound heterozygosity in cma1 in an infant with severe, infantile food protein-induced enterocolitis. Functional analysis in these families is on-going.

Conclusion: Rare, loss of function genetic variants play a strong role in VEO-IBD and extreme gastrointestinal disease although therapeutic implications remain to be determined.

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Poster Number 51

Higher Body Mass Index is Associated with Methylation and Suppression of 5-alpha reductase 2 in Adult Prostate Tissues

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Introduction: 5-alpha reductase 2 (SRD5A2) plays a central role in prostate development and growth. Finasteride, a specific inhibitor of the SRD5A2, is used for treatment of lower urinary tract symptoms due to bladder outlet obstruction secondary to benign prostatic hyperplasia (BPH). However, many patients are resistant to the therapeutic effect of Finasteride. We have found that 30% of human adult prostatic tissues do not express 5AR2, which may account for resistance to Finasteride. We have postulated that absent expression of SRD5A2 could be secondary to methylation of the CpG island in the promoter region of SRD5A2. Our aim is to investigate the association of SRD5A2 gene methylation and absence of SRD5A2 protein expression in patients who have undergone transurethral resection of the prostate (TURP).

Methods: We analyzed 80 benign prostate samples after TURP. Methylation of the SRD5A2 promoter was assessed using Methylated CpG Island Recovery Assay. According to CpG methylation status, patients were grouped as M-positive or M-negative. The expression of SRD5A2 protein was assessed as absent (<10%),weak (11-30%), medium (31-60%) or strong (>60%), using the immunohistochemical assays. Associations between methylation status, protein expression, prostate volume (PV), prostate specific antigen (PSA), body mass index (BMI) were examined. Wilcoxon rank sum, chi-square and Spearman rank tests were used for statistical analyses.

Results: We found that methylation of SRD5A2 strongly correlated with absence of SRD5A2 protein expression (p=0.001). Higher BMI correlated with methylation of

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Poster Number 52

Rare APOC3 loss-of-function variants lower plasma triglycerides and protect against coronary heart disease

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Plasma triglyceride concentrations are heritable and correlated with risk for coronary heart disease (CHD). Sequencing the protein-coding regions of the human genome ('the exome') has the potential to discover rare mutations with a large effect on a phenotype. We sequenced the protein-coding regions of 18,666 genes in each of 3,734 participants of European and African ancestries from the U.S. National Heart, Lung, and Blood Institute's Exome Sequencing Project. We tested whether rare coding sequence mutations, individually or aggregated within a gene, were associated with triglycerides. For mutations associated with triglycerides, we subsequently evaluated association with risk for CHD in 110,970 individuals. We found an aggregate of rare mutations in the apolipoprotein C-III (APOC3) gene was associated with lower plasma triglycerides. Of four mutations that drove this association result, three were annotated as loss-of-function (R19*, splice site mutation IVS2+1 G>A, and splice site mutation IVS3+1 G>T) and a fourth as a missense mutation (A43T). About one in 300 individuals was a heterozygous carrier of at least one of these four mutations. Carriers had 39% lower triglycerides (P < 1 x 10-20) and 46% lower concentration of circulating apoC-III protein (P = 8 x 10-10). APOC3 rare mutation carriers were at 40% reduced risk for CHD (OR 0.60, 95% CI 0.47 - 0.75, P=4 x 10-6 among 110,472 non-carriers and 498 carriers). In conclusion, rare mutations that disrupt APOC3 gene function protect against CHD, with lower plasma triglycerides and apoC-III protein serving as biomarkers of reduced gene function.

Poster Number 53

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Rescue of Cellular Phenotypes in a Novel NCL Human iPS System

Investigators: Alexandra M. Simas, BA; John F. Staropoli, MD, PhD; Scott Coppel, BA; Anton Petcherski, BA; Dolan Sondhi, PhD; Ronald G. Crystal, MD; Susan L. Cotman, PhD

We have recently developed new human iPSC models for two forms of neuronal ceroid lipofuscinoses (NCL): classic late-infantile and juvenile NCL, caused by mutations in TPP1 and CLN3, respectively. We also established differentiation protocols and demonstrated progressive accumulation of NCL-type storage material as the cells were differentiated into a neuronal lineage. Phenotyping revealed primary defects in the endosomal-lysosomal system, as well as accumulating abnormalities in other systems upon neuronal differentiation. Here, we have performed proof-of-concept studies using the hallmark NCL storage material as an end-point to test potential therapies including, 1) gene therapy vectors, and 2) candidate small molecules. The TPP1 neuronal progenitor cells (TPP1-NPCs) and CLN3-NPCs were infected with adeno-associated viral (AAV) vectors containing the respective nonmutated human transgenes, and expression of the lysosomal proteins was confirmed by immunofluorescence. For the TPP1-NPCs, these results were corroborated by western blot analysis and TPP1 enzyme assay. For both TPP1-NPCs and CLN3-NPCs, treatment with the gene therapy vectors substantially rescued storage material accumulation. Treatment of TPP1-NPCs with small molecules previously reported to upregulate TPP1 expression and enzyme activity, however, gave mixed results. Fenofibrate and gemfibrozil, reported to induce TPP1 activity in control human and mouse cells, failed to increase TPP1 activity in the patient NPCs. Conversely, nonsense suppression by PTC124 resulted in both an increase of TPP1 expression and enzyme activity, and in attenuation of storage material. This study therefore documents the high value of this new set of tools for improved drug screening and for investigating early mechanisms driving NCL pathogenesis.

Poster Number 54

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Products of Murine Cytochrome P450 Cyp2j Locus Regulate Pulmonary Vascular Response to Alveolar Hypoxia.

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Human CYP2J2 synthesize epoxyeicosatrienoic acids (EETs) with potent vasoactive effects. However, genetic analysis of the contributions of CYP2J2 to physiologic processes in humans has been confounded by the allelic expansion in rodents: mice have 8 Cyp2j genes similar to CYP2J2.

To investigate the role of CYP2J2 in cardiovascular function, we created mice bearing a 626-kb murine Cyp2j locus deletion using a combination of homologous and site-directed recombination strategies. Two bacterial artificial chromosomes (BACs) covering the two ends of Cyp2j locus were modified to allow an integrase-mediated site-specific recombination event to form a deletion replica. The replica was introduced into mouse ES cells to ultimately enable generation of Cyp2j-/- mice. RT-MLPA revealed that all Cyp2j gene expressions were eliminated in Cyp2j-/- mice. A mouse strain with the complement of human CYP2J2 was also created (Cyp2j-/-tg).

Systemic and pulmonary hemodynamic parameters did not differ in anesthetized WT, Cyp2j-/-, and Cyp2j-/-tg mice at baseline. Hypoxic pulmonary vasoconstriction (HPV) was monitored by measuring left pulmonary vascular resistance (LPVR) and systemic arterial oxygenation (PaO2) before and during left main stem bronchial occlusion (LMBO). LMBO increased LPVR 2.0- and 2.3-fold in WT and Cyp2j-/-tg mice ($P<0.05$). LMBO did not change LPVR in Cyp2j-/- mice consistent with impaired HPV. During LMBO, PaO2 was greater in WT and Cyp2j-/-tg mice than in Cyp2j-/- mice ($P<0.05$) demonstrating that Cyp2j deficiency impairs matching of ventilation with perfusion (V/Q). These data suggest that products of Cyp2j epoxygenase contribute to the pulmonary vasoconstriction produced by alveolar hypoxia and preservation of V/Q matching.

IMMUNOLOGY & INFLAMMATION

Poster Number 55

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Microfluidic Platform for the Quantitative Analysis of Leukocyte Migration Signatures

Investigators: Leo Boneschansker, MD; Jun Yan, PhD; Elisabeth Wong, BS; David M. Briscoe, MD; Daniel Irimia MD, PhD

Leukocyte migration into tissues is characteristic of acute and chronic inflammation. It is usually measured in vitro as the average displacement of populations of cells towards various biochemical gradients; however, current assays rarely acknowledge other patterns of leukocyte migration. Here, we developed and validated a microfluidic migration platform for the quantitatively analysis of bi-directional migration signatures at the single cell level that simultaneously analyzes the four basic leukocyte migration patterns: chemo-attraction, -repulsion, -kinesis and –inhibition. We found that all basic patterns coexist in heterogeneous populations of human neutrophils and lymphocytes and a homogenous clonal cell line. We found that interleukin-8 (IL-8) and complement component 5a (C5a), known chemoattractants, induce both attraction and repulsion migratory patterns in equal proportions in human neutrophils. Also, we found that lymphocyte migration is characterized by non-persistent migratory patterns; and that twice as many lymphocytes migrate faster and with higher persistence away from than towards SDF-1. Moreover, we found that Slit2, a known chemoinhibitor, has notable effects only on the size of migrating human neutrophil subpopulations while it does not alter their migration patterns. Our data provides a new paradigm that defines chemokine-induced leukocyte migration as the summation of time-dependent chemo-attraction, -repulsion, –kinesis and –inhibition patterns. We suggest analysis of leukocyte migration signatures is important for advancing our understanding of the actions of chemokines and could have important therapeutic implications for design and development of anti-inflammatory agents.

Poster Number 56

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CARMA3 Links the Innate and Adaptive Immune Response in Allergic Asthma

Investigators: Benjamin Causton, PhD; Ravisankar A. Ramadas, PhD; Khristianna Jones, BS; Benjamin D. Medoff, MD

Innate immune responses by airway epithelial cells (AECs) help initiate and propagate the adaptive immune response associated with allergic airway inflammation in asthma. Activation of NF-κB in AECs by allergens or secondary mediators via GPCRs is an important component of this multifaceted inflammatory cascade. The CARMA family of proteins (CARMA1, 2 and 3) are molecular scaffolds required for the assembly of multi-protein complexes which mediate cell-specific NF-κB activation. Whereas CARMA1 and CARMA2 are predominantly expressed in lymphocytes and placenta respectively, CARMA3 is highly expressed in AECs where it mediates activation of NF- B in response to GPCR stimulation.

We demonstrate that lentiviral knockdown of CARMA3 in NHBEs reduces the production of pro-asthmatic mediators IL-8, TSLP, CCL20/MIP-3α and GM-CSF in response to a panel of asthma-relevant GPCR ligands, including house dust mite and Alternaria alternata. We have developed a genetically modified mouse whereby CARMA3 is deleted specifically in AECs (CARMA3AEC). Utilizing murine models of allergic airways disease, we show that CARMA3AEC mice have reduced airway eosinophilia and pro-inflammatory cytokine production compared to control mice. In addition, there were reduced numbers of mature myeloid dendritic cells (DCs) in the lung and lung draining lymph nodes in CARMA3AEC mice compared to control mice and lung DCs from CARMA3AEC mice have impaired antigen presentation.

In conclusion CARMA3 helps mediate allergic airway inflammation and is a critical signaling molecule bridging the innate and adaptive immune responses in the lung. These data suggest that CARMA3 could be a promising therapeutic target to reduce airway inflammation in asthma.

Poster Number 57

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A Novel Tissue Engineered Construct for Wound Treatment and Skin Immunity Research

Investigators: Mihail Climov, MD; Abraham J. Matar, BS; Erika Medeiros, BS; Jizeng Qiao, MD; Evan A. Farkash, MD; Raimon Duran-Struuck, DVM, PhD; Ashley Gusha, BS; Edward Harrington, BS; David H. Sachs, MD; Mark Randolph, MS; Thomas Bollenbach, PhD; Christene A. Huang, PhD

Purpose: This study was performed to evaluate the viability, persistence and immunogenicity of a novel self-assembled porcine bilayered cellular construct (SA-PBCC) when transplanted across MHC barriers in a preclinical swine model.

Methods: Fibroblast and keratinocyte cell banks were generated from skin punches of neonatal SLAcc and SLAdd MGH MHC-defined miniature swine. A dermal matrix was prepared from fibroblasts stimulated to generate extracellular matrix, and then seeded with keratinocytes to form the epidermal layer. The resulting SLAdd SA-PBCC were grafted on split thickness wounds in SLAcc (n=5) and SLAdd (autologous control) animals. Engraftment and/or rejection were evaluated by gross examination and biopsy histology. Mixed lymphocyte reactions, alloantibody flow cytometry, and complement-dependent cytotoxicity assays were used to assess immunogenicity of the grafts. Animals received a second grafting of full MHC mismatched SA-PBCC 7 weeks following the first set of grafts together with MHC matched SA-PBCC to assess for clinical sensitization.

Results: Autologous SA-PBCC engrafted and persisted similarly to autologous skin grafts. All fully mismatched SA-PBCC successfully engrafted with histologic evidence for neovascularization of the fibroblastic component by day 4. By day 6, perivascular inflammatory infiltrates were present in the fibroblastic layer; some with extension to the epidermal component (grades 1-2 by Banff classification of skin-containing composite tissue allograft pathology). Complete cellular rejection and tissue loss occurred by day 8 for most grafts. Following the second application, both SA-PBCC (full MHC mismatched and MHC matched) underwent accelerated rejection (<4 days). MHC-specific cytotoxic alloantibody could be detected within 10-18 days post first grafting, with increased titer post re-grafting.

Summary and Conclusions: These data demonstrate that SA-PBCC engraft, undergo vasculogenesis, and mature into structures resembling normal skin. Despite the lack of donor hematopoietic-derived cells, MHC-mismatched SA-PBCC are rejected in a similar time course to allogeneic skin and sensitize across MHC barriers. Since the absence of professional donor antigen presenting cells does not appear to prolong graft survival, sensitization to minor histocompatibility antigen likely occurred through indirect antigen presentation following vascularization and host cell infiltration into the construct. The fact that autologous SA-PBCC permanently engrafts could have important implications for wound treatment and may someday complement the use of tissue expanders in reconstructive surgery.

IMMUNOLOGY & INFLAMMATION

Poster Number 58

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Bone marrow hematopoietic stem cells give rise to inflammatory innate immune cells after ischemic stroke

Investigators: Gabriel Courties, PhD; Fanny Herisson, MD; Ying Wei, MD; Timo Heidt, MD; Filip Swirski, PhD; Ralph Weissleder, MD, PhD; Michael Moskowitz, MD; Matthias Nahrendorf, MD, PhD

Stroke induces an acute systemic inflammatory response and increases circulating numbers of innate immune cells. Myeloid cells are recruited in large numbers to the ischemic brain, where they pursue critical roles in wound healing but also contribute to reperfusion injury. While we know that circulating leukocyte numbers change after stroke and that the bone marrow is the site of leukocyte production, there are limited data available on the crosstalk between the central nervous, immune and hematopoietic systems. We hypothesized that after depletion of the body's leukocyte reservoirs, increased leukocyte numbers after stroke are the result of accelerated cell production by the hematopoietic system. Experimental stroke induced by a transient middle cerebral artery occlusion in mice increased bone marrow stem cell activity, expanded the number of downstream leukocyte progenitors and instigated a hematopoietic system bias towards the myeloid lineage, resulting in increased bone marrow output of monocytes and neutrophils. We investigated two potential mechanisms that may instruct the bone marrow to produce more leukocytes. Pretreatment of mice with 6-hydroxydopamine was used for the blockade of the sympathetic nervous system, however hematopoietic output was unchanged. We next investigated the bone marrow of mice lacking MyD88, involved in signal transduction downstream of toll like receptors. In these mice, we found a significant lack of bone marrow stem cell activation and reduced myelopoiesis after stroke, indicating that soluble danger signals, e.g. toll-like receptor ligands, may have a role in activating the bone marrow production of inflammatory leukocytes after ischemic brain injury.

Poster Number 59

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A unique inflammatory signature that tracks with the development of bNAbs in the absence of high viremia

Investigators: A.S. Dugast, PhD; M. Hoffner; K. Arnold, PhD; E. Brown, PhD; M. Ackerman, PhD; F. Pereyra, MD; M. Seaman, PhD; B. Walker, MD; D. Lauffenburger, PhD; G. Alter, PhD

Background: Over the past decade, there has been an exponential increase in the discovery of a number of new broadly neutralizing Abs (bNAbs), however the mechanism by which bNAbs are induced remains unclear. Here we sought to dissect the inflammatory signals that tracks with bNAb evolution in a large cohort of subjects that spontaneously control viral replication (Controllers), to define whether viremia and inflammation can be unlinked as predictors of the induction of bNAbs.

Methods: Neutralizing activity, 20-plex cytokine analyses, B cell profile, antibody responses including subclass titers, functionality (ADCC, ADCP, ADCDC) and Fc-receptor binding were performed in a group of 300 Controllers with and without bNAbs.

Results: A unique inflammatory profile was observed among controllers that generated neutralizing breadth compared to controllers that did not, including: a) higher plasma levels of CXCL13, sCD40L and IP10; b) an expansion of tissue-like memory B cells expressing BAFF-receptor and CXCR5; c) increased titers of IgG1 gp140-specific antibodies, d) skewed antibody Fc-glycosylation with a preferrential capacity to engage FcgR2A, FcgR3A, and complement activation.

Conclusion: Overall, these data suggest that undetectable viral replication in tissue reservoirs and/or a unique inflammatory profile in controllers may drive the evolution of neutralizing antibody breadth in the absence of highly diversified plasma viral replication. Therefore it is plausible that vaccine adjuvanting approaches potentially able to elicit these profiles may equally play a key role in driving protective humoral responses.

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Poster Number 60

Activation of CCR2+ hematopoietic stem cells after myocardial infarction

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Background: Myocardial infarction (MI) results in leukocytosis and massive infiltration of myeloid cells into the injured heart. The high demand for leukocytes must be met by hematopoietic organs. However, most hematopoietic stem cells (HSC) are quiescent, and it is currently unknown how hematopoiesis responds to ischemic injury.

Hypothesis: We hypothesized that myocardial infarction activates a specific HSC subset.

Results: Bone marrow HSC cycled more vigorously on day 2 after myocardial infarction ($p<0.01$). Interestingly, a CCR2+ HSC subset proliferated at 4-fold higher rates when compared to CCR2- HSC. Gene set enrichment analysis of whole transcriptome microarray data revealed a role for myeloid translocation gene 16 (Mtg16) in the emergence of CCR2+ HSC. In Mtg16-/- mice, paucity of CCR2+ HSC compromised monocyte and macrophage supply to infarcted hearts ($p<0.01$). Gene set enrichment analysis and a competitive bone marrow reconstitution assay showed that CCR2- HSC are hierarchically primitive. CCR2+ HSC produced more myeloid cells than CCR2- HSC ($p<0.01$), consistent with a higher expression of myeloid transcription factors PU.1 and Cebpa in CCR2+ HSC. After myocardial infarction, the bone marrow preferentially released CCR2+ HSC, and this release was inhibited by siRNA treatment silencing CCR2 ($p<0.01$).

Conclusions: Here we show CCR2 expression identifies a uniquely active and migratory stem cell subset, and marks the most upstream activation point of the hematopoietic lineage after myocardial infarction, providing a new therapeutic opportunity to fundamentally change the course of the disease.

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Poster Number 61

Ubiquitous MHC Class II Peptides Shape Regulatory T Cell Development

Investigators: Sharon Germana, MS; Christian LeGuern, PhD

T cell auto- and allo-reactivity is primarily tamed in the periphery by thymus-derived CD4 regulatory T cells (Tregs) that mature in the medullar compartment. We have achieved Treg-dependent tolerance of fully allogeneic transplants via the transfer of donor MHC class II genes (MHCII) that induced newly made Tregs specific of donor MHCII peptides. Given that gene therapy for tolerance was effective with MHCII but not MHCI genes, the goal of the study was to identify natural MHCII peptides able to impact Treg development and function. Using experimental models that either recapitulated MHCII peptide presentation – the IEα 52-68 peptide presented on I-Ab molecules – or promoted Treg-mediated tolerance to heart grafts via MHCII gene transfer, we confirmed that high amounts of IEα peptide were presented by MHCII from most if not all I-Ab+ CD11c+ dendritic cells of the thymic medulla, suggesting its role in Treg maturation. This hypothesis was confirmed by demonstrating that the introduction of the IEα peptide through hematopoietic chimerism converted host IEα-specific CD4 T cells into CD25hiFoxp3+ suppressive Tregs. Treg suppression for tolerance to transplants required prior activation by cognate pMHCII complexes as MHCII-treated recipients rejected MHCII-deficient while accepting MHCII-sufficient grafts. These data are consistent with a prominent role of "MHCII self-presentation" in shaping thymic Treg TCR specificities and controlling Treg function in the periphery.

IMMUNOLOGY & INFLAMMATION

Poster Number 62

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The Effect of Intestinal Alkaline Phosphatase on Abscess Fluid Induced Inflammation

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Introduction: Intestinal alkaline phosphatase (IAP) is an intestinal brush border enzyme with the ability to detoxify pro-inflammatory bacterial components. IAP irrigation has shown beneficial effects in a mouse model of intra-abdominal sepsis. We sought to determine whether IAP could inhibit the inflammatory effects of human abscesses. Methods: Paracolic, pelvic, liver and thigh samples (n=11) were collected from patients undergoing abscess drainage by Interventional Radiology. Different dilutions of the supernatants were pre-incubated +/- IAP (200 Units) for 1 hour and then applied to human monocytic THP-1 cells in triplicate. Endotoxin-free water (EFW) and IAP were applied to the cells as controls. Interleukin-8 (IL-8) levels were measured after 6 hours by sandwich ELISA and microbiology analysis performed on all samples. Results: Samples alone induced dramatic IL-8 release from THP1 cells, whereas little IL-8 was seen in the case of EFW or IAP alone. Abdominal and pelvic abscesses showed the highest levels of IL-8 and pre-incubation with IAP reduced the inflammatory response by an average of 60% (400.6 ± 93.2 vs. 152.8 ± 47.0 pg/ml, p= 0.039). Abscesses with gram positive bacteria induced high IL-8 levels and there was a 70% reduction with IAP treatment. IAP also reduced the inflammatory response to samples with gram negative bacteria (56% reduction, 473.2 ± 52.4 vs. 208.2 ± 22.5 pg/ml, p= 0.043). Conclusions: IAP is able to inhibit the inflammatory response associated with a variety of human abscesses. Local irrigation with IAP may represent a novel adjuvant therapy in the management of established human infections.

Poster Number 63

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Retrotaxis of Human Neutrophils during Mechanical Confinement inside Microfluidic Channels

Investigators: Bashar Hamza, MS; Elisabeth Wong, BS; Sachin Patel, BS; Hansang Cho, PhD; Joseph Martel, PhD; Daniel Irimia, MD, PhD

The current paradigm of unidirectional migration of neutrophils from circulation to sites of injury in tissues has been recently challenged by observations in zebrafish showing that neutrophils can return from tissues back into the circulation. However, the relevance of these observations to human neutrophils remains unclear, the forward and reversed migration of neutrophils is difficult to quantify, and the precise conditions modulating the reverse migration cannot be isolated. Here, we designed a microfluidic platform inside which we observed human neutrophil migration in response to chemoattractant sources inside channels, simulating the biochemical and mechanical confinement conditions at sites of injury in tissues. We observed that, after initially following the direction of chemoattractant gradients, more than 90% of human neutrophils can reverse their direction and migrate persistently and for distances up to 1000 micrometers away from chemoattractant sources (retrotaxis). Retrotaxis is enhanced in the presence of lipoxin A4 (LXA4), a well-established mediator of inflammation resolution, or Tempol, a standard antioxidant. Retrotaxis stops after neutrophils encounter targets which they phagocytose or on surfaces presenting high concentrations of fibronectin. Our microfluidic model suggests a new paradigm for neutrophil accumulation at sites of inflammation, which depends on the balance of three simultaneous processes: chemotaxis along diffusion gradients, retrotaxis following mechanical guides, and stopping triggered by phagocytosis.

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Poster Number 64

Ikaros mutation confers integrin-dependent pre-B cell survival and progression to acute lymphoblastic leukemia

Investigators: Ila Joshi, PhD; Toshimi Yoshida, PhD; Nilamani Jena, PhD; Xiaoqing Qi; Jiangwen Zhang, PhD; Richard A. Van Etten, MD, PhD; Katia Georgopoulos, PhD

Deletion of the Ikaros DNA-binding domain generates dominant-negative isoforms that interfere with Ikaros family activity and correlate with poor prognosis in human precursor B cell acute lymphoblastic leukemias (B-ALL). Here, we show that conditional inactivation of the Ikaros DNA binding domain in early pre-B cells arrests their differentiation at a stage where integrin-dependent niche adhesion augments mitogen-activated protein kinase signaling, proliferation, and self-renewal, and attenuates pre-B cell receptor signaling and differentiation. Transplantation of polyclonal Ikzf1 mutant pre-B cells results in long-latency oligoclonal pre-B-ALL, demonstrating that loss of Ikaros contributes to multistep B-leukemogenesis. These results explain how normal pre-B cells transit from a highly proliferative and stromal-dependent to a stromal-independent phase where differentiation is enabled, providing potential therapeutic strategies for IKZF1 mutant B-ALL.

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Poster Number 65

Near-infrared laser adjuvant for influenza vaccine

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Safe and effective immunologic adjuvants are often essential for vaccines. However, the choice of adjuvant for licensed vaccines is limited, especially for those that are administered intradermally. We show that non-tissue damaging, near-infrared (NIR) laser light given in short exposures to small areas of skin, without the use of additional chemical or biological agents, significantly increases immune responses to intradermal influenza vaccination without augmenting IgE. The NIR laser-adjuvanted vaccine confers increased protection in a murine influenza lethal challenge model as compared to unadjuvanted vaccine. We show that NIR laser treatment induces the expression of specific chemokines in the skin resulting in recruitment and activation of dendritic cells and is safe to use in both mice and humans. The NIR laser adjuvant technology provides a novel, safe, low-cost, simple-to-use, potentially broadly applicable and clinically feasible approach to enhancing vaccine efficacy as an alternative to chemical and biological adjuvants.

Poster Number 66

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Elevated levels of interferon-production by memory T cells do not promote transplant tolerance resistance in aged recipients

Investigators: James I. Kim, PhD; Ryan T. Stott, BS; Julie Soohoo, BA; Kang Mi Lee, PhD; Gaoping Zhao, MD; Heidi Yeh, MD; Shaoping Deng, MD; James F. Markmann, MD, PhD

Immunosenescence predisposes the elderly to infectious and autoimmune diseases and impairs the response to vaccination. We recently demonstrated that ageing also impedes development of transplantation tolerance. Unlike their young counterparts (8-12 weeks of age) aged male recipients (greater than 12 months of age) transplanted with a full MHC-mismatched heart are resistant to tolerance mediated by anti-CD45RB antibody. Surprisingly, either chemical or surgical castration restored tolerance induction to levels observed using young recipients. Based on the strong impact of endocrine modulation on transplant tolerance, we explored the impact of ageing and castration on the immune system. Here we report a significant increase in the percentage of T cells that produce interferon-γ (IFN-γ) in aged male versus young male animals and that the overall increase in IFN-γ production was due to an expansion of IFN-γ-producing memory T cells in aged animals. In contrast to IFN-γ production, we did not observe differences in IL-10 expression in young versus old male mice. We hypothesized that endocrine modulation would diminish the elevated levels of IFN-γ production in aged recipients, however, we observed no significant reduction in the percentage of IFN-γ+ T cells upon castration. Furthermore, we neutralized interferon-γ by antibody and did not observe an effect on graft survival. We conclude that while elevated levels of interferon-γ serves as a marker of tolerance resistance in aged mice, other as yet to be identified factors are responsible for its cause. Defining these factors may be relevant to design of tolerogenic strategies for aged recipients.

Poster Number 67

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Sequence-specific alterations of epitope production and presentation by HIV Protease Inhibitors

Investigators: Georgio Kourjian, MS; Yang Xu, MS; Ijah Mondesire-Crump, MD; Mariko Shimada, MS; Pauline Gourdain, PhD; Sylvie Le Gall, PhD

Epitopes displayed by MHC-I are generated from intracellular degradation of proteins by proteasome and aminopeptidases and the cross-presentation of exogenous antigens degraded by lysosomal cathepsins. We hypothesize that due to structural homologies HIV protease-inhibitors (PIs) used in antiretroviral-therapies may affect activities of cellular peptidases involved in epitope processing and alter epitope presentation to immune cells.

Four out of seven HIV-1 PIs tested variably altered proteasome, aminopeptidase and/or cathepsin hydrolytic activities in PBMCs, CD4 T and dendritic cells. To assess how PI-induced activity changes affect epitope production, cytosolic or lysosomal extracts preincubated with various PIs were used to degrade HIV, HCV, EBV, Influenza and MAGE3 peptides and degradation products were defined and semi-quantified by mass-spectrometry. These PI-induced changes in protease activities were sequence-dependent leading to enhanced or reduced cleavage of specific residues and resulting in alteration of epitope production kinetics and intracellular or lysosomal half-life of peptides prior to MHC-I loading.

Finally we assessed the impact of PIs on the endogenous processing and presentation of epitopes by HIV-infected cells and the cross-presentation of exogenous antigens to CD8 T-cells using a fluorescence-based cytotoxicity-assay. HIV PIs altered (from 2.2fold decrease to 1.5fold increase, $p<0.01$) the presentation of HIV epitopes and recognition by epitope-specific CD8 T-cells.

These findings suggest that in HIV-infected patients an antiretroviral-therapy including PIs might -by altering host proteases function- modify the pattern of epitope presentation, possibly leading to the elicitation of additional CTL responses against HIV and potentially against other pathogens co-infecting HIV+ persons.

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Poster Number 68

A novel approach to the quantification and characterization of cell mediated cytotoxicity

Investigators: GM La Muraglia II, BSc; ML Madariaga, MD; SG Michel, MD; KS Mordecai, BA; IM Hanekamp, PhD; JC Madsen, MD, DPhil

Cell mediated lysis (CML) is an established in vitro assay to assess the lysis of target cells by cytototoxic T lymphocytes. Current methods employed in the detection of CML are based upon the quantification of traceable elements (51Cr) released from target cells. In this study, we propose a novel approach to the detection of CML by providing a comprehensive analysis of effector-target cell interaction that combines both the statistical power of flow cytometry and the qualitative benefits of cell imaging. Our model tested the levels of CML in effector lymphocytes to full MHC-disparate primary porcine lymphocyte targets. Effector cells were generated by priming responder peripheral blood mononuclear cells (PBMCs) with irradiated target-type stimulators for 6 days. Effector PBMCs were then harvested and stained with CD8 and CD4. Lymphocyte targets were labeled with eFluor670 membrane dye. The classic chromium-based assay was performed in parallel to provide verification of results. We found that the use of image-stream technologies provides data in line with that of the classic chromium-based assay, such as the state of donor-responsiveness or -unresponsiveness. Unlike traditional approaches, this method allows for more qualitative and quantitative analysis of effector-target cell interactions. We demonstrated the presence of other helper cells, changes in morphology and apoptotic indices for specific immune cell subsets. Here we establish a novel image-stream technology that allows for the quantification and qualification of cell-mediated lysis. This method has wide applicability for transplantation models of tolerance and rejection.

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Poster Number 69

"Fibronucleation" and stiffness gradients drive tissue fibrosis by promoting fibroblast recruitment and activation through cell "durotaxis"

Investigators: David Lagares, PhD; Fei Liu, PhD; Vera Auernheimer, MSc; David Adams, PhD; Vincent Ludovic, MSc; Mohit Kapoor, PhD; Melissa Suter, PhD; Ben Fabry, PhD; Wolgang Goldmann, PhD; Adam Engler, PhD; Daniel Tschumperlin, PhD; Andrew Tager, MD

Increased matrix stiffness has been shown to be a cause as well as a consequence of fibrosis progression in idiopathic pulmonary fibrosis (IPF) patients. The molecular mechanisms through which increased tissue rigidity drives disease progression remain obscure. To characterize the spatial alterations in matrix stiffness produced in vivo, we performed atomic force microscopy (AFM) studies on tissues from animal models of lung fibrosis. AFM elastograms revealed that matrix stiffness, rather than being uniformly elevated in fibrotic tissues, rises and falls in spatial gradients. We hypothesize that the stiffness "peaks" represent areas in which the fibrotic process was initiated, or "nucleated", and that initial focal increases in stiffness activates local fibroblasts to myofibroblasts in a process we term "fibronucleation". We further hypothesize that once fibrosis is nucleated, the stiffness gradients leading to these fibrotic peaks are amplified by fibroblast "durotaxis," – the directed migration of cells from regions of lower to higher stiffness that occurs in the absence of chemoattractant. Using hydrogels that recapitulate the stiffness gradients observed in vivo, we demonstrated durotaxis of lung fibroblasts in time-lapse microscopy studies in vitro. Fibronucleation was confirmed by using optical coherence tomography (OCT) to construct 3D birefringence maps on mouse lung fibrotic tissues. OCT birefringence is increased by augmented collagen crosslinking and correlates with tissue stiffness. In humans studies, AFM revealed nucleated areas and stiffness gradients within lung tissues from IPF patients. Our study suggests that targeting fibroblast durotaxis has potential to be a new therapeutic strategy for the treatment of pulmonary fibrosis.

Poster Number 70

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Gliadin is a chemoattractant factor for neutrophils and induces migration via engagement of the formyl peptide receptor, FPR1

Investigators: Karen M. Lammers, PhD; Marcello Chieppa, PhD; Lunhua Liu, PhD; Carole A. Parent, PhD; Alessio Fasano, MD

Gluten-containing cereals (wheat, rye and barley) form a substantial part of the human diet but are increasingly associated with disease. Our recent studies point out that the innate immune system is a critical driving force in the host's early response to gliadin, the main component of gluten.

Duodenal tissues of gliadin-exposed C57BL/6 and Lys-GFP mice showed increased intestinal permeability and a rapid recruitment of neutrophils. Subsequent in-vitro studies using the EZ-Taxi-scan assay allowed monitoring of murine and human neutrophils chemotaxis to gliadin and the primary bacterial-derived product, N-formyl-Methionine-Leucine-Phenylalanine (fMLF). Gliadin induced neutrophil chemotaxis with similar potency as fMLF. Given that the formyl peptide receptor (FPR)-1 specifically recognizes fMLF, we studied its involvement in gliadin-induced neutrophil chemotaxis by pretreating human neutrophils with cyclosporine H, a specific inhibitor of FPR1. This pretreatment abolished fMLF-mediated neutrophil chemotaxis, while leukotriene B4-mediated chemotaxis that does not involve FPR1, remained unaffected. Remarkably, gliadin-induced chemotaxis was completely abrogated after cyclosporine H pretreatment. To exclude possible LPS contamination of the gliadin preparation, 25 peptides of our alpha-gliadin synthetic peptide library were tested. Thirteen peptides induced neutrophil migration in a FPR1-dependent manner, confirming the specificity of the gliadin-induced neutrophil migratory response and its binding to FPR1.

These novel data emphasize an emerging concept that gliadin is interpreted and handled by the host mucosa as a danger signal similar to that provided by fMLF, likewise exerting direct and robust chemoattractant effects on neutrophils.

These observations provide new insight into our understanding of how gliadin triggers inflammatory and autoimmune responses.

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Poster Number 71

Gene Regulatory Network Modeling Host-Pathogen Interaction of Caenorhabditis elegans and Pseudomonas aeruginosa

Investigators: Lucy Eunju Lee; Javier E. Irazoqui, PhD; Erel Levine, PhD

The course of infection is a complex control process of gene transcription within a pathogen and its host. The regulatory mechanisms of two organisms react recursively to each other's responses, resulting in a spectrum of final consequences. Therefore, a thorough understanding of such an interaction is essential for effective diagnosis and treatment of infectious diseases. In this study, we hypothesized that the genes of host and pathogen compose an integrated network of transcriptional control, which continually evolves by interaction among genes over the time course of infection.

We studied the interaction between a nematode Caenorhabditis elegans and its pathogen Pseudomonas aeruginosa to study how host gene expression influences that of the pathogen, and vice versa. To test our hypothesis, the time-course gene expression data were obtained by collecting RNA from the infected worms at multiple time points. Then activities of genes of both the pathogen and the host were investigated by transcriptome profiling using an RNA-Seq technique. Using quantitative data analysis methods including network clustering and inference, we identified potentially important regulatory gene groups of the host-pathogen network.

Ultimately, we would like to develop a computational method to predict and change the course of infection by analyzing the critical gene groups and key regulatory connections of the network. Considering that C. elegans defense pathways have mammalian counterparts and that P. aeruginosa virulence factors are common in human infections, the results of this study are also expected to have implications in our understanding of human diseases.

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Poster Number 72

Endogenous CD4+ T cells specific for self-antigens survive negative selection and are controlled by tissue-specific mechanisms of tolerance in the periphery

Investigators: Francois Legoux, PhD; André Han, BA; Jong-Baeck Lim, MD; James Moon, PhD

CD4+ T cells specific for self-antigens are involved in most autoimmune disorders, but the steady-state mechanisms maintaining tolerance in these cells remain poorly understood. Growing evidence suggests that some CD4+ T cells with specificity to tissue-restricted self-antigens routinely escape the thymus and populate the body. Little is known about these potentially harmful T cells because of their low frequency and tolerant phenotype in normal animals.

In this study, we used Cre recombinase as a model self-antigen in mice expressing Cre in various tissues. We have mapped a CD4+ T cell epitope in the Cre protein and generated the corresponding peptide: MHCII tetramer. Using magnetic enrichment of tetramer-stained T cells, we directly detected naive populations of Cre-specific CD4+ T cells.

We found that negative selection of Cre-specific T cells was efficient in mice expressing Cre ubiquitously, but very inefficient in mice expressing Cre in a tissue-restricted fashion. In mice expressing Cre exclusively in the pancreas, immunization with Cre peptide induced strong expansion of specific CD4+ T cells, suggesting immune ignorance to the pancreatic self-antigen. In contrast, tolerance to self-antigens expressed in mucosal tissues, such as the gut and lung, relied on increased steady-state frequencies of Cre-specific Tregs. While these Tregs were very efficient at controlling primary autoimmune responses, we found that only negative selection of self-antigen specific T cells could maintain tolerance over successive antigenic challenges.

In summary, our data show that steady-state tolerance to tissue-restricted self-antigens relies on non-deletional tissue-specific mechanisms that can be over-ruled by successive antigenic stimulations.

IMMUNOLOGY & INFLAMMATION

Poster Number 73

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Heart Allograft Tolerance Induced by Kidney Cotransplantation is Dependent on MHC Matching Between the Donor Heart and Kidney Parenchyma

Investigators: M.L. Madariaga, MD; S.G. Michel, MD; G.M. La Muraglia II, BS; V. Villani, MD; M. Sejikima, MD; D.A. Leonard, MD; H.J. Powell, BS; W.J. Selkirk, BA; J.G. Connolly, BA; E.A. Farkash, MD; R.B. Colvin, MD; J.S. Allan, MD, MBA; C.A. Huang, PhD; D.H. Sachs, MD; K. Yamada, MD; J.C. Madsen, MD, DPhil

We have previously shown that kidney allografts have the ability to induce cardiac allograft tolerance across a full MHC barrier in miniature swine after a 12-day course of high-dose tacrolimus. However, the renal element responsible for kidney-induced cardiac allograft tolerance (KICAT) is unknown. Here we tested whether parenchymal cells or hematopoietic-derived cells within the kidney are necessary for KICAT. Heart and kidney allografts were transplanted into MHC-mismatched recipients treated with high-dose tacrolimus for 12 days. Group 1 animals (n=3) received a kidney allograft that was fully MHC-mismatched to the heart. Group 2 animals (n=3) received a kidney allograft whose parenchyma was MHC-mismatched to the heart. Group 3 animals (n=2) received a kidney allograft whose passenger leukocytes were MHC-mismatched to the heart. Five out of six cardiac allografts in groups 1 and 2 were rejected within 35 days. In contrast, cardiac allografts in group 3 survived >160 days with no evidence of rejection on serial cardiac biopsies. In vitro CML and MLR assays showed initial donor-specific unresponsiveness in all groups; some animals regained donor-specific responsiveness at time of rejection. Allo-antibody formation was detected in one animal in group 1 and transiently in all animals in group 3. Two of three kidney allografts in group 1 recipients showed severe rejection suggesting that the process of cardiac allograft rejection interfered with the induction of renal allograft tolerance. These data suggest that the mechanisms underlying KICAT is more dependent on cells associated with the renal parenchyma rather than with kidney passenger leukocytes.

Poster Number 74

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Effect of Irradiation on Incidence of PTLD following Haploidentical Cell Transplantation in Miniature Swine

Investigators: Abraham J. Matar, BS; Aarti R. Patil, MD; Ahmad Al-Musa, MD; Isabel Hanekamp, PhD; Christene A. Huang, PhD; Raimon Duran-Struuck, DVM, PhD.

Post-transplant lymphoproliferative disease (PTLD) is a major complication of clinical organ and cell transplantation. Conditioning and immunosuppressive regimens that significantly impair T cell immunity, including depleting antibodies and calcineurin inhibitors, increase the risk of PTLD following transplantation. Swine PTLD has been shown to closely resemble human PTLD in morphology, histology, and viral-driven reactivation of B cells. Previously, we reported high incidences of PTLD following hematopoietic cell transplantation in miniature swine recipients conditioned with thymic irradiation (TI) in addition to T cell depletion and cyclosporine monotherapy following transplantation. Replacement of TI with 100 cGy of total body irradiation (TBI) markedly decreased the incidence of PTLD. Furthermore, elevated levels of serum lactate dehydrogenase (LDH) were determined to be reliable markers for the diagnosis of PTLD in swine, similarly to humans. Results from this large cohort of animals provide valuable insight on the effect of irradiation and T cell immunity on the incidence of PTLD following hematopoietic cell transplantation (HCT) and reinforce the pig model as a valuable tool for the study of PTLD and HCT.

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Poster Number 75

VaxCelerate: the Use of MTBhsp70-Avidin as an Adjuvant to Rapidly Generate Self-Assembling Vaccines With Biotinylated, Antigen-Specific Peptides Targeting Emerging Pathogens

Investigators: Pierre Leblanc, PhD; Timothy Brauns, MBA; Cybelle Luza; Kanawat Chantaralawan, MD; Lynchy Lezeau, BS; Daniel Richer, BS; Christine Boyle, PhD; Lenny Moise, PhD; Anne De Groot, MD; Bill Martin, PhD; Jordan B. Fishman, PhD; Eric A. Berg, PhD; David Baker, PhD; Brandon Zeigler, PhD; Dale E. Mais, PhD; William Taylor; Russell Coleman, PhD; Shaw Warren, MD; Jeff Gelfand, MD; Mark C. Poznansky, MD, PhD

Development of effective vaccines against emerging infectious diseases(EID) can take years to progress from pathogen isolation/identification to clinical approval. Therefore, conventional approaches fail to produce field-ready vaccines before the EID has spread extensively. VaxCelerate's goal is to address the need for rapid vaccine development by creating a platform capable of generating and pre-clinically testing a new vaccine against a specific pathogen target in less than 120 days. A self-assembling vaccine is at the core of the approach which consists of a fusion protein composed of the immunostimulatory Mycobacterium tuberculosis heat shock protein 70(MTBhsp70) and the biotin-binding protein, avidin. Mixing the resulting protein(MAV) with biotinylated pathogen specific immunogenic peptides yields a self-assembled vaccine(SAV). To meet the constrained time requirement for this project we used a distributed R&D model involving experts in the fields of protein engineering and expression, bioinformatics, peptide synthesis/design and GMP/GLP manufacturing and testing standards. This approach was first tested in a model system, Ovalbumin in C57Bl/6 mice, and then progressed to testing Influenza(H1N1) specific peptides and ultimately a Lassa fever virus(LFV) specific vaccine in transgenic HLA-DR3 mice. Using a GLP-validated assay we demonstrated that the assembled LFV vaccine induced significantly increased class-II peptide specific interferon-γ CD4+ T cell responses in transgenic mice compared to peptide or MAV alone controls. VaxCelerate may also facilitate accelerated regulatory review by using an identical design for each vaccine reducing review requirements for subsequent vaccines, and by developing safety assessment tools that are more relevant to human vaccine responses than current preclinical models.

Poster Number 76

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PTEN: A Metabolic Switch Controlling Regulatory T Cells

Investigators: Bhavana Priyadharshini, PhD; Alexandria Huynh, BS; Valerie Gerriets, BS; Jeff Rathmell, PhD; Laurence Turka, MD

Regulatory T cells (Tregs), are essential to prevent autoimmunity, inflammation mediated tissue damage, and promote transplantation tolerance. Conversely, Tregs are a barrier for cancer immunotherapy. Recent studies indicate that inflammation can destabilize Tregs, and convert them into effector T cells, thereby contributing to transplant rejection or autoimmunity. Therefore, understanding the cellular mechanisms that control Treg homeostasis is important for designing better therapeutics in a variety of immune settings. Over-activation of the phosphoinositide 3 kinase (PI3K/Akt) pathway disrupts Treg development, suggesting that Treg homeostasis is tightly controlled at the level of PI3K. Here, we have created mice with Treg-specific deletion of the phosphatase and tensin homolog on chromosome 10 (PTEN), a primary negative regulator of PI3K/Akt pathway that is highly expressed in Tregs, as a tool to study the role of PI3K/Akt pathway in Treg homeostasis. Metabolic programming directs T cell fate determination. Specifically, T effector cells require glycolytic metabolism that is dependent upon PI3K/Akt signaling, whereas Tregs favor fatty acid oxidation through mitochondrial oxidative phosphorylation (OXPHOS), a state favored by low PI3K/Akt signaling. Our data suggests that excessive PI3K activity leads to Treg dysfunction and instability, characterized by a high glycolytic rate and loss of CD25 (the α chain of the high-affinity IL-2R). Moreover, preliminary studies show that inhibition of glycolysis restores CD25 expression in Tregs. These findings suggest that elevated PI3K signals, such as occurring during inflammatory/autoimmune diseases, can reprogram Tregs towards higher glycolytic metabolism leading to the loss of CD25 and disruption of Treg homeostasis.

Poster Number 77

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CARMA1 differentially regulates thymic and peripheral regulatory T cells

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Background: CARMA1 is essential for T cell activation and effector functions via T-cell receptor signaling. We have previously shown that CARMA1 is necessary for development of thymic and peripheral regulatory T cells (Tregs). However, it is unknown if CARMA1 regulates Treg function. In this study we investigated whether CARMA1 regulates thymic and peripheral Treg suppressive activity.

Methods: We crossed mice with floxed CARMA1 alleles (CARMA1F/F) with mice that express Cre recombinase under control of the Foxp3 promoter (Foxp3Cre). Foxp3Cre/CARMA1F/F mice delete CARMA1 from Foxp3-expressing T cells after differentiation into Tregs and thus have normal thymic and peripheral Treg development. We studied the suppressive capacity of CARMA1-deficient thymic Tregs in vitro using a T cell proliferation assay and of CARMA1-deficient peripheral Tregs in vivo using a murine model of airway tolerance to a foreign antigen (ovalbumin).

Results: Thymic Foxp3Cre/CARMA1F/F Tregs had enhanced suppression of T cell proliferation compared to wild type (WT) Tregs. However, Foxp3Cre/CARMA1F/F mice failed to suppress eosinophilic airway inflammation in the in vivo model, consistent with a failure of peripheral Treg-induced airway tolerance. Importantly, the number of Tregs in the lung was higher in Foxp3Cre/CARMA1F/F mice compared to WT.

Conclusions: CARMA1 inhibits the suppressive activity of thymic Tregs but enhances suppression in peripheral Tregs. These data suggest that CARMA1-mediated signaling regulates Treg function and has differential effects in peripheral and thymic Tregs. Since peripheral and thymic Tregs serve different roles in disease, therapeutic targeting of CARMA1 could have divergent effects depending on the nature of the Treg response.

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Poster Number 78

Expression of FOXP3 isoforms in gluten related disorders

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Celiac disease is an autoimmune enteropathy triggered by gluten. Non-celiac gluten sensitivity is a gluten related disorder that differentiates from celiac disease for the absence of autoimmune mechanisms and the lack of enteropathy.

Dysfunction in T regulatory cells has been associated to celiac disease. However the mechanisms underlying their loss of function remain unknown.

FOXP3 regulates the function of T regulatory cells. Two isoforms of FOXP3 have been described. The full length and the Delta2 that is unable to inhibit Th17 cells proliferation. We hypothesize that gluten related disorders may be associated with analtered expression of FOXP3 isoforms.

We detected FOXP3 isoforms expression by RT-PCR in intestinal and blood samples from celiac, gluten sensitive and non-celiac patients. We cultured mononuclear cells from celiac and non-celiac patients with IFNg and run RT-PCR to detect FOXP3 isoforms expression.

At intestinal level FOXP3 full length was equally expressed in all three groups. However the expression of Delta2 was significantly increased in celiac patients. Gluten sensitive patients also showed an increase in FOXP3 Delta2 expression, but this difference didn't rich statistical significance. We didn't see any difference in FOXP3 expression in the peripheral blood of all groups. Furthermore our stimulation experiments showed that the overexpression of Delta2 is not influenced by IFNg. For the first time we demonstrate that celiac patients are characterized by intestinal overexpression of the defective FOXP3 Delta2. We suggest that factors other than IFNg in the intestinal microenvironment of these patients may lead to an oversexpression of Delta2.

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Poster Number 79

Haptoglobin 2-2 genotype causes increased susceptibility to intestinal inflammation: A mouse model

Investigators: Craig Sturgeon, BA; Jinggang Lan, PhD; Alessio Fasano, MD

Zonulin, a protein that regulates intestinal permeability through reversible disassembly of tight junctions, has been identified as the precursor to haptoglobin (HP)-2. HP has two genotypes, HP-1 and HP-2, the latter being linked to immune mediated diseases. HP2 homozygosis is associated with more severe clinical outcome. Our aim is to use a knock-in HP2 murine model to establish whether HP2 homozygosis increases susceptibility to gut inflammation by using the DSS protocol model. After 7 days the C57Bl/6 wild type mice on DSS had an average weight of 90.5% their starting weight and the HP2 an average of 86.5% ($p>0.05$). Histological analysis of the colon of the HP2 animals showed more damage and inflammation compared to C57Bl/6 mice but did not reach statistical significance. After an additional 7 days recovery the C57Bl/6 animals showed good recovery with a final weight of 92.1% their starting weight while the HP2 mice saw a final weight of 81.5% and a low of 66.9% on day 12. We can conclude that HP2 mice are more susceptible to intestinal inflammation compared to C57Bl/6 wild type mice.

IMMUNOLOGY & INFLAMMATION

Poster Number 80

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Diphtheria toxin-based murine IL-2 fusion toxin for depleting murine Tregs in vivo

Investigators: Min Wei, MSc; Jose Marino,MD; Aaron Trowell; Huiping Zhang; Jaclyn Stromp Peraino, BSc; Priyani V. Rajasekera,BSc; Joren C. Madsen, MD, PhD; David H. Sachs, MD; Christene A. Huang, PhD; Gilles Benichou, PhD; Zhirui Wang, PhD

Regulatory T cells (Tregs) are a very important CD4+ T cell sub-population that suppresses the immune response thereby protecting the host against autoimmune disease development, inducing transplantation tolerance, and impeding the effective immune response against tumor cells. An effective in vivo Treg depletion agent is needed for Treg-associated studies across many research areas. In this study we have developed diphtheria toxin based monovalent and bivalent murine IL-2 fusion toxins for depleting murine CD25+ cells in vivo. Their potencies were assessed by in vitro protein synthesis inhibition and cell proliferation inhibition assays using a murine CD25+ CTLL-2 cell line. Surprisingly, in contrast to other recombinant fusion toxins, the monovalent isoform (DT390-mIL-2) was approximately one log more potent than its bivalent counterpart (DT390-bi-mIL-2). Binding analysis by flow cytometry demonstrated that the monovalent isoform bound stronger than the bivalent version. In vivo Treg depletion with the monovalent murine IL-2 fusion toxin was performed using C57BL/6J (B6) mice. Spleen Tregs were significantly depleted with a maximum reduction of ~70% and detectable as early as 12 hours after the treatment. The spleen Tregs numbers were reduced until day 3 and returned to control levels by day 7. The levels of other leukocyte populations, including CD4+, CD8+, CD19+ (B cells) and NK-1.1+ (NK cells) cells remained unchanged. We believe that this monovalent murine IL-2 fusion toxin will be a species-specific and effective in vivo murine Treg depleter.

Poster Number 81

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Bone cells govern T lymphopoiesis by regulating thymic emigrants from bone marrow

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Mesenchymal cell direction of parenchymal cell activity is hypothesized to be critical for adult tissue function, but remains poorly defined. We examined how specific subsets of osteolineage mesenchymal cells control parenchymal hematopoietic cells in the bone marrow using selective cell depletion genetic murine models. Unexpectedly, mature bone cells expressing osteocalcin (Ocn+) were found to be critical for T lymphopoiesis through the modulation of cells destined for thymic emigration. Specific depletion of Ocn+ cells reduced the number of adult bone marrow T-lymphoid biased progenitors by reduced endosteal DLL4 production and corresponding hematopoietic progenitor Notch activation. Thymic emigrants were compromised in association with reduced chemotactic molecules CCR7 and PSGL1, yet were capable of normal T lineage differentiation upon adoptive transfer to the thymus. Other hematopoietic lineages and hematopoietic stem cell numbers were unperturbed. Therefore, mature osteolineage cells have a highly constrained effect on T lymphoid cells, altering T cell production by regulation of thymic emigrants and their ability to translocate to the site of maturation. These data reveal a new role for bone in the homeostasis of the immune system and demonstrate how perturbation of bone cells can critically impact adult immunity.

Poster Number 82

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Complement factor B plays an essential role in the pathogenesis of polymicrobial sepsis

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The complement system is activated via classical, lectin and alternative pathways. Complement factor B (cfB) and factor D are essential components of alternative pathway (AP) activation. Previous studies have shown that cfB mediates endotoxin-triggered cardiomyocyte sarcolemmal injury and ischemia-induced apoptosis in the kidney. However, the role of cfB and AP in bacterial sepsis is unknown. Here, we demonstrated that sepsis, created by cecal ligation and puncture (CLP) in a mouse model, augmented cfB in the blood, the peritoneal cavity and major organs including the kidney and heart. Sepsis also led to the AP activation, C3 fragment deposition in the kidney and heart, and cfB-dependent C3dg production. Bacteria isolated from septic mice activated the serum AP ex vivo in a factor D-dependent manner. Deletion of MyD88, a key signaling molecule of Toll-like receptors (TLRs), attenuated cfB/C3 up-regulation as well as cleavage during bacterial infection. Importantly, during sepsis, absence of cfB conferred a protective effect with improved survival and cardiac function, and attenuated acute kidney injury. cfB deletion also led to increased neutrophil migratory function during the early phase of sepsis, decreased local and systemic bacterial load, attenuated cytokine production and reduced neutrophil reactive oxygen species production. Together, our data indicate that cfB is markedly up-regulated during bacterial infection probably via TLRs and may play an essential role in the pathogenesis of severe sepsis.

NEUROSCIENCES

Poster Number 83

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Avoiding the Regional Bias in Non-Rigid Image Registration via an Adaptive Quasi-Volume-Preserving Constraint: A Basic Approach to Registration Symmetry

Investigators: Iman Aganj, PhD; Martin Reuter, PhD; Mert R. Sabuncu, PhD; Bruce Fischl, PhD

Image registration is a crucial step in numerous clinical and neuroscientific imaging studies involving the comparison of images, such as population investigations and longitudinal analyses. The choice of a reference image influences the results of non-rigid image registration, thereby making it asymmetric. Given that symmetry is often an intrinsic attribute of the desired registration, e.g., pairwise image alignment, asymmetry can be a sign of unreliability. A non-uniform weighting in the objective function integral is the source of such error, resulting in general registration inaccuracy, a consequence of which is asymmetry. Current approaches to restore symmetry to non-rigid registration, although successful in achieving inverse-consistency, do not eliminate the regional bias that is the source of the error. In this work, instead of symmetrizing the objective function, we address the root of the problem: the non-uniformity of the integral in both the asymmetric and the symmetrized implementations. We introduce a new quasi-volume-preserving constraint that allows for volume change only in areas with well-matching image intensities, and show that such a constraint puts a bound on the error arising from non-uniformity, hence both the registration symmetry and increased accuracy. We demonstrate the advantages of our method through experiments on synthetic images, and real X-ray and 2D/3D brain MRI data. We observed that adding the proposed nonlinear registration constraint to standard diffeomorphic demons results in image alignment with better image intensity matching, less registration-induced distortion, more accurate matching of manually defined neuroanatomical structures, and higher stability.

Poster Number 84

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Acute and chronic increases in neuronal sodium concentrations during post-traumatic epileptogenesis

Investigators: Trevor Balena, PhD; Kevin J. Staley, MD

Post-traumatic increases in intracellular Cl- can shift the action of GABA from hyperpolarizing to depolarizing, which could lead to disinhibition and early post-traumatic seizures. Increases in intracellular Cl- anions are likely to be balanced by an increase in intracellular cations, and the consequent salt accumulation could underlie cytotoxic edema. We investigated changes in intracellular cation concentration beginning with Na+. Acute hippocampal slices and organotypic hippocampal slice cultures were prepared from wild-type C57BL/6J mice and incubated with SBFI (10μM). Two-photon imaging was used to excite SBFI and ratiometrically determine the intracellular Na+ concentration ([Na+]i). Here we show that, in acute slices, hippocampal neurons demonstrated a significantly higher [Na+]i than has been reported in undamaged neurons, particularly in neurons closest to the cut surface of the slice. In organotypic slice cultures, [Na+]i returned to low levels by 2 days after slice trauma. At longer incubation times, during which slices become epileptic, [Na+]i increased to levels seen immediately post-trauma, but by 20 days after trauma had returned to low levels. These trends were mirrored in the percentage of propidium iodide (PI)-positive neurons. PI staining was also higher closer to the cut surface, and PI-positive neurons had very high [Na+]i. 10μM of the Na+/K+ ATPase inhibitor ouabain and 100μM of the KCC2/NKCC1 antagonist furosemide increased [Na+]i. 10μM of the NKCC1 antagonist bumetanide increased [Na+]i during the first 10 days after trauma, and thereafter decreased [Na+]i. These findings suggest that both trauma and seizures are associated with increased [Na+]i.

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Poster Number 85

Functional interrogation of a neural pathway underlying generalization of fear

Investigators: Antoine Besnard, PhD; Nannan Guo, PhD; Tomer Langberg, BS; Amar Sahay, PhD

New neurons are continuously generated throughout life in the hippocampus. Recent evidence argues for a role of adult hippocampal neurogenesis in pattern separation, a cognitive process by which we distinguish between similar experiences and events. Given the crucial role of hippocampus in cognition and emotion, we hypothesized that pattern separation may represent a mechanism to distinguish between threatening and safe environments. Importantly, a failure in such a mechanism may contribute to increased generalization of fear as seen in post-traumatic stress disorder. Using a recently established genetic system by which we can inducibly enhance adult hippocampal neurogenesis and pattern separation, we found that increasing the number of adult-born neurons exerts widespread changes in hippocampal connectivity. Next, we sought to identify neural pathways by which encoding functions of adult-born neurons link with extra-hippocampal circuits that subserve fear responses. Combining immediate early gene based analysis of neural activation during a contextual fear discrimination learning task, we identified a CA3-dorsolateral septum (DLS) pathway as a candidate pathway that responds to ambiguity. Optogenetic dependent light-silencing of this non-canonical neural pathway enhanced the discrimination of fear without interfering with innate anxiety. These ongoing studies argue for a prominent role of a non-canonical extrinsic CA3 pathway for rapid transfer of pattern separation computations by adult-born neurons to fear circuits to generate context-appropriate behavioral responses. Collectively, our results provide new insights on how adult-born neurons influence intrinsic and extrinsic hippocampal activity to link cognition with emotion.

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Poster Number 86

Inhibiting the purinergic receptor P2x7 suppresses spreading depolarization

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Spreading depolarization (SD) is a wave of neuroglial depolarization. It is the electrophysiologic event underlying migraine, and ischemia-triggered SDs are associated with neurological deterioration in patients after stroke or trauma. P2x7 is a purinergic receptor on neurons and astrocytes affecting cerebral excitability and neuroinflammation. It facilitates the release of glutamate and potassium, with potential relevance to SD susceptibility and nociception. P2x7 exhibits two possible conformations depending on its activation status – a channel form selective for small cations like calcium, and, upon sustained stimulation with ATP, a pore form permeable to molecules up to 900 Da. We hypothesized that p2x7 antagonists suppress SD and consecutive inflammasome activation in rats. Here we show that p2x7-antagonists selectively targeting the pore form (brilliant blue FCF) or both channel and pore form (brilliant blue G and A438079) inhibit cortical SD frequency by 32-41% upon topical continuous 1M KCl ($p<0.05$). Similarly, A438079 reduces the number of SDs provoked by electrical stimulation (800μC stimulus every 6 min.; 3.9±1.3 vs. 6.0±2.1 in controls, $p=0.03$). Treatment with A438079 also reduces SD-triggered IL-1β production by two-third ($p=0.03$), when assessed by quantitative reverse-transcriptase polymerase chain reaction. In contrast, selectively inhibiting the channel form of p2x7 by calmidazolium did not affect SD susceptibility when administered intracerebroventricularly or topically. None of the antagonists altered the electrical threshold for CSD induction. We conclude that p2x7 blockade inhibits re-initiation of SD and consecutive inflammasome activation, without affecting the electrical threshold for SD induction. The potential of p2x7 antagonists as therapeutics suppressing SD deserves exploration.

NEUROSCIENCES

Poster Number 87

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3-dimensional Blood-Brain-Barrier Model in Microfluidics for Study of Neurodegeneration

Investigators: Hansang Cho, PhD

Blood–brain barrier (BBB) in vivo models are too complex to implement and current in vitro models have limited functionality. Here, our platform technology accomplishes for the first time 3-dimentional (3D) in vitro BBB model, which enables physiologically relevant studies, real-time imaging of penetration of blood cells, and easy integration with other components. The tightness of our BBB was validated by imaging localized membrane proteins: VE-cadherin and ZO-1 along cellular boundary and monitoring the reduced transmigration of innate immune cells, neutrophils through our BBB in a real time along a gradient of IL-8 presented through the BBB. The tight sealing of our BBB model could be disrupted under the treatment of TNF-α and an ischemia condition, turning into the uniform distribution of ZO-1 proteins, which represented the breakdown of the BBB. We envision that our model can easily add more functionality including the capability of co-culturing other brain cells for the understanding on neuro-diseases and screening drug candidates.

Poster Number 88

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Hypercapnia evokes amygdalo-thalamic and brainstem activity: A 7-Tesla fMRI study in healthy humans

Investigators: Karleyton C. Evans, MD, MSc; Boris Keil, PhD; Donald G. McLaren, PhD; Marta Bianciardi, PhD; Jared P. Zimmerman, BS; Tian-Yue Song, BS; Michael J. Gustin; Christina Triantafyllou, PhD

The neural mechanisms underlying reflex breathing and anxiety responses to hypercapnia (elevated end-tidal partial pressure of carbon dioxide (PCO2)) remain poorly understood. The present ultra-high resolution BOLD-fMRI study sought to examine neural responses to hypercapnia in candidate subcortical and brainstem structures.

Eight healthy subjects breathed spontaneously while inspired CO2 was systematically changed to yield 40 s conditions of elevated PCO2 (~4 mmHg and ~9 mmHg > resting PCO2) alternating with an 80-120 s baseline condition (1 mmHg < resting PCO2). Subjects underwent two hypercapnic trials in a Siemens 7-Tesla system using the following parameters: N scans/TE/TR/GRAPPA=296/25 ms/2500 ms/3, coronal slices centered on the brainstem and 1.5 mm3 voxels. Image pre-processing included the removal of cardiac and respiratory motion artifacts (RETROICOR) and standard SPM8 processing techniques. PCO2 was convolved with a hemodynamic response function to serve as the primary regressor in image analyses.

Group image analysis identified significant BOLD signal increases in the bilateral medial-dorsal thalamus, amygdala and brainstem ($p > 0.05$, FWE small volume corrected) during hypercapnia. Brainstem activity was localized to ventrolateral foci at the ponto-medullary junction, in close proximity to the Bötzinger/pre-Bötzinger complex (Böt/pre-BötC).

The high spatial resolution conferred by 7-Tesla fMRI enabled the first in vivo identification of the BötC/pre-BötC brainstem respiratory nuclei (only recently identified in humans via post-mortem histological staining). The findings also provide evidence for a distributed amygdalo-thalamic and brainstem chemosensitive network. Thus, we speculate that the amygdala plays a significant role in the modulation of reflex breathing and affective responses to hypercapnia.

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Poster Number 89

Non-monotonic pharmacodynamic model for the high-frequency oscillations induced by ketamine.

Investigators: Francisco J. Flores, PhD; ShiNung Ching, PhD; Katharine Hartnack, BSc; Patrick Purdon, PhD; Matthew A. Wilson, PhD; Emery N. Brown, MD, PhD

The actions of anesthetic drugs are usually associated with the instantaneous power of the brain electrical activity using monotonic pharmacodynamic (PD) functions. This approach works well when the time course of the instantaneous power has a single rise and decay phase. However, ketamine produces a high-frequency oscillation (HFO) between 120 and 160 Hz, which completely disappears during ketamine-induced loss of consciousness. The HFO therefore exhibits a biphasic profile in its instantaneous power, which cannot be explained by monotonic PD functions. Therefore, we derive a non-monotonic PD function, which we term Difference Interaction Model. In this model, the HFO is produced by NMDA receptor (NMDAR) action of ketamine, which negatively interact with the non-NMDAR actions of the drug that are responsible for loss of consciousness. To test the model, we recorded local field potentials across the cortical surface of freely behaving rats, during the injection of anesthetic and subanesthetic doses of ketamine. We show that the Difference Interaction Model fits the instantaneous HFO power at both doses of ketamine, even when the negative effect is lesser at the subanesthetic dose. The model suggests that the instantaneous power of the HFO is attenuated by the non-NMDAR actions of ketamine, which become more prominent at the concentrations relevant to produce loss of consciousness. The model also provides a general framework to study complex neurophysiological endpoints produced by drugs with multiple pharmacological actions.

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Poster Number 90

Synaptic dysfunction and early neuropathology in the mouse model of Mucolipidosis type IV

Investigators: Yulia Grishchuk, PhD; Weiyuan Ma, PhD; Nikita Rudinskiy, PhD; Sarmi Sri, MS; Matthew Cottle, BS; Katherine Stember, BS; Ellen Sapp, BS; Marian Difiglia, PhD; Rebecca Betensky, PhD; Bradley Hyman, MD, PhD; Raymond Kelleher, MD, PhD; Jonathan Cooper, PhD; Susan Slaugenhaupt, PhD

Mucolipidosis type IV (MLIV) is an autosomal-recessive disease with severe cognitive impairment, motor decline and progressive loss of vision. It is caused by mutations in MCOLN1 gene, which encodes a lysosomal/late endosomal cation channel TRPML1. The mechanism of how loss of TRPML1 leads to severe CNS pathology remains currently unknown and there is no therapy. Examination of the MLIV mouse model (TRPML1 knock-out mouse) revealed the first signs of motor and cognitive decline at the age of 2 months using open field testing. These early behavioral phenotypes were accompanied by activation of astrocytes and microglia and dysmyelination. Histopathological analysis showed no changes of volume in the cortex, striatum, thalamus and hippocampus and neuronal loss in Mcoln1-/- brains even at the late stage of the disease. Evaluation of resting Ca2+ activity by multiphoton imaging of a genetically encoded calcium sensor in the 3 month-old Mcoln1-/- brains in vivo showed no difference between knockouts and littermate controls, implying normal calcium homeostasis at this age. Evaluation of the synaptic transmission and plasticity in the Schaeffer collateral pathway revealed elevated paired-pulse facilitation (PPF) and long-term potentiation (LTP) in Mcoln1-/- hippocampi. Ultrastructural analysis revealed changes in synaptic morphology, reduced thickness of myelin sheaths and accumulation of abundant aggregates of inclusion bodies. Altogether, here we report for the first time synaptic dysfunction and cognitive deficits associated with TRPML1 loss and the early involvement of glial cells in MLIV disease pathophysiology, suggesting a new avenue towards the development of therapies for this devastating disease.

NEUROSCIENCES

Poster Number 91

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Molecular re-engineering of excitation-inhibition balance in memory circuits to reverse cognitive impairments

Investigators: Nannan Guo, PhD, Paoyan Lin, MD; Amar Sahay, PhD

Episodic memory formation requires a balance of two distinct mnemonic processes, pattern separation and pattern completion in the dentate gyrus (DG)-CA3 circuit of the hippocampus. Whereas, pattern separation in DG is essential to distinguish between similar experiences by minimizing interference, pattern completion in CA3 facilitates the retrieval of memories based on partial cues. Rodent studies suggest that pattern separation-completion balance is disrupted in aging and this manifests as inflexible remapping and elevated firing of place cells in CA3. Interestingly, aged humans and individuals with mild cognitive impairment also show increased activation of CA3 and excessive pattern completion. Analysis of DG-CA3 wiring suggests a role for filopodia on DG mossy fiber terminals (MFTs) that modulate levels of feed-forward inhibition (FFI) and MFTs onto CA3 neurons that mediate feed-forward excitation (FFE). Here, we hypothesize that reduced FFI and increased FFE underlies excessive pattern completion and memory imprecision in aging. Screening for regulators of synaptic connectivity, we identified a factor that we refer to as Negative Regulator of MFT Filopodia (NeRF). Extensive localization and expression analysis suggests that NeRF acts as molecular brake on MFT structural plasticity and gates learning induced changes in FFI. Viral-based bidirectional modulation of NeRF levels was sufficient to increase FFI or FFE in DG-C3 circuitry. Importantly, down regulation of NeRF in mature dentate granule neurons in aged mice reinstated FFI, restrained pattern completion and restored memory precision. These studies identify a novel molecular mechanism by which age-related changes in connectivity can be reversed to restore memory precision.

Poster Number 92

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Transplantation of enteric neuronal precursors for the treatment of Hirschsprung disease

Investigators: Sarah Miller; Alyssa Thomas; Weihua Pan, MD, PhD; Jaime Belkind-Gerson, MD; and Allan M. Goldstein, MD

Purpose: Cell therapy offers a potential treatment to replace enteric neurons missing in children with congenital colorectal aganglionosis, Hirschsprung disease, which affects 1 in 5000 newborns. We tested whether enteric neuronal stem/progenitor cells (ENSPC) can survive, migrate, and differentiate into neurons and glial cells following transplantation in vivo.

Methods: ENSPC were isolated from dissociated postnatal colon of Actb-DsRed mice in which all cells express red fluorescent protein (RFP). RFP+ ENSPC were expanded in culture and transplanted microsurgically into ganglionic and aganglionic rectum of wild-type and Ednrb-/- mice, respectively. Recipient mice (n=15, 11 wild-type and 4 Ednrb-/- mice) were sacrificed 1-2 weeks following surgery and the distal colon examined immunohistochemically.

Results: After 7 days in culture, ENSPC formed neurospheres containing cells immunoreactive to neural crest (P75), enteric neuronal (Ret), and neuronal stem cell (Nestin) markers. These cells retained the ability to form neurospheres after multiple passages and gave rise to neurons and glial cells in vitro. A subset of TuJ1+ neurons exhibited immunoreactivity to neuronal subtypes expressing nitric oxide synthase (NOS) or choline acetyltransferase (ChAT). Following transplantation, ENSPC survived, with cells migrating longitudinally along the myenteric layer and extending neuronal processes. Transplanted cells gave rise to enteric neurons and glial cells in vivo, including NOS+ and ChAT+ neurons.

Conclusions: Transplanted ENSPC can give rise to neurochemically appropriate enteric neurons in the aganglionic gut in vivo. Current studies aim to establish whether these cells can improve gut motility, laying the groundwork for cell-based therapy for the treatment of Hirschsprung disease.

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Poster Number 93

Brain-Network Reconfiguration and Perceptual Decoupling during Trance

Investigators: Michael Hove, PhD

Trance is a state of consciousness characterized by narrowed awareness of external surroundings and long used by shamans to gain valued insight. Shamans often induce trance by listening to rhythmic drumming. However, little is known about the underlying brain mechanisms. Using fMRI, we examined the brain networks associated with trance. Experienced shamanic practitioners (n=15; average shamanic experience = 9.3 years) listened to rhythmic drumming with their eyes closed, and either entered a trance state or remained in a non-trance state, during 8-minute runs. We analyzed network functional connectivity using a data-driven approach called eigenvector centrality mapping, and a seed-based approach with seeds placed in the default network and the auditory pathway. Participants reported a robust trance state in the scanner. fMRI results revealed that trance was associated with higher Eigenvector Centrality (i.e., stronger hubs) in three distinct regions: posterior cingulate cortex (PCC), anterior cingulate cortex (ACC), and left insula/operculum. Seed-based analysis revealed co-activation of the PCC seed (a key 'default' network hub involved in internally-oriented states) and ACC and insula (key regions of the 'salience' network important for selecting and amplifying relevant neural streams). Thus an internally-oriented neural stream could be amplified by the salience network. Additionally, during trance, seeds in the auditory brainstem and auditory cortex were less connected, suggesting perceptual decoupling and suppression of the monotonous auditory stimuli. In sum, trance involved co-active default and salience networks, and dampened sensory processing, perhaps allowing an extended internal train of thought wherein integration and moments of insight can occur.

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Poster Number 94

Wireless MR miniature coil based brain PET motion correction in simultaneous PET-MR

Investigators: Chuan Huang, PhD; Jerome L. Ackerman, PhD; Yoann Petibon, MS; Marc D. Normandin, PhD; Georges El Fakhri, PhD, DABR; Jinsong Ouyang, PhD

Brain Positron Emission Tomography (PET) plays an important role in the diagnosis, prognostication and monitoring of many brain diseases. Artifacts from both voluntary and involuntary head motion are one of the major challenges in brain PET. We investigated the use of wireless magnetic resonance (MR) miniature coils to track head motion during simultaneous PET-MR brain scans and incorporate the measured motion fields in the list-mode PET reconstruction to remove the motion artifacts.

Several wireless MR miniature coils (diameter < 5mm) with sealed doped water samples inside, and a dedicated tracking MR pulse sequence were built. Data were acquired on a phantom containing multiple spheres with varying size (to mimic brain lesion) and a non-human-primate with and without motion. Motions were measured with the miniature coils using the MR sequence; each measurement takes less than 12ms. List-mode PET reconstruction was performed on the motion PET data with and without motion correction. Static PET images were acquired as gold standard.

Here we show the motion artifacts, which were prominent without motion correction, were eliminated by the miniature coil based motion correction in both the phantom and the monkey experiments. Quantitatively, the motion correction reduced the PET contrast (similar to specific uptake value) bias of the spheres by 17% to 64%. The channelized Hotelling observer signal-to-noise-ratios of the spheres were improved by 1.2 to 6.9.

The proposed wireless MR miniature coil based motion correction removes motion artifacts in brain PET images and yields accurate quantitative values from data acquired in simultaneous PET-MR scanner.

NEUROSCIENCES

Poster Number 95

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Gene transfer of Human APOE isoforms differentially modulates the course of Alzheimer's disease in transgenic mouse models

Investigators: Eloise Hudry, PhD; Jonathan Dashkoff, BS; Alysson D. Roe, BS; Shuko Takeda, MD, PhD; Robert M. Koffie, MD, PhD; Tadafumi Hashimoto, PhD; Maria Scheel, BS; Michal Arbel-Ornath, PhD; Rebecca Betensky, PhD; Beverly L. Davidson, PhD; Bradley T. Hyman, MD, PhD

Inheritance of the ε4 allele of apolipoprotein E (APOE) is the strongest genetic risk factor associated with the sporadic form of Alzheimer's disease (AD), whereas the rare APOE ε2 allele has an opposite effect. However, the mechanisms whereby APOE confers risk and protection remain uncertain. We hypothesize that APOE isoforms differentially influence soluble species of amyloid β (Aβ) in the interstitial fluid (ISF), which have been implicated in plaque-associated neuritic destruction and synaptic loss, as well as stabilization of amyloid aggregates. Using a gene transfer approach to bathe the cortex in virally-expressed APOE, we monitored Aβ with longitudinal multiphoton imaging, in vivo microdialysis, and post mortem array tomography to study in detail the kinetics of APOE mediated changes in Aβ-related neurotoxicity. We observe that APOE4 increases the levels of oligomeric Aβ species within the ISF and exacerbates plaque deposition, which was reversed by exposure to APOE2. Peri-plaque synapse loss and neuritic dystrophies around individual plaques were also worsened by APOE4 or attenuated by APOE2. Surprisingly, we find that egress of Aβ from the CNS into the plasma is diminished by APOE3 and APOE4 compared to APOE2, in accord with accumulation of Aβ in the CNS. Overall, our data argue for a profound differential effect of APOE isoforms on both amyloid deposition and clearance, and, more importantly, on Aβ mediated synaptotoxicity. These results suggest that APOE genetic risk is mediated via Aβ, and that therapeutic approaches aiming at decreasing APOE4 or increasing APOE2 may be beneficial even in established AD.

Poster Number 96

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Towards improved methods of electric stimulation

Investigators: Maesoon Im, PhD; Shelley I. Fried, PhD

For improved retinal prosthetics, it is important to understand the relationship between the stimulation parameters and the resulting neural activity. Presumably, the ability to create spiking patterns that match the light responses for each type of retinal ganglion cell (RGC) would create the highest quality vision. Surprisingly, the similarities between light- and electrically-elicited response patterns have not been well studied. Here, we measured the response patterns elicited by epiretinal electric stimulation in RGCs and explored correlations between the electrically- and the light-elicited spikes.

We found that electric response patterns had clear distinctions across cell types. ON brisk transient (BT) cells always (n=18/18) contained three or more bursts of spikes while ON brisk sustained (BS) cells typically (n=13/17) responded with two bursts. Both ON types generally showed strong correlations between electrically- and light-elicited responses in terms of strength and timing. In contrast, the electric response patterns from OFF cells did not neatly differentiate into two distinct groups. OFF cells (n=46) had poorer correlations to light responses than did ON cells. There were further differences between ON and OFF cells when the responses to repetitive stimulation were examined: each new stimulus strongly suppressed responses to previous stimuli in ON cells. This unique behavior of ON cells created differences between ON and OFF cells in response to repetitive stimulation with various inter-stimulus intervals.

Our results indicate that electric stimulation elicits responses that closely match physiological responses in ON but not OFF cells.

Poster Number 97

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A Combined FDG-PET and fMRI Based Investigation of Brain Arousal Control

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In the United States, more than 60,000 patients receive general anesthesia daily to safely undergo most surgical and many non-surgical procedures. Despite the central role of anesthesiology in modern healthcare, the mechanisms underlying general anesthesia are still considered a major mystery of modern medicine. In humans, the prevalent approaches to studying the mechanisms of action of anesthetics have been instrumental in identifying the common molecular and pharmacological principles that underlie anesthetic drugs. However, these molecular and pharmacological studies do not precisely inform us on how anesthetics alter arousal.

Hence, our approach was to use a novel functional imaging technique (integrated PET/MR imaging) to address this fundamental question. This technique allowed us to simultaneously estimate cerebral glucose metabolism, cerebral blood flow, and alterations in resting state networks (cortical and subcortical) in healthy volunteers, either in the awake state, or in an anesthesia (dexmedetomidine) induced altered arousal state. Or results show that altered arousal was associated with reduced flow as well as glucose metabolism in the thalamus, and in regions belonging to the Default Mode, and the Frontoparietal Networks.

Importantly the use of the integrated PET/MR also allowed us to image and describe flow-metabolism coupling in the human brain, an investigation that previously has never been accomplished using simultaneous recording of flow and metabolism. In summary, our study offers multi-modal derived insights into the neural circuit mechanism of anesthesia. In so doing, we present a new fundamental understanding of brain arousal control.

NEUROSCIENCES

Poster Number 98

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A novel ontogenetic phospho-switch of GABA activity during brain development

Investigators: Kristopher T. Kahle, MD, PhD; Perrine Friedel, PhD; Liliya Silayeva, PhD; Stephen J. Moss, PhD; Igor Medina, PhD; David E. Clapham, MD, PhD

GABA is the main inhibitory neurotransmitter in the adult central nervous system, exerting its fast synaptic hyperpolarizing effect via the activation of ligand-gated, Cl--permeable GABAA receptors (GABAARs). However, in developing neurons, GABAAR-mediated responses are often depolarizing and excitatory, which is critical for Ca2+-dependent neuronal proliferation and migration. This so-called GABA "developmental switch" has been attributed to an increased functional expression of the K+-Cl- co-transporter KCC2 (mediating Cl- efflux) in adult relative to immature neurons, but the mechanisms triggering KCC2 activation in this context are unknown. Here, we show WNK1/HSN2 kinase, mutated in a Mendelian form of congenital pain insensitivity, interacts with KCC2 in vivo and inhibits KCC2-mediated Cl- extrusion in immature but not adult neurons by promoting the inhibitory phosphorylation of KCC2 at a C-terminal di-threonine (Thr) phospho-switch (Thr906/Thr1007). shRNA knockdown, dominant-negative over-expression, or chemical genetic inhibition of WNK1/HSN2 in immature but not adult neurons stimulates KCC2 activity by promoting KCC2 Thr906/Thr1007 dephosphorylation. In utero silencing of WNK1 activity is sufficient to shift the depolarized GABA equilibrium potential of the developing rodent brain to more hyperpolarized values by relieving inhibitory T906/T1007 phosphorylation. Accordingly, in vivo KCC2 T906 phosphorylation in cortex shows a dramatic > 50-fold reduction from birth to adulthood. These data reveal the ontogenetic change in GABAAR-mediated responses from depolarizing to hyperpolarizing is coupled to a developmental induction of KCC2 activity via relief of tonic WNK1/HSN2-mediated inhibitory phosphorylation, and identify a novel druggable kinase pathway capable of modulating endogenous GABA function, with implications for diseases featuring neuronal hyperexcitability.

Poster Number 99

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Striatal Stimulation for Enhancement of Recovery in a Rodent Traumatic Brain Injury Model

Investigators: Husam A. Katnani, PhD; Joshua P. Aronson, MD; Jimmy Yang, BA; Emad N. Eskandar, MD

Behavioral and physical therapy after traumatic brain injury (TBI), to rehabilitate cognitive and motor function, is limited by lack of information optimizing neural recovery at the cellular and systems level. The striatum plays a critical role in learning and motivation, which are key aspects of recovery from TBI. We hypothesized that intermittent stimulation of the ventral striatum could enhance behavioral performance in a rodent TBI model. Forty-eight C57BL/6 mice were used in this study. Thirty-two mice underwent right parietal craniotomy and controlled cortical impact over the right parietal cortex and hippocampus. Sixteen mice underwent craniotomy but did not undergo cortical impact (Control Group). Seven days later, electrodes were implanted in all mice ipsilateral to the TBI, with the contact in the Nucleus Accumbens (NAcc). Mice were tested in the Morris Water Maze for five consecutive days. Sixteen mice received 5 seconds of 50 μA of biphasic stimulation (Stimulation group) upon finding the platform (130 Hz, 160 μs pulse width) and sixteen did not receive any stimulation (Sham group). All mice demonstrated decreased escape latency over a 5-day testing period. Mean latencies at days 3-5 was significantly improved ($p<0.01$) in the Stimulation group as compared with the Sham group. Percentage improvement from days 1 to 5 was 63.5±4.4% (±SE) in the control group, 46.1±3.2% in the stimulation group, and 27±4.2% in the sham group. Intermittent stimulation of the NAcc can enhance behavioral performance in a rodent model of TBI, with a significant improvement in performance compared to non-stimulated TBI control mice.

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Poster Number 100

Mass spectrometry analysis of phospholipids from wild-type and knock-in Q140/Q140 Huntington's disease mouse brains

Investigators: Karin Green, PhD; Ellen Sapp; Antonio Valencia, PhD; Hollis McClory; Ghazeleh Sadri-Vakili, PhD; Neil Aronin, MD, PhD; Scott Shaffer, PhD; Marian DiFiglia, PhD; Kimberly B. Kegel-Gleason, PhD

Huntington's disease (HD) is a neurodegenerative disease caused by a CAG expansion in the HD gene, which encodes the protein Huntingtin. Huntingtin is a membrane-associated protein that can interact directly with phospholipids. Microarray studies from several groups have identified changes in mRNA levels for genes that regulate phospholipid and sterol metabolism. Although early experiments in human tissue implicated changes in membrane phospholipids, little is known about their role in HD. Previously, we showed that full-length mutant Huntingtin from brain of a knock-in HD mouse model (Q140/Q140) had altered association with phospholipids compared to wild-type, with increased binding to the same phospholipids or promiscuous binding to additional phospholipids. Here we analyzed lipid profiles from brains of wild-type and Q140/Q140 HD knock-in mice (11 months, male, 3 per group). Polar lipids from cerebellum, cortex, and striatum were extracted and analyzed by liquid chromatography and tandem mass spectrometry analysis (LC/MS/MS). Small changes were measured between wild-type and HD samples for numerous lipid groups in all three regions of the brain. In particular, levels of specific phosphatidic acid (PA) species were changed with more species being changed in cortex and striatum than cerebellum. Moreover, mRNA or protein levels for two enzymes that synthesize phosphatidic acid were changed in striatum of HD mice compared to wild-type. These novel results identify a potential site of molecular pathology caused by mutant Huntingtin that may impart early changes in HD and underscore the need for complete lipidomic analysis in HD.

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Poster Number 101

Identification and rescue of α-Synuclein toxicity in Parkinson patient iPS cell-derived neurons

Investigators: Vikram Khurana, MBBS, PhD; Chee-Yeun Chung, PhD; Pavan K. Auluck, MD, PhD; Daniel F. Tardiff, PhD; Joseph R. Mazzulli, PhD; Frank Soldner, MD; Sukhee Cho; Alison E. Mungenast, PhD; Nathan T. Jui, PhD; Birgitt Schüle, MD; Stephen L. Buchwald, PhD; Rudolf Jaenisch, MD; Susan Lindquist, PhD

The induced pluripotent stem (iPS) cell field promises a new era for in vitro disease modeling, but has been hindered to date by the absence of adequate controls and inability to identify robust innate pathologic phenotypes. Here, we utilize mutation correction of iPS cells and the extraordinary conservation of proteotoxic mechanisms from yeast to human to discover and reverse conserved phenotypic responses to α-Synuclein (αSyn), a key protein involved in Parkinson's disease (PD). We generated cortical neurons from iPS cells of patients harboring αSyn mutations, who are at particularly high risk of developing PD dementia. Genetic modifiers from unbiased screens in a yeast model of αSyn toxicity led to the identification of early pathogenic phenotypes in patient neurons compared to mutation-corrected controls. These included nitrosative stress, accumulation of ER-associated degradation (ERAD) substrates and ER stress. A small molecule identified in a yeast screen, and the ubiquitin ligase Nedd4 it activates, reversed pathologic phenotypes in these neurons. Our approach establishes a functional genomics and small molecule probe discovery platform for neurodegenerative diseases.

NEUROSCIENCES

Poster Number 102

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Platelet TSC1 is an Important Mediator of Thrombus-Mediated Ischemic Stroke

Investigators: Yong-Joo Ahn, MD; Min Ju Kim, MD; Eo Jin Kim, MD, PhD; Maya H. Kim, BS; Hyung-Hwan Kim, PhD

Background: The activation of platelets is the final common pathway for most ischemic strokes. The tuberous sclerosis complex 1 (TSC1) are one of important mediators of the actin cytoskeleton. Because changes in the actin cytoskeleton underlie platelet activation and aggregation, we hypothesize that TSC1 in platelets may play an important role in thrombus-mediated ischemic stroke.

Methods and Results: Platelet specific TSC1-deficient (TSC1Plt–/–) mice showed increased platelet size compared to those of WT mice. To determine the role of platelet TSC1 in thrombosis-mediated ischemic stroke, we generated a mouse strain with platelet-specific TSC1 deficiency (TSC1Plt–/–) using conditional TSC1flox/flox mice and platelet-specific PF4-Cre mice. One day before ischemic onset, arterial blood was withdrawn from donor mice (WT or TSC1Plt–/– mice), into PE-50 tubing, stored at room temperature for 2 h, and then kept at 4 °C for 22 h. Middle cerebral artery (MCA) occlusion was induced by injection of the coagulated blood from donor mice (WT or TSC1Plt–/–) into recipient WT mice. Following clot injection, an embolus of varying size was found in the proximal MCA of all mice at 24 h. Compared to WT→WT mice, TSC1Plt–/– →WT exhibited greater improvement in relative cerebral blood flow. This correlated with decreased cerebral infarct volume (28.3 ± 14.1 mm3 (TSC1Plt–/– →WT) vs. 100.1 ± 24.4 mm3 (WT→WT), $p<0.05$, $n=7$) and improved neurological deficit score (1.5 ± 0.3 (TSC1Plt–/– →WT) vs. 3.0 ± 0.4 (WT→WT), $p<0.05$, $n=7$).

Conclusion: These finding indicate that TSC1 in platelets is critical for thrombus formation and thrombus-mediated ischemic stroke.

Poster Number 103

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Identification of novel effectors of excitatory synapse scaling by high-throughput RNAi screening

Investigators: Jasmin Lalonde, PhD; Surya A. Reis, PhD; Thomas Nieland, PhD; Wen-Ning Zhao, PhD; Stephen J. Haggarty, PhD

To prevent instability, neuronal circuits must keep a tight control over excitation and inhibition. To achieve this, neurons have bidirectional homeostatic mechanisms that allow them to adjust their membrane excitability, postsynaptic neurotransmitter receptor expression, and/or presynaptic neurotransmitter release. Using these forms of homeostatic plasticity, neuronal cells can counterbalance the destabilizing influence of other types of synaptic plasticity and maintain this way their activity within a stable firing range.

In recent years, significant progress has been made to identify conditions and treatments that promote homeostatic adaptations; however, our molecular understanding of these distinct mechanisms remains limited. Interestingly, although there is currently no direct association between a dysfunction in homeostatic plasticity and the pathophysiology of brain disorders like schizophrenia and autism, emerging evidence suggest that the function of several susceptibility genes implicated in the etiology of these impairments may be related to homeostatic responses. At this point, there is thus a need to systematically assess the contribution of these genes to this form of neuroplasticity.

Here, we present a high-throughput RNAi screening strategy designed to evaluate the requirement of individual genes to BDNF-induced downscaling of excitatory synapses—a form of homeostatic response that involves the activity-dependent immediate-early gene Arc/Agr3.1. For this study, we are screening an shRNA library targeting 615 distinct genes that are functionally related to actin cytoskeleton dynamics, synapse development, calcium signaling, and the etiology of various brain disorders (ex. schizophrenia, Alzheimer's disease). Our analysis represents the most extensive screening effort to date to identify novel factors implicated in synaptic downscaling.

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Poster Number 104

Klf9 is a novel transcriptional regulator of resilience to chronic stress

Investigators: Antoine Besnard, PhD; Tomer Langberg, BS; Sally Levinson, BS; Kimberly M. Scobie, PhD; Rene Hen, PhD; Amar Sahay, PhD

Major depressive disorder (MDD) affects multiple neural circuits subserving anhedonia, encoding, and stress-coping behaviors and has greater prevalence in women. Although MDD arises from an interaction of genes that moderate vulnerability and environmental factors such as stress, how these factors converge upon neural circuitry to alter behavior is poorly understood. Identification of molecular mechanisms that moderate the effects of stress on neural circuits to influence behavior may catalyze the generation of novel antidepressants. Here, we identify Kruppel-like factor 9 (Klf9) as a novel regulator of synaptic connectivity that moderates the effects of chronic stress on depression-like behaviors. Previous work showed Klf9 expression to be upregulated by glucocorticoids and in the hippocampus of patients with MDD. Using novel transgenic tools by which Klf9 expression can be inducibly and reversibly silenced in the forebrain, we investigated the effects of Klf9 down-regulation on depression-like behaviors in a mouse model of chronic restraint stress (CRS). Silencing Klf9 in adulthood did not affect contextual encoding or depression-like behaviors at baseline, but produced antidepressant-like behavioral responses in the sucrose preference and forced-swim tests and prevented stress-induced enhancement of fear memory following CRS. Interestingly, these effects were only seen in females and not males, mirroring Klf9 silencing dependent-reversal of CRS induced changes in dendritic spines in ventral CA1. Furthermore, Klf-9 downregulation blunted the corticosterone response to an acute stressor challenge in CRS female mice. Together, these observations suggest that Klf9-silencing promotes resilience to chronic stress and targeting Klf9 may harbor therapeutic potential for MDD.

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Poster Number 105

Suppression of Subthalamic Nucleus Neurons by Microcoil-Induced Magnetic Stimulation

Investigators: Seung Woo Lee, PhD; Shelley I. Fried, PhD

Magnetic stimulation delivered via 0.5 mm diameter coils was recently shown to activate retinal neurons; the small coil size raises the possibility that micro-magnetic stimulation (μMS) could underlie a new generation of implanted neural prosthetics. Such an approach has several inherent advantages over conventional electric stimulation, including the potential for selective activation of neuronal targets as well as less susceptibility to inflammatory responses. The viability of μMS for some applications, e.g. DBS, may require suppression (rather than creation) of neuronal activity however and therefore, we explored here whether (μMS) could in fact suppress activity. While single pulses elicited weak and inconsistent spiking in neurons of the mouse sub-thalamic nucleus (in vitro), repetitive stimulation effectively suppressed activity in ~70% of targeted neurons. This is the same percentage suppressed by conventional electric stimulation; with both modalities, suppression occurred only after an initial increase in spiking. The latency to the onset of suppression was inversely correlated to the energy of the stimulus waveform: larger-amplitudes and lower frequencies had the fastest onset of suppression. These findings support the viability of μMS as a next-generation implantable neural prosthetic.

NEUROSCIENCES

Poster Number 106

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Polyglutamine Expansion in Huntingtin Disturbs the Function of a Complex containing rabGEF TRAPP and rac1GEF kalirin

Investigators: Hollis McClory, BA; Mary McKee, BA; Ellen Sapp, BA; Antonio Valencia, PhD; Marian DiFiglia, PhD; Xueyi Li, PhD

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a mutation in huntingtin. Exactly how mutant huntingtin causes neuropathology is still under exploration. We demonstrate in a series of studies that the HD mutation impairs endosomal recycling by compromising a factor called guanine nucleotide exchange factor (GEF) for activating rab11, a GTPase necessary for sustaining the homeostatic abundance of receptors and transporters on the cell surface. HD cells display deficient production of small vesicles for transporting endocytosed proteins to cell surfaces. We found that inadequate activity of rab11 is a basis for oxidative stress and reduced glucose utilization in HD neurons. We and others have shown that rab11 enhancers may be effective in treating HD. Critical to developing rab11-targeted therapy for HD is the identification of the rab11GEF that was compromised in the context of HD. Here we describe a rab11-interacting protein complex that contains huntingtin, Transport Protein Particle (TRAPP), a rab1GEF and a debated rab11GEF, and kalirin, a rac1GEF. TRAPP isolated from 293T cells expressing 6xHis-tagged Trappc4 (a TRAPP subunit) activated rab11, but not rab5. Cryo-immunogold labeling detected co-distribution of Trappc4 with rab11 and/or with kalirin at tubulovesicular membranes. We showed that huntingtin and Trappc4 bound to different domains in kalirin. Polyglutamine expansion in huntingtin altered the interaction of huntingtin with kalirin and reduced the activity of anti-kalirin precipitated protein complex in activating rab11 and rac1. Our data suggest that a complex containing huntingtin, kalirin, and TRAPP activates rab11 and rac1, and is disturbed by mutant huntingtin.

Poster Number 107

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Alternative Approaches for PET Radiotracer Development in Alzheimer's Disease: Imaging Beyond Plaque

Investigators: Steven Liang, PhD; Jason Holland, PhD; Benjamin Rotstein, PhD; Nicheisha Stephenson, PhD; Timothy Shoup, PhD; Lee Collier, PhD; Keith Johnson, MD; Thomas Brady, MD; Neil Vasdev, PhD

Alzheimer's disease (AD) and related dementias show increasing clinical prevalence, yet our understanding of the etiology and pathobiology of disease-related neurodegeneration remains limited. In this regard, non-invasive imaging with radiotracers for positron emission tomography (PET) presents a unique tool for quantifying spatial and temporal changes in characteristic biological markers of brain disease, and for assessing potential drug efficacy. PET radiotracers targeting different protein markers, receptors and enzymes are being developed to address questions pertaining to the molecular and/or genetic heterogeneity of AD and related dementias. For example, radiotracers including [11C]-PiB and [18F]-AV-45 (Florbetapir) are being used to measure the density of amyloid-beta plaques in AD patients and to interrogate the biological mechanisms (such as the amyloid cascade hypothesis) of disease initiation and progression. Our focus is on the development of novel PET imaging agents, targeting proteins beyond amyloid-beta plaques, which can be used to investigate the broader mechanism of AD pathogenesis. In this presentation, we will describe the chemical basis of various radiotracers which show promise in either preclinical or clinical studies for use in evaluating either the phenotypic or biochemical basis of AD. Radiotracers for PET imaging of targets including neuroinflammation, metal ion association with amyloid-beta plaques, tau protein, cerebral amyloid angiopathy, cholinergic and cannabinoid receptors, as well as various enzymes including glycogen-synthase kinase 3beta and monoamine oxidase B amongst others, and their connection to AD will be highlighted.

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Poster Number 108

Cortical pathology is associated to proximal underlying white matter injury in multiple sclerosis: a multimodal 7T and 3T MRI study using surface based and tract based analysis

Investigators: C. Louapre, MD; S.T. Govindarajan, MSc; C. Gianni, MD; R.P. Kinkel, MD; C. Mainero, MD, PhD

Cortical damage and diffuse normal appearing white matter (NAWM) appear to be hallmarks of physical and cognitive disability in Multiple Sclerosis (MS). Neuropathological studies have provided evidence of degenerative process (demyelination, inflammation, neuronal loss) that primarily targets the cortex, independent from white matter pathology. Additionally, recent in vivo finding that showed a correlation between cortical lesion load and diffuse NAWM injury, have lead to speculate that cortical lesions are implied in NAWM pathogenesis. This question is highly relevant for clinical purposes as so far, disease-modifying therapies are targeting only the white matter component while having no effect on cortical degeneration.

The aim of our study is to analyze if cortical degeneration as assessed by cortical thickness and intracortical laminar mapping of T2* relaxation decay (acquired at 7 Tesla) is spatially associated with underlying white matter diffuse pathology as assessed by diffusion tensor imaging (DTI).

In 33 MS patients, we have found that cortical thickness decrease and intracortical T2* increase correlate with juxta-cortical white matter diffusion abnormalities, in the postcentral gyrus, supramarginal gyrus and precuneus mainly. Moreover, cortical T2* increase correlatates with diffusion abnormalies of tractographically connected white matter within the cortico-spinal tract and the cingulum, in their proximal portions closest to the cortex only.

Our results demonstrate a spatial relationship between cortex and subcortical white matter pathology at the overall brain level, and as a function of the depth from the white matter surface within white matter tracts.

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Poster Number 109

Histology studies by Optical Coherence Tomography

Investigators: Jean Augustinack, PhD; David Boas, PhD; Bruce Fischl, PhD; Caroline Magnain, PhD

Spectral domain optical coherence tomography (SD-OCT) is a three dimensional high resolution (in the order of microns) imaging technique that generates excellent contrast based on intrinsic optical properties of the tissue, such as neurons and fibers. The SD-OCT data acquisition is performed directly on the tissue block, entirely removing the need for mounting and staining, which is necessary when performing gold standard histology studies (Nissl, Gallyas...). This protocol considerably reduces the deformations often encounter in histology, such as tears, mispositioning which impair registration within modality and inter modality (with MRI for example). Here, we first demonstrate that OCT distinguishes the laminar structure in isocortex, which is qualitatively and quantitatively comparable to the what is observed with a Nissl staining protocol. The optical properties of the different layers are assessed. Then, by increasing the resolution of the OCT system to under than a micron, we performed optical histology slices by projecting the maximum intensity over 50um and registering the resulting images with the corresponding 50um-thick Nissl stained slices. We show that individual neurons are imaged and their locations closely overlapping with the ones see in the Nissl images. Given these results, we propose that SD-OCT can be used to reliably generate 3D reconstructions of multiple cubic centimeters of cortex that can be used to accurately and automatically perform standard histological analyses when coupled with our imaging rig with xyz translation stage and a vibratome.

NEUROSCIENCES

Poster Number 110

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Differential muscle recruitment using functional electrical stimulation with cuff electrodes placed around peripheral nerves

Investigators: Wasim Q. Malik, PhD; Jonathan M. Winograd, MD; Neelakantan Sunder, MD; Neil Fairbairn, MD; Robert Ajemian, PhD; Emilio Bizzi, MD, PhD; Emery N. Brown, MD, PhD

Neuroprosthetics offer a potential rehabilitative intervention for people with tetraplegia and locked-in syndrome. By using cortical activity to directly control assistive devices or natural limbs, neuroprosthetics bypass lesions in the central nervous system and provide an artificial neural pathway for downstream motor signals. A major challenge in neuroprosthetics is devising an artificial means for activating muscles in paralyzed limbs. Previously, electrodes inserted into muscles have been used, with problems related to long-term tolerance and reliability. Here we show that flexible polyimide-based cuff electrodes placed around the periphery of rodent sciatic nerves, including nerves as small as 0.65 mm in diameter, can be used for selectively activating and recruiting the gastrocnemius and tibialis muscles. The key to our approach is to have several electrical contacts spaced uniformly around the nerve, so that independent channel stimulation causes current to spread locally, thereby exploiting the anatomical segregation of fascicles occurring at that level. We show that rectangular pulses of short duration (roughly 50 microseconds) achieve better selectivity than those of longer puslewidth and shorter amplitude. Further, we establish that these cuff electrodes function in a chronic preparation exhibiting similar operating characteristics weeks after the initial implant. The demonstration of this technology in nerves of such small diameter is important as a preclinical proof-of-concept because many of the nerves innervating the human arm muscles also have small diameters. In our future work, we will connect signals from the motor cortex to independent channels of the cuff electrode for peripheral nerve stimulation in non-human primates.

Poster Number 111

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A novel method to assess basal dopamine function using simultaneous PET and fMRI

Investigators: Joseph B. Mandeville, PhD; Christin Y. Sander, BS; Jacob Hooker, PhD; Bruce Rosen, MD, PhD

New methods for non-invasive assessments of basal dopamine levels could have a major impact on our understanding of dopamine system dysfunction, which has been implicated in numerous neuropathologies. In this study, we infused graded doses of drugs that specifically target dopamine D2 receptors in non-human primates, and we simultaneously monitored changes in receptor occupancy using PET and changes in brain function using an fMRI method sensitive to changes in cerebral blood volume. We observed dose-dependent changes in PET and fMRI signals within the basal ganglia: changes in fMRI signal correlated with increases in receptor occupancy by drug. D2 antagonists and agonists elicited changes in cerebral blood volume that were positive and negative, respectively, consistent with the known negative functional effect of D2 receptor stimulation. Using an antagonist, PET and fMRI responses were roughly matched in time, consistent with a simple interpretation through a classical receptor competition model. Conversely, an agonist produced a temporal dissociation between PET and fMRI responses, implicating desensitization mechanisms like receptor internalization. For matched occupancies using an antagonist, fMRI responses in putamen were significantly larger than those observed in caudate, consistent with a physiological model in which the antagonist-induced fMRI response is a simple function of the occupancy by drug at D2 receptors and the basal occupancy by dopamine at D2 receptors. Overall, results suggest that simultaneous PET/fMRI using a D2 receptor antagonist will provide a method to non-invasively assess basal dopamine levels in human brain.

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Poster Number 112

Modulation of neural stem cell activation and neuronal competition homeostasis in the adult dentate gyrus to enhance memory precision

Investigators: Kathleen McAvoy, PhD; Kimberly M. Scobie, PhD; Stefan Berger, PhD; Nannan Guo, PhD; Hugo Vega-Ramirez, BS; Sreyan Chowdhury; Sam Miake-Lye, BA; Mark Nelson, PhD; Rene Hen,PhD; Amar Sahay, PhD

Adult hippocampal neurogenesis is a unique form of circuit-plasticity that generates new neurons in the dentate gyrus (DG) throughout life. Work by us and others have suggested a role for adult-born neurons in disambiguating similar representations, a process known as pattern separation. Previous work has shown that adult-born neurons compete with mature dentate granule neurons for synaptic inputs in an activity dependent manner to functionally integrate. Here, we developed a novel genetic system by which we reversibly eliminate a subset of dendritic spines of mature granule neurons, biasing integration robustly in favor of young adult-born neurons. Remarkably, restoration of mature dentate granule neuron dendritic spines restores neuronal competition-homeostasis and returns the numbers of integrating adult-born neurons to baseline levels. Furthermore, we found that both the neural stem cell and progenitor populations are bidirectionally sensitive to alterations in synaptic inputs onto mature dentate granule neurons, via as yet-unidentified paracrine signaling mechanisms distinct from activity-dependent competition. Harnessing our system to probe hippocampal functions, we found that genetic expansion of the reservoir of 4-8 weeks old adult-born neurons does not affect anxiety or depression-like behaviors, but enhances pattern separation in both adult and during aging. Thus, multiple feedback loops within the hippocampal stem cell lineage mediate nuanced adaptation to changes in activity of mature neurons to govern memory precision. These studies may inform therapeutic strategies to reverse pattern separation impairments in depression, anxiety disorders, and during aging by rejuvenating the DG.

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Poster Number 113

Spinal IL-33/ST2 signaling contributes to the pathogenesis of neuropathic pain by regulating astroglial JAK2-STAT3 and neuronal CaMKII–CREB cascades

Investigators: Shenbin Liu, BA; Ping Han, PhD; Jing Zhao, MA; Wenli Mi, PhD; Yanqing Wang, PhD ; Jianren Mao, MD

The inability to reverse chronic pain is due in part to lack of knowledge of its underlying mechanism. Interleukin-33 (IL-33), a novel member of IL-1 family, has attracted growing interest since its discovery in 2003. In the present study, we found that the spinal IL-33 and its ST2 receptor were involved in the development of inflammatory (in a formalin induced acute inflammatory pain mouse model), neuropathic (in a spared nerve injury (SNI) induced neuropathic pain mouse model) and also cancer pain (in a 4T1 mammary carcinoma cells induced bone cancer pain mouse model). And we also further explored the underlying cellular signaling pathways in the SNI induced neuropathic pain. In detail, after the SNI surgery, the mice developed obvious mechanical and cold allodynia. Intrathecal administration of ST2 antibody or knockout of ST2 gene significantly attenuated the SNI-induced neuropathic pain. Furthermore, the effects of IL-33/ST2 signaling in neuropathic pain were mediated by spinal NMDA receptor through the activation of the astroglial JAK2–STAT3 cascade and the neuronal CaMKII–CREB cascade. These results suggested that spinal IL-33/ST2 signaling contributes to the pathogenesis of neuropathic pain and provided a novel therapeutic target for the treatment of neuropathic pain.

Keywords: Interleukin-33; ST2; Signaling; N-methyl-D-aspartate receptor; Pain

NEUROSCIENCES

Poster Number 114

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Creation of a new mouse model for the mRNA splicing disease Familial Dysautonomia

Investigators: Elisabetta Morini, PhD; Paula Dietrich, PhD; Ioannis Dragatsis, PhD; Monica Salani, PhD; Fabio Urbina; Susan A. Slaugenhaupt, PhD

Familial dysautonomia (FD) is a recessive neurodegenerative disease caused by a splice mutation in the IKBKAP gene which leads to variable skipping of exon 20. We found that kinetin can correct the IKBKAP splicing defect and increase the amount of normal mRNA and protein in FD cell lines. We have also shown that kinetin can increase the level of functional IKAP protein in mice following oral dosing in all tissues tested, including brain. Despite these remarkable advances we lacked animal model in which to test the effect of targeting mRNA splicing to increase IKAP protein on FD phenotype.

In order to create a phenotypic model of FD in which we could also manipulate mRNA splicing we introduced an FD transgene (TgFD9), which contains the human IKBKAP gene with the major FD splice mutation, into the Ikbkap delta20/flox mouse model by sequential mating. The introduction of the human IKBKAP transgene attenuates the severe FD phenotype that we observed in the Ikbkap delta20/flox mouse and recreates the same tissue-specific mis-splicing defect.

FD9/Ikbkap delta20/flox mice recapitulate several phenotypic features observed in FD patients like reduction of fungiform papillae, poor peripheral skin innervations, reduction of volume of stellate ganglia and cervical DRGs. Our results demonstrate that the new TgFD9/Ikbkap delta20/flox mouse accurately models both the disease phenotype and the tissue-specific mRNA mis-splicing defect seen in FD patients. The creation of this new model has allowed us to initiate a clinical trial of kinetin and will permit testing of other strategies for targeting mRNA splicing.

Poster Number 115

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An in vitro screen for antiepileptogenic compounds utilizing organotypic hippocampal slice cultures

Investigators: Yero Saponjian, PhD; Yevgeny Berdichevsky, PhD; Waldemar Swiercz, PhD; Kevin Staley, MD

The accelerated course of epileptogenesis in the in vitro organotypic hippocampal slice culture model of severe traumatic brain injury and subsequent post-traumatic epilepsy was utilized to conduct a moderate-throughput screen of an array of drugs to study their antiepileptic and neuroprotective effects and ultimately find antiepileptogenic compounds. The lactate and lactate dehydrogenase concentrations in spent culture medium are strongly correlated with electrographic seizure activity and with propidium iodide-stained neuron counts, respectively, and were used as assays for seizure activity and neuroprotection. Our approach enables one researcher to screen 9 conditions per week with 3 replications per condition. We investigated antiepileptogenic effects of over 150 high-interest drugs. Drugs were typically tested at 3 concentrations: the most likely effective concentration as well as 1 log above and below this concentration. Positive screens were repeated to confirm the findings. Drugs exhibiting significant anticonvulsant activity were further analyzed in wash-out experiments to differentiate anticonvulsant from antiepileptogenic effects. Several drugs exhibited dose-dependent anticonvulsive or proconvulsive effects, as well as neuroprotective or neurotoxic effects. The timing of drug application was an important determinant of the action of several drugs, supporting the idea that epileptogenesis results from a sequence of processes, each of which may respond to unique interventions. Anticonvulsant and neuroprotective effects were strongly correlated, presumably due to reductions in ictal cell death. This technology comprises a promising tool for the rapid investigation of drug efficacy in chronic epilepsy, and could be further scaled with available robotic technologies.

Poster Number 116

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Dynamic fPET/fMRI of the human visual system

Investigators: Marjorie Villien, PhD; Joseph B. Mandeville, PhD; Hsiao-Ying Wey, PhD; Ciprian Catana, PhD; Jonathan R. Polimeni, PhD; Christin Y. Sander, BSc; Nicole R. Zürcher, PhD; Daniel B. Chonde, BSc; Joanna S. Fowler, PhD; Bruce R. Rosen, PhD; Jacob M. Hooker, PhD

Brain mapping of task-associated changes in hemodynamics and metabolism with positron emission tomography (PET) has been accomplished in the past by subtracting scans acquired during two distinct static states. Here we show that PET can provide truly dynamic information on cerebral energy metabolism using concepts common to functional magnetic resonance imaging (fMRI). Using the widely available radiotracer, 2-[18F]-fluoro-deoxyglucose (FDG), we show that quantitative glucose utilization changes during multiple visual stimuli can be determined from neuroimaging data acquired during FDG constant infusion.

The mean percent increase in glucose utilization derived from fPET-FDG for our three subjects in the V1 region of the visual system was 25% for the full-field checkerboard, 26% for the left hemi-field checkerboard and 28% for the right hemi-field checkerboard. The mean percent increase in cerebral blood flow and BOLD signal in our three subjects during the full-field checkerboard activation in the V1 region was 21% and 4% respectively and largely colocalized with fPET-FDG activations.

This study demonstrated for the first time that multiple stimuli can be measured dynamically during a single FDG PET scan. The absolute changes in FDG utilization as measured by fPET-FDG are consistent with previous studies measuring single response in a two-scan paradigm. The complementary nature of fPET-FDG to fMRI capitalizes on the emerging technology of hybrid MR-PET scanners. fPET-FDG, combined with quantitative fMRI methods, will allow us to simultaneously measure dynamic changes in glucose utilization, hemodynamics and oxygen consumption, addressing vital questions about neuronal and neurovascular relationships across tasks and disease states.

NEUROSCIENCES

Poster Number 117

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Synaptic alterations during post-traumatic epileptogenesis in Vitro

Investigators: Zemin Wang, MD, PhD; Kevin Staley, MD

Axon sprouting and rewiring of neural networks after brain injury underlie both functional recovery and, in some patients, epileptogenesis. However, the nature of these changes in network connectivity is poorly understood. To investigate the progressive synaptic changes in a neural network during post-traumatic epileptogenesis, we combined dual whole-cell patch clamp recording with two-photon imaging in a well-established hippocampal organotypic slice culture model.

We found that hippocampal organotypic slices became epileptic over a predictable time course of several days after traumatic slicing injury, with interictal spikes preceding ictal activity. We analyzed the cross-correlation of activities in pairs of neurons versus the inter-neuron distance for 1) ictal activity 2) interictal activity, and 3) postsynaptic currents at different stages of epileptogenesis. We found that interictal and postsynaptic currents were synchronized locally shortly after slicing. The correlations of postsynaptic activities in distant pyramidal cell pairs (> 200 um apart) increased steadily with number of days in culture, and most closely paralleled the appearance of ictal activity. Pyramidal neurons' morphological changes were also measured by two photon imaging after Alexa Fluor 594 Hydrazides was micro-injected through the recording pipette, showed that the dendritic arborization is continuously during epileptogenesis.

Thus, during post-traumatic alterations in neuronal network connectivity, the appearance of increased long-distance correlations in synaptic activities is most closely associated with the appearance of ictal activity in this preparation. This suggests either that synaptic connections between distant neurons are necessary for seizure activity, or that ongoing seizure activity promotes the development of these longer-range synaptic connections.

Poster Number 118

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The migraine trigger caffeine increases susceptibility to spreading depolarization

Investigators: Nilufer Yalcin, MD; Esther Sori Yu, MS; Cenk Ayata, MD; Katharina Eikermann-Haerter, MD

Migraine is an episodic painful headache disease, and the most common neurological disorder. Cortical spreading depolarization (CSD), a wave of neuroglial depolarization, is the underlying electrophysiologic event. Many factors modulating migraine also alter susceptibility to CSD. We here test whether the migraine trigger caffeine enhances CSD susceptibility in mice as a potential mechanism of action.

C57BL6 mice were treated once acutely or chronically (2x/day over 1 week) with caffeine (60mg/kg i.p., equivalent to 5 cups of coffee in humans) or vehicle. Withdrawal experiments were performed 24 hours after last administration of chronic caffeine treatment. CSD susceptibility was assessed by measuring the electrical threshold for CSD induction, and by analyzing CSD evoked by continuous application of KCl (300mM for 30 minutes) onto occipital cortex. Mice were ventilated, and blood pressure and blood gas monitored and maintained within normal range. Experiments were done in a blinded fashion.

Here we show that acute caffeine administration decreases the electrical threshold for CSD induction compared to vehicle. A weaker stimulus applied to the occipital cortex was sufficient to trigger CSD ($p=0.035$). CSD parameters upon topical application of KCl (CSD frequency, speed, amplitude and duration) were not affected. Chronic treatment or withdrawal did not influence CSD susceptibility after topical KCl or electrical stimulation. Systemic physiologic parameters did not differ between groups.

Our data show that acute administration of caffeine enhances CSD susceptibility as a possible mechanism to explain its effectiveness to trigger migraine attacks and promote the migraine chronification process.

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Poster Number 119

Chronic treatment with anesthetic propofol improves cognitive function and attenuates caspase activation in aged and Alzheimer's disease transgenic mice

Investigators: Yiying Zhang, MD; Haijun Shao, MD; Yuanlin Dong, MD; Zhongcong Xie, MD, PhD

Objective: Alzheimer disease (AD), an aging associated disorder with an incidence of 13% in people over 65 years of age, has been no effective treatments so far. A recent study showed that AD patients may have the loss of a functional GABA receptor. Propofol, an intravenous anesthetic, is also a GABA receptor agonist, has been reported to potentially enhance cognitive function in humans. However, the in vivo relevance, the underlying mechanisms, and the functional consequences of these findings remain largely to be determined.

Methods: We therefore set out to assess the effects of propofol on old WT and AD Tg mice on mitochondrial function, cytotoxicity, and learning and memory using flowcytometry, Western blot analysis, water maze and ELISA.

Results: Here we showed that propofol ameliorated the aging-associated cognitive impairment in both 18 month-old WT and AD Tg mice. And propofol attenuated the aging-associated caspase-3 activation through the mitochondrial pathway of apoptosis in both 18 month-old WT and AD Tg mice. Finally, propofol mitigated the Aβ42-induced opening of the mitochondrial permeability transition pore, and activation of caspase-9 and caspase-3 through the GABA receptor in the H4 naïve cells and N2A cells.

Interpretation: These data have suggested that propofol may improve aging-associated cognitive impairment via attenuating the Aβ-induced mitochondria dysfunction and caspase activation. These findings would promote more studies to determine whether propofol and other GABA receptor agonists can be potentially used to prevent and treat AD.

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Poster Number 120

Chronic modulation of metabotropic glutamate receptors in 6-OHDA-induced Parkinson's disease (PD) rat model

Investigators: Aijun Zhu, PhD; Kun-Eek Kil, PhD; Ji-Kyung Choi, PhD; Bruce Jenkins, PhD; Sreekanth Kura, MS; Kumudu Kuruppu, PhD; Chunyu Gong, MD; Zhaoda Zhang, PhD; Anna-Liisa Brownell, PhD

6-OHDA-induced PD-like rat model was used to explore interplay of dopaminergic and glutamatergic systems. Metabotropic glutamate receptors were investigated using PET-CT imaging with [18F]FPEB and [11C]ML128 for detecting modulations of mGluR5 and mGluR4 in striatum and hippocampus in rat brain during progressive degeneration.

5 male Sprague-Dawley rats received a left-sided lesion at substantia nigra by 6-OHDA. 4 weeks later following PET studies were performed: 11C-labeled 2β-carbomethoxy-3β-(4-fluorophenyl)tropane ([11C]CFT) for dopamine transporters; 11C-labeled N-(Chloro-3-methoxyphenyl)-2-picolinamide ([11C] ML128) for mGluR4; and 18F-3-fluoro-5-[(pyridin-3-yl)ethynyl]benzonitrile ([18F]FPEB) for mGluR5. 6 weeks later, the studies were repeated. Binding potentials (BP) were calculated on the striatum and hippocampus for CFT and FPEB using cerebellum as reference. %ID (injected dose) per gram was calculated for mGluR4 data due to lack of reference region. The differences between left and right sides of BPs or %ID/g were compared on the two imaging points.

Large differences of BP of [11C]CFT were observed between the left and right striatum due to the abolished dopamine transport from the nigra to the striatum. 4 weeks after the lesioning, BP of [18F]FPEB was decreased in the left lesion-side striatum while increased in the left lesion-side hippocampus; 6 weeks later striatum had non-significant change but BP of FPEB was enhanced in hippocampus on the lesion side. 4 weeks after the lesioning, BP of mGlu4 expression ([11C]ML128) was enhanced in the striatum and the hippocampus but reduced 6 weeks later.

This study demonstrates enhanced presynaptic mGluR4 expression as an immediate response to dopaminergic degeneration changing later to dominantly enhanced mGluR5 expression.

Poster Number 121

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Mechanisms of Caspase-1 Mediated Schwannoma Regression

Investigators: Sherif G. Ahmed, PhD; Mehran Taherian, MD; Shilpa Prabhakar, MSc; Giulia Fulci, PhD; Miguel Sena-Esteves, PhD; Xandra O. Breakefield, PhD; Gary J. Brenner, MD, PhD

Schwannomas are tumors composed of Schwann-lineage cells that form along peripheral, spinal and cranial nerves. These tumors can cause pain, sensory/motor dysfunction, and death through compression of peripheral nerves, the spinal cord, and/or the brain stem. We have developed an experimental model in which human schwannoma cells are implanted into the sciatic nerve of nude mice and used this model to test a gene therapy strategy developed in our laboratory. Our strategy involves delivering the inflammatory, apoptotic protein, caspase-1 (ICE, IL1-converting enzyme) under the Scwann cells P0 promoter, through an adeno-associated vector 1 (AAV1) injected directly into the tumors. Our gene therapy vector was denominated AAV-P0-ICE.

Our data indicate that AAV1-P0-ICE efficiently debulks schwannomas and resolves schwannoma associated pain, without any neuronal toxicity. Additional data indicate that AAV-P0-ICE induces micro-environmental changes that prevent re-growth of a second lesion implanted in the location of the primary tumor. These micro-environmental changes cause apoptosis of newly re-implanted tumor cells without causing any neuronal damage, thus proving the stability, efficacy, and lack of toxicity of this treatment strategy. These microenvironmental changes may be mediated by the Caspase-1 pro-inflammatory components which in turn trigger immune responses mediated by innate and adaptive immune cells.

Altogether these data show that our caspase-1 mediated gene therapy approach has the potential to generate new therapeutics for schwannomas. Not only by inducing apoptosis, but the resulting tumor killing and associated activation of host immune responses may generate a vaccination effect that could control the development and growth of subsequent tumors.

Poster Number 122

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Safety and Efficacy of Oncolytic HSV in Sciatic Nerve Malignant Peripheral Nerve Sheath Tumor Models

Investigators: Slawomir J. Antoszczyk, PhD; Samuel D. Rabkin, PhD

Malignant peripheral nerve sheath tumors (MPNST) are highly aggressive sarcomas arising in peripheral nerves. There is currently no effective therapy for patients with neurofibromatosis type 1 (NF1) who develop MPNST. NF1 is a tumor suppressor gene that encodes a RAS-GAP and is frequently mutated in solid tumors. Oncolytic herpes simplex viruses (oHSVs) are genetically engineered to replicate selectively in and kill cancer cells.

Here we describe two new orthotopic MPNST sciatic nerve (SN) tumor models: mouse MPNST cell lines derived from spontaneously-arising tumors in Nf1/Trp53 heterozygous mice implanted in syngeneic mice; and human S462 MPNST stem-like cells (MSLCs) implanted in immunodeficient mice. We evaluated the efficacy of oHSV G47Δ in mouse immunocompetent and human immunodeficient models. To evaluate tumor growth, we used external tumor size, neurological deficit score, and survival. Treatment was initiated when mice begin to exhibit hind limb deficits due to tumor growth. A single injection of G47Δ into human S462 MSLC SN or mouse M2 SN tumors significantly delayed tumor growth and prolonged mouse survival. The safety of G47Δ and preservation of nerve function in infected nerves was demonstrated by EM analysis and neurologic scoring after virus injection directly into the SN (non-tumor bearing) of immunodeficient mice.

These studies demonstrate the efficacy of G47Δ in treating orthotopic MPNST tumors in the SN of both immune-competent and -deficient mice. The MPNST models of human MSLCs and mouse M2 isolated from transgenic spontaneously arising MPNSTs are useful for evaluating other oncolytic viruses and therapeutics.

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Poster Number 123

Blockade of PIGF/NRP1 Signaling Inhibits the Growth and Spread of Pediatric Medulloblastoma

Investigators: Ana Batista, PhD; Matija Snurdel, MD; Nathaniel Kirkpatrick, PhD; Carmen Almodovar, PhD; Lars Riedemann, MD; Teresa Peterson, BS; Lei Xu, MD, PhD; Peter Carmeliet, MD, PhD; Rakesh Jain, PhD

Medulloblastoma (MB), a cerebellar tumor, is the most common pediatric brain cancer and a heterogeneous disease. Several studies have characterized tumor genetic heterogeneity with the hope of identifying targets for personalized therapy. In fact, inhibitors of Sonic Hedgehog (Shh)- a developmental pathway aberrantly activated in a subset of MBs- are currently being evaluated. Unfortunately, mutations and potential developmental adverse effects in the pediatric population can limit these therapies. We propose targeting the tumor stroma as an efficacious alternative treatment option. Our goal is to identify and disrupt factors that mediate critical tumor-stroma signaling crosstalk that sustains viable cancer cells. We have identified placental growth factor-PIGF, a member of the vascular endothelial growth factor (VEGF) family as a stromal protein with a crucial role in MB growth and progression. Here, we show that: 1) PIGF is expressed by 90% of primary pediatric Medulloblastomas, regardless of their genetic subtype; and high expression of PIGF receptor NRP1 correlates with poor overall survival of patients; 2) Medulloblastoma cells release Shh ligands that stimulate paracrine production of stromal PIGF by cerebellar granule neurons; 3) PIGF signals through NRP1 to activate the Erk/MEK pathway and sustain tumor survival; 4) Blockade of PIGF with specific antibody causes dramatic Medulloblastoma regression, decreases spinal metastatic burden, and prolongs survival in in vivo orthotopic and spontaneous mouse models. This work identifies PIGF as the first common target across Medulloblastoma subgroups as a new and safer potential target option for the treatment of this pediatric disease.

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Poster Number 124

Modeling Heterotypic Communication in Tumor Growth and Treatment Response: The Role of Tumor Endothelial Cells and Stromal Fibroblasts

Investigators: Imran Rizvi, PhD; Emma Briars, BA; Arnav Chandra; Sriram Anbil, BA; Jonathan P. Celli, PhD; Heather F.M. Gudejko, PhD; Shazia Khan, PhD; William R. Hanna, BS; Dustin P. Jones, BS; Tayyaba Hasan, PhD

The tumor microenvironment plays a critical role in the biological characteristics and response to therapy of metastatic disease. Understanding the therapeutic implications of heterogeneities that result from crosstalk between tumor cells and stromal partners is critical for designing more effective therapy regimens. Stromal partners such as tumor endothelial cells (TEC) and tumor-associated fibroblasts (TAF) are emerging as important biological modulators of many cancers including ovarian cancer (OvCa) and pancreatic cancer (PanCa). Heterocellular 3D tumor arrays that restore communication with stromal partners may be increasingly important complements to existing systems. However, these models lack the ability to differentiate between distinct cell populations within the tumor, which may vary in their susceptibility to therapy. Here we adapt and characterize a fluorescent dye-labeling method for extended longitudinal monitoring and evaluation of differential responsiveness to therapy between tumor and stromal cells.

Human OVCAR-5 (OvCa), HUVEC-C (TEC), MiaPaCa-2 (PanCa), and MRC-5 (TAF) cells were labeled with VybrantTM dyes at concentrations ranging from 5μM (recommended) to 50μM. Fluorescence confocal microscopy revealed that 25μM is optimal for imaging labeled cells both seven days in monolayer, and over two weeks in 3D. There was no significant toxicity from cell labeling, except in MRC-5 at concentrations of 25 and 50μM ($p < 0.0001$). Labeled OVCAR-5 and HUVEC-C cells showed no significant increase in susceptibility to photodynamic therapy, an emerging light-based modality, compared to unlabeled controls. These findings suggest the applicability of this approach for ongoing and future 3D treatment response studies, in addition to the development of rational treatment combinations.

Poster Number 125

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Prevention and Reversion of Multidrug Resistance (MDR) in Cancer by NSC23925

Investigators: Xiaoqian Yang, MD; Pei Yang, MD, PhD; Jacson Shen, BA; Eiji Osaka, MD, PhD; Edwin Choy, MD, PhD; Gregory Cote, MD, PhD; David Harmon, MD; Zhan Zhang, MD, PhD; Henry Mankin, MD; Francis J. Hornicek, MD, PhD; and Zhenfeng Duan, MD, PhD

The major limitation to the success of chemotherapy in cancer is the development of multidrug resistance (MDR). Preventing the emergence of MDR during chemotherapy treatment has been a high priority of clinical and investigational oncology, but remains an elusive goal. NSC23925 has recently been identified as a novel and potent MDR reversal agent. However, whether NSC23925 can prevent the development of MDR in cancer is unknown. Therefore, this study was to evaluate the effects of NSC23925 on prevention and reversion of MDR. Human osteosarcoma cell line U-2OS and Saos were exposed to increasing concentrations of taxol alone or in combination with NSC23925 for 6 months. The results showed that cells selected with increasing concentrations of taxol alone developed MDR with resistance to taxol and other Pgp substrates, while cells cultured with taxol-NSC23925 simultaneously prevented the development of MDR and cells remained sensitive to chemotherapeutic agents. Taxol selected resistant cells showed high expression and activity of drug transporter P-glycoprotein (Pgp), whereas taxol-NSC23925 combination treatment prevented the expression of Pgp. Our findings suggest that NSC23925 may prevent the development of MDR by specifically preventing the overexpression of Pgp. Moreover, NSC23925 can reverse MDR in drug resistant cells by modulating Pgp activity. Given the significant incidence of MDR in cancer and the lack of effective agents for prevention and revision of MDR, NSC23925 and derivatives hold the potential to improve the outcome of cancer patients with poor prognosis due to drug resistance.

Poster Number 126

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Transforming growth factor-beta inhibitor synergizes with oncolytic herpes simplex viruses to kill cancer stem cells isolated from recurrent glioblastoma

Investigators: Shinichi Esaki, MD, PhD; Samuel D. Rabkin, PhD; Robert L. Martuza, MD, FACS; Hiroaki Wakimoto, MD, PhD

Glioblastoma (GBM) inevitably recurs despite current standard therapies. The culprit may be a subset of tumor cells with stem-like properties, termed GBM stem cells (GSCs), which have been shown to be resistant to radiation and chemotherapy. Oncolytic herpes simplex viruses (oHSVs) have been safely tested in GBM patients and are efficacious against GSCs isolated from newly diagnosed GBM. In this study, we isolated GSCs from recurrent GBM, investigated the therapeutic activity of oHSV against these GSCs, and explored a combinatorial approach with a small molecule inhibitor of transforming growth factor beta (TGF-beta), which plays a role in maintaining GSC stemness.

We established 6 neurosphere cultures from surgical specimens of GBMs that had recurred after surgery, radiotherapy, and temozolomide (TMZ) chemotherapy (recurrent GSCs). All of these recurrent GSCs generate tumors in the mouse brain, and were resistant to TMZ. The TGF-beta pathway is active in these GSCs, and TGF-beta inhibitor SB431542 suppressed clonogenicity and proliferation of recurrent GSCs. Genetically engineered oHSVs, G47delta [(ICP34.5(-), ICP6(-), LacZ(+), ICP47(-)] and MG18L [US3(-), ICP6(-), LacZ(+)] replicated, spread well, and were cytotoxic at low multiplicities of infection in the recurrent GSCs, with MG18L being more potent. The combination of oHSV and TGF-beta inhibitor synergistically killed the recurrent GSCs in vitro.

In conclusion, recurrent GSCs are susceptible to oHSV and the combination with TGF-beta inhibitor can enhance activity. This combination strategy is promising for the treatment of TMZ-resistant recurrent GBM.

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Poster Number 127

Combined treatment with photodynamic therapy and irinotecan is effective in pancreatic cancer in vivo

Investigators: Huang-Chiao Huang, PhD; Srivalleesha Mallidi, PhD; Zhiming Mai, PhD; Ruth Goldschmidt, PhD; Joyce Liu; Dmitriy Timerman; Imran Rizvi, PhD; Tayyaba Hasan, PhD

The genetic complexity and heterogeneity of pancreatic cancer (PanCa) make it extremely difficult for any single treatment to impact outcome. This study strategically combines two clinical-relevant, nanotechnology-based therapies to facilitate rapid clinical translation and immediately improve on the dismal statistics of PanCa patients. We hypothesized that benzoporphyrin derivative (BPD)-based photodynamic therapy (PDT) (Phase I/II clinical study) destroys tumor efflux transporters, which may help maintain high intracellular concentrations of Irinotecan (CPT-11) (Phase III clinical trial) to reduce tumor burden and prolong survival. Moreover, we hypothesized that CPT-11, inhibiting the accumulation of HIF-1alpha and overcoming tumor hypoxia, may sensitizer tumors to PDT. We test these hypotheses in two orthotopic PanCa models (MIA PaCa-2 and AsPC-1). Two types of nanoliposomes were fabricated: (i) Liposome with BPD in lipid bilayer (LBPD) and (ii) Liposome encapsulating CPT-11 in aqueous core (LCPT-11). Treatments were initiated when the tumors reach ~35 mm cube. Tumor bearing mice were intravenously injected with low doses of LBPD and LCPT-11 1h before light administration. PDT was performed on the exteriorized pancreas of the anesthetized animal, and then followed by wound closure. The longitudinal, non-invasive ultrasound monitoring of orthotopic tumor volume in response to combination treatment was carried out with appropriate controls. We observed a synergistic tumoricidal effect of LBPD-PDT and LCPT-11, which significantly inhibited tumor growth up to at least 3 weeks post-treatment, without systemic toxicities. We anticipate these findings, based on two clinically relevant treatments, will form the basis for rapid translation of a novel combination regimen for PanCa patients.

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Poster Number 128

Imaging drug resistance to Eribulin at the single cell level in vivo

Investigators: Ashley Laughney, PhD; Eunha Kim, PhD; Melissa Sprachman, PhD; Rainer Kohler, PhD; Katy Yang, PhD; Ralph Weissleder, MD, PhD

The novel mitosis inhibitor, Eribulin, was recently FDA approved for the late stage treatment of taxol refractory breast tumors. Despite it's approval, little is known about its distribution in tumors, uptake by tumor cells and pharmacodynamic effects at the single cell level. We hypothesized that a fluorescent, companion imaging drug could be used to quantify these parameters and also allow comparison between wild type and multidrug resistant tumors (mediated via the multi-drug resistance (MDR1) P-glycoprotein). We therefore fluorescently labeled eribulin with BOPIDY to perform high-resolution and longitudinal intravital imaging in multidrug resistant cancers characterized by fluorescent expression of MDR1. Specifically we asked the following questions: What are the cellular influx and efflux rates of eribulin in human cells? How do these rates depend on MDR1 expression level? Do MDR1 inhibitors actually increase tumor specific drug accumulation in vivo? How can cellular accumulation of drug be maximized? Remarkable heterogeneity in cellular drug accumulation and a strong inverse relationship of efficacy with MDR1 expression was observed for Eribulin, as was the case for taxol. Heterogeneity was characterized according to MDR1 expression and distance from tumor vasculature. Most important we show that MDR1 inhibition (via HM30181, a small molecule P-glycoprotein inhibitor) can completely reverse the resistance phenotype and restore drug efficacy in vitro. This was possible in vivo, but delivery of the new MDR1 inhibitor was the main limiting factor of efficacy.

Poster Number 129

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Tumor Cell Radiosensitivity, not Tumor Vasculature, is the Principle Determinant of Radiocurability

Investigators: Wende Li, MD; Peigen Huang, MD; Yujiao Liu, MS; Leo E. Gerweck, PhD

Aim: To evaluate the roles of tumor cell radiosensitivity and tumor vasculature as determinants of the dose of radiation required to achieve permanent local control.

Methods: We cloned a DNA-Pkcs-/- radiosensitive cancer stem cell line in SCID mice and transfected the same line with a competent DNA-Pkcs+/+ gene, which increased the cells' radioresistance via restitution of DNA double-strand break repair. The fraction of cells killed by radiation in vitro, i.e., in the absence of tumor stroma, and the fraction of cells killed in tumors initiated by the same isogenic lines and irradiated in vivo, was evaluated by clonogenic assays. Functional tumor vascular density was assessed prior to and following irradiation. The dose of radiation resulting in permanent local of 50% of treated tumors was evaluated by the TCD50 assay.

Results: The fraction of cells killed by the same dose of radiation was invariant regardless of whether the cells were irradiated in vitro or in vivo. The ratio of the repair deficient and proficient cells' surviving fraction was also similar whether irradiated in vitro or in vivo. The tumors' TCD50s was directly proportional to the difference in the intrinsic radiosensitivity of their constituent tumor cells. The functional vascular densities of the sensitive and resistant tumors did not differ, in spite of the difference in the tumors' radiocurability.

Conclusion: The number of stem cells per tumor and their radiosensitivity, is the principle determinant of radiocurability.

Poster Number 130

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A large-scale transgenic screen in zebrafish identifies TOX as a novel oncogene in T-cell acute lymphoblastic leukemia

Investigators: Riadh Lobbardi, PhD; Nouran Abdelfattah, MS; Barbara Martinez, PhD; Jordan Pinder, PhD; Deborah Toiber, PhD; Jessica S. Blackburn, PhD; Manon De Waard, MS; Graham Dellaire, PhD; Raul Mostoslavsky, MD, PhD; David M. Langenau, PhD

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of thymocytes. To identify molecular pathways underlying T-ALL progression, a zebrafish transgenic screen was performed in which 38 amplified and over-expressed genes found in human T-ALL were assessed for accelerating leukemia onset in the zebrafish T-ALL model. From this analysis, thymocyte specific HMG-box protein (TOX) was identified as a potent collaborating oncogene with both MYC and NOTCH to enhance T-ALL aggression. TOX is genomically amplified in both human and mouse T-ALL and highly expressed in a majority of human T-ALL. shRNA knock down of TOX resulted in a block in late S- phase and an associated increase in apoptosis, confirming a critical role for TOX in T-ALL maintenance and continued growth. Immunoprecipitation and Tandem Mass Spectrometry analysis identified Ku70 and Ku80 as key binding factors with TOX, both of with are required for double strand break repair (DSBR). Full-length TOX, when transfected into 3T3 cells, efficiently inhibited non-homologous end-joining (NHEJ), while mutants lacking either the nuclear localization signal or the DNA-binding HMG-box fail to alter DSBR. Moreover, Ku80 recruitment to sites of DNA damage was reduced in TOX-overexpressing cells as assessed by laser-induced DNA damage and real-time imaging analysis. TOX knockdown in T-ALL cells accelerated DBSR as assessed by NHEJ assays, comet assay, and 53BP1 and γH2A.X foci counting following irradiation. Our data support a role for TOX in accelerating T-ALL onset by suppressing DSBR though inhibition of Ku70/80 function resulting in accumulation of DNA alterations and genomic instability.

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Poster Number 131

Computing Proton Dose to Irregularly Moving Targets

Investigators: Justin Phillips, PhD; Gueorgui Gueorguiev, PhD; James A. Shackleford, PhD; Stephen Dowdell, PhD; Clemens Grassberger, PhD; Harald Paganetti, PhD; Gregory C. Sharp, PhD

While 4DCT and deformable registration can be used to assess proton dose delivered to regularly moving lung tumors, there are currently few methods available for those exhibiting irregular motion. This study describes a method for computing the dose delivered to irregularly moving tumors based on 1D or 3D waveforms captured at the time of delivery.

The procedure requires 4DCT images for dose calculation, and 1D or 3D respiratory waveforms to estimate target position at time of delivery. Dose volumes are converted from their Cartesian geometry into a beam-specific radiological depth space, parameterized in 2D by the beam aperture, and longitudinally by the radiological depth. In this water-equivalent depth space, the

proton doses are translated according to the motion found in the 1D or 3D trajectory. These translated dose volumes are weighted and summed, then transformed back into Cartesian space, yielding an estimate of the dose that includes the effect of the measured breathing motion.

The method was validated with two respiratory motions functions in a synthetic lung phantom and a single representative patient CT. Gamma evaluation (3 mm, 3%) above 90% was achieved for each tested breathing function in the phantom and patient data.

We have demonstrated a method for accurately reproducing proton dose to an irregularly moving target from a single CT image. We believe this algorithm could prove a useful tool to study the dosimetric impact of baseline shifts either before or during treatment.

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Poster Number 132

The biology of endogenous tumor-derived extracellular vesicles

Investigators: Ferdinando Pucci, PhD; Christopher Garris, BS; Charles Lai, PhD; Andita Newton, BS; Xandra Breakefield, PhD; Ralph Weissleder, MD, PhD; Mikael Pittet, PhD

Extracellular vesicles (EVs) are nanometer-sized vesicles containing lipids, cellular proteins, mRNAs, and non-coding RNAs. EVs are released by cells and can travel long distances in the body to deliver undegraded and undiluted material to distant recipient cells, thereby facilitating inter-cellular communication without direct cell-to-cell contacts. A decade of work dedicated to understanding tumor-derived EVs (tEVs) indicates that they can interact, and thus affect, several immune cell subsets involved in promoting cancer growth. Yet most of this knowledge has been obtained from studies in which tEVs were manipulated in vitro; therefore the biodistribution routes and cellular tropism of tEVs in vivo, as well as the nature and impact of tEV interactions with target cells, remain largely unknown. Here, we will present new genetic approaches that allow one to track endogenously-produced tEVs and their recipient cells directly in vivo and at the organismal, cellular and molecular levels. By combining these technological advances, we are discovering where tEVs traffic in vivo, which cells they interact with, and how they communicate and transfer information. We identified a specific subset of tumor-draining lymph node (tdLN) macrophages as a major "acceptor" of tEVs outside the tumor microenvironment. Macrophages are commonly viewed as tumor-promoting cells; however, here we will discuss whether this tdLN macrophage subset provides anti-tumor immune function by preventing the dissemination of tumor-derived material and, by extension, progression of disease, or whether they conform as tumor-promoting cells by mediating tumor immune escape and tolerance toward tumor-derived antigens.

Poster Number 133

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Targeting Physical and Stromal Determinants of Tumor Heterogeneity in Bioengineered 3D Models

Investigators: Imran Rizvi, PhD; Umut Gurkan, PhD; Sriram Anbil, BS; Savas Tasoglu, PhD; Jonathan P. Celli, PhD; Nermina Alagic, MD; Lawrence B. Mensah, PhD; Emma Briars, BA; Zhiming Mai, PhD; Shazia Khan, PhD; Ruth Goldschmidt, PhD; Iqbal Massodi, PhD; Michael Glidden, BS; Utkan Demirci, PhD; Tayyaba Hasan, PhD

Tumor metastases develop in a complex milieu of microenvironmental cues that include flow-induced stress and stromal cross-talk. Ovarian cancer (OvCa) disseminates as shed tumor cells and aggregates move along ascitic currents and communicate with the local microenvironment to initiate peritoneal implants. Growth of these implants leads to advanced stage disease, which prognosticates the poorest outcomes for patients with this lethal gynecologic malignancy. Although metastatic OvCa nodules colonize distant sites under the influence of flow, the role of ascitic currents as physical modulators of OvCa heterogeneity remains poorly understood. Here, we describe the development of a bioengineered 3D tumor platform that integrates hydrodynamic and marix-based cues to elucidate the roles of flow and stromal communication as determinants of tumor heterogeneity. A flow-induced increase in epithelial-mesenchymal transition is observed, with a concomitant post-translational upregulation of epidermal growth factor receptor expression and activation. These changes in the morphologic features of, and biomarker expression in, 3D tumor nodules grown under continuous flow are indicative of a motile and aggressive tumor phenotype. Future studies will enhance the biological relevance of the system by incorporating heterotypic cellular partners such as tumor endothelial cells, which are emerging as dynamic regulators of metastatic progression and mediators of treatment susceptibility. This approach supports a framework for developing therapeutic strategies that target the effects of physical and stromal cues for OvCa and other lethal malignancies.

Poster Number 134

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Adeno-associated Virus 9 (AAV-9) delivered human Mullerian Inhibiting Substance (MIS) as a novel, minimally toxic treatment for ovarian cancer.

Investigators: Amanda B. Sosulski, MD; David Pepin, PhD; Leo Andrew Benedict, MD; Katie Hendren; Li Hua Zhang; Katia Meirelles, MD; Guangping Gao, PhD; Robert H. Brown, MD; Patricia K. Donahoe, MD

Introduction: Mullerian Inhibiting Substance (MIS) has been shown to inhibit ovarian cancer cells both in-vitro and in-vivo and particularly target a putative ovarian cancer progenitor cell population enriched by a panel of CD44+, CD24+, Ep-CAM+, and E-cadherin- surface markers. Gene therapy with AAV9-MIS constructs was successfully used to extend the life of SOD1 mutant ALS mice after a single injection; therefore, we examined whether AAV9-MIS constructs could be used to treat ovarian cancer xenotransplants as a single intraperitoneal injection.

Methods: Each of three different AAV9 viral vectors carrying modified MIS transgenes compared to a GFP construct control were injected into female nude mice (5/group), and blood levels were monitored weekly thereafter using a sensitive ELISA for human MIS. When peak levels of MIS were reached (21 days), ovarian cancer (OVCAR5) cells were xenografted into the mice, tumor volumes were monitored and liver and cardiac enzymes and MIS levels measured.

Results: Gene therapy using an AAV9 viral vector carrying the modified MIS resulted in high blood concentrations, and inhibition of tumor growth in the group manifesting the highest MIS levels in a xenograft model of ovarian cancer, compared with AAV9-GFP injected controls ($p = 0.01$).

Conclusion: AAV9-MIS induces sustained levels of MIS sufficient to inhibit growth of ovarian cancer xenografts after a single intraperitoneal injection. Single injection therapy could provide a patient friendly treatment option for this deadly gynecologic disease.

Poster Number 135

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Effects of Anti-VEGF therapy combined with radiation on NF2 vestibular Schwannoma

Investigators: Lei Xu, MD, PhD; Yingchao Zhao, MD; Xing Gao, MD; Ruo Xu, MD; Weixin Guo, MD, PhD; Chong Liu, MD, PhD; Scott Plotkin, MD, PhD; Rakesh Jain, PhD

The recent landmark study of bevacizumab treatment in patients with neurofibromatosis type 2 showed that bevacizumab treatment is associated with a reduction in the volume of growing Vestibular Schwannomas and improved hearing in patients. However, anti-VEGF therapy alone is unable to effectively achieve a long-term therapeutic effect. For patients with sporadic vestibular Schwannomas, radiation therapy is associated with long-term tumor control, but hearing loss is its main limitation. Here we determined whether combining anti-VEGF with radiation therapy can achieve a better tumor control and minimize toxicity compared with each therapy alone.

Here we showed, in two animal models, tumor growth delay is most significant with the combined anti-VEGF and radiation therapy. Anti-VEGF treatment creates a "normalization window"- during which vessel density decreased and the remaining vessels are less tortuous and less dilated. Immunohistochemistry study showed anti-VEGF therapy increased pericyte coverage of the tumor vessels. As a result of this normalized structure, vessel perfusion function improved significantly leading to an increased tumor tissue oxygenation, which is known to enhance radiation response. Indeed, when radiation is applied during the normalization window, but not before or after it, it is most effective. Furthermore, we observed that anti-VEGF treatment decreased tumor tissue edema and improved neuromuscular function.

Our study is the first to demonstrate that combining radiation therapy and anti-VEGF treatment is more effective for treatment of NF2-related Schwannomas, providing rationale for testing the benefits of combined anti-angiogenic therapy and radiotherapy in the management of Schwannomas.

PHYSIOLOGY & METABOLISM

Poster Number 136

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Inhibition of calcium-mediated podocyte injury as a strategy to prevent kidney failure

Investigators: Philip M. Castonguay, BS; Frank Dubois; Sookyung Kim; Constantine Tarabanis; Dequan Tian, PhD; Astrid Weins, MD, PhD; Anna Greka, MD, PhD

Our recent work showed that inhibition of TRPC5 ion channels is protective in two acute models of kidney disease. Here we turn our attention to a model of chronic proteinuric kidney disease, the podocyte specific Angiotensin Type 1 Receptor (AT1R) transgenic (Tg) rat. AT1R Tg rats develop progressive proteinuria and die of kidney failure by 50 weeks of life. Treatment of rats with the calcineurin inhibitor Cyclosporine A (CsA) for one week was sufficient to abrogate proteinuria and thus mitigate disease progression. This is line with our in vitro findings, whereby TRPC5 activity drives disease-associated calcineurin activity in podocytes. Furthermore, treatment with a TRPC5 specific inhibitor may be effective at suppressing proteinuria in AT1R Tg rats. We conclude that calcium-mediated podocyte toxicity may drive chronic kidney disease, and thus strategies to block these pathways may offer new strategies to prevent progressive disease and kidney failure.

Poster Number 137

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Fluorescent Exendin-4 Derivatives for Pancreatic-Cell Analysis

Investigators: Susan M. Clardy, PhD; Edmund J. Keliher, PhD; James F. Mohan, PhD; Christophe Benoist, MD, PhD; Diane Mathis, PhD; Ralph Weissleder MD, PhD

The ability to reliably identify pancreatic beta cells would have far reaching implications for a greater understanding of beta cell biology, measurement of beta cell mass in diabetes, islet transplantation, and drug development. The glucagon-like peptide-1 receptor (GLP1R) is highly expressed on the surface of insulin producing pancreatic beta cells. Exendin-4 based peptides are one of the most effective GLP1R antagonists known to date with nM binding affinities and excellent therapeutic profiles. Using systematic modifications of exendin-4, we screened over 25 compounds and identified a palette of fluorescent exendin-4 with high GLP1R binding affinity. Here we show considerable differences in affinity, as well as utility of the top candidates for flow cytometry and microscopy of beta cells. Some of the developed compounds should be particularly useful for basic and translational beta cell research.

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Poster Number 138

Anesthesia induced skeletal muscle autophagy

Investigators: Aki Kashiwagi, MD, PhD; Sachiko Hosokawa, PhD; Jeevendra A. Martyn, MD; Shingo Yasuhara, MD, PhD

Autophagy is known to exhibit cell protective effect and its defects play an important role in the pathophysiology of neurodegenerative disease. Recently, abnormalities in the regulation of autophagy have been shown in myopathies and muscular dystrophies. On the other hand, malignant hyperthermia, which is a significant adverse condition induced by anesthesia, frequently occurs to patients with myopathy. These observations indicate a link between anesthesia and metabolic dysregulations in skeletal muscles. We therefore focused on the study of the impact of anesthesia on the regulation of autophagy in the skeletal muscles.

We anesthetized mice with pentobarbital, ketamine, isoflurane, propofol or xylazine and isolated skeletal muscles in 2 hours. Western blot analysis showed that the expression of LC3II, autophagy marker protein increased in all experimental groups compared to control. We performed intravital confocal imaging of the sternomastoid muscle under anesthesia using LC3-GFP transgenic mice and determined the effect of anesthetics on the autophagosome formation. The LC3 dot formation, representing autophagosomes, increased at 2-3 hours and sustained for 6hours, consistent with the chronological data by Western blotting. This increase of autophagosome formation induced by anesthesia was partially blocked by train of four muscle stimulation (TOF) as determined by Western blot analysis and intravital confocal imaging. Furthermore the autophagosome formation was rescued after termination of TOF, indicating that inhibition of muscular contraction plays a role in induction of LC3. We conclude that anesthesia-induced muscular up-regulation of autophagy is mediated at least partially by the lack of muscle contraction.

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Poster Number 139

TRPC5 channels are a therapeutic target for kidney disease

Investigators: Sookyung Kim; Constantine Tarabanis; Dequan Tian, PhD; Frank Dubois; Lisa Buvall, PhD; Philip Castonguay; Thomas Schaldecker; Samy Hakroush, MD; Astrid Weins, MD, PhD; Anna Greka, MD, PhD

In the current worldwide epidemic of diabetes and obesity, proteinuria heralds cardiovascular disease and kidney failure. Podocytes are highly specialized cells in the kidney glomerulus, whose injury and loss leads to proteinuria and progressive kidney disease. Thus, there is currently great unmet need for podocyte-protective treatments. Our laboratory recently showed that Transient Receptor Potential Classical (TRPC5) channels drive the activity of the cytoskeletal modulator Rac1 leading to podocyte cytoskeletal collapse. Here, we show in vivo that absence of TRPC5 as well as pharmacologic inhibition of TRPC5 prevents proteinuria and filter barrier damage in two acute models of disease. Using advanced Ca2+ imaging of intact isolated glomeruli, we show ex vivo TRPC5-mediated Ca2+ influx driven by Lipopolysaccharide (LPS) and Protamine Sulfate (PS). Similarly, mice treated with LPS develop proteinuria and mice treated with PS show foot process effacement (FPE), a hallmark of early kidney damage. Furthermore, the podocyte-specific inducible expression of TRPC5 in podocytes in vivo leads to proteinuria within four weeks of induction. Our study reveals TRPC5 as a novel therapeutic target for kidney disease.

Poster Number 140

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Aminoisobutyric Acid Induces Browning of White Fat and Hepatic-oxidation and is Inversely Correlated with Cardiometabolic Risk Factors

Investigators: Lee D. Roberts, PhD; John F. O'Sullivan, MD, PhD; Pontus Boström, PhD; Robert T. Schinzel, PhD; Gregory D. Lewis, MD; Andre Dejam, MD PhD; Youn-Kyoung Lee, PhD; Melinda J. Palma, PhD; Sondra Calhoun, PhD; Anastasia Georgiadi, PhD; Ming-Huei Chen, PhD; Vasan S. Ramachandran, MD, PhD; Martin G. Larson, PhD; Claude Bouchard, PhD; Tuomo Rankinen, PhD; Amanda L. Souza, PhD; Clary B. Clish, PhD; Thomas J. Wang, MD, MPH; Jennifer L. Estall, PhD; Alexander A. Soukas, MD, PhD; Chad A. Cowan, PhD; Bruce M. Spiegelman, PhD; Robert E. Gerszten, MD

Introduction: The transcriptional co-activator peroxisome proliferator-activated receptor-gamma co-activator-1 α (PGC-1α) regulates metabolic genes in skeletal muscle, and contributes substantially to the response of muscle to exercise. Muscle specific PGC-1α transgenic expression and exercise both increase the expression of thermogenic genes within white adipose. How the PGC-1α mediated response to exercise in muscle conveys signals to other tissues remains incompletely defined.

Methods: We employed a metabolic profiling approach to examine metabolites secreted from myocytes with forced expression of PGC-1α. We then performed functional studies in cell-based and animal models of metabolic disease, and imaging of animal body fat stores. We tested the human relevance of our findings by assessing metabolite levels in two prospective cohorts, and used genome wide scans to identify the genes regulating metabolite levels in humans.

Results: We identified β-aminoisobutyric acid (BAIBA) as a novel small molecule myokine. BAIBA increases the expression of brown adipocyte-specific genes in white adipose tissue and fatty acid -oxidation in hepatocytes both in vitro and in vivo through a PPARα mediated mechanism, induces a brown adipose-like phenotype in human pluripotent stem cells, improves glucose homeostasis in mice, and increases whole body energy expenditure and decreases body fat percentage in mice. In humans, plasma BAIBA concentrations are increased with exercise and inversely associated with metabolic risk factors.

Conclusion: BAIBA may serve as a disease biomarker in humans and may contribute to exercise-induced protection from metabolic diseases.

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Poster Number 141

Novel GLP-1-derived Pentapeptide Attenuates the Development of Metabolic Syndrome in Diet-induced Obese Mice by Modulating the Expression of UCP-1 and UCP-3 in Brown Adipose Tissue

Investigators: Eva Tomas, PhD; Violeta Stanojevic; Joel F. Habener, MD

Infusions of GLP-1(32-36)amide for sixteen weeks in diet-induced obese(DIO) mice curtailed body weight gain, attenuated the development of metabolic syndrome by lowering plasma glucose, insulin, glycerol, and triglyceride levels, and preventing hepatic steatosis(65% decrease in liver triglyceride accumulation). Moreover, basal energy expenditure, determined by the rates of oxygen consumption (VO2) in mice infused with GLP-1(32-36)amide, was increased by 25% and 31% during the light and dark cycles, respectively. Changes in VO2 were independent of physical activity. To address whether these effects on energy expenditure were associated with changes in mRNA and protein expression of uncoupling proteins (UCPs), levels for UCP-1 and UCP-3 were measured in brown adipose tissue (BAT) and UCP-3 was measured in skeletal muscle. UCP-1 mRNA and protein expression levels were significantly increased in BAT from mice receiving GLP-(32-36)amide as well as, but to a lesser extent, expression levels of UCP-3 . Likewise, UCP-3 expression in skeletal muscle was increased. These findings suggest that the attenuation of the manifestations of the metabolic syndrome by GLP-1(32-36)amide in DIO mice is due to increased basal energy expenditure mediated, at least in part, by changes in UCP-1 and UCP-3 expression in BAT and possibly also by changes in UCP-3 expression in skeletal muscle.

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Poster Number 142

The efficacy of glycyrrhizin against production of mitochondria-derived reactive oxygen species in the skeletal muscles in the hindlimb direct burn injury model

Investigators: Ryusuke Ueki, MD, PhD; Jeevendra Martyn, MD, FRCA, FCCM; Shingo Yasuhara, MD, PhD

Introduction: Skeletal muscle wasting in the severely burned patients has been considered one of the major risk factors affecting the patient's prognosis. In this study, using the direct burn on hindlimb (tibialis anterior, TA) muscles, we directly measured the amount of mitochondria-derived reactive oxygen species (ROS) at the local site, and examined the protective effect of glycyrrhizin.

Methods: Under anesthesia with pentobarbital, a third degree burn injury was inflicted on the TA muscle. The fluorescent dye for mitochondrial ROS, MitoSOX, was injected intramuscularly. The TA muscle was analyzed under the in vivo fluorescent microscope. The specificity of the signal was confirmed by signal elimination with anti-oxidant, ascorbic acid. In the treatment group, 50 μg/ml of glycyrrhizin was injected.

Results: In the examination on the same day (day 0), muscles with burn injury showed clearly red stained signal with MitoSOX compared with those from sham-burn group. Increase in the red fluorescence was reduced by treatment with glycyrrhizin to 40.6% compared to the non treatment burn model after 60 minutes from MitoSOX administration (n = 5).

Conclusions: Using a novel in vivo microscopic analysis, we have documented mitochondria-derived ROS production in the skeletal muscle of direct burn injury model. The observed signal was specific to the oxygen radical produced in vivo, because ascorbic acid treatment diminished the signal. The feasibility and usefulness of this new method for evaluating the drug efficacy was demonstrated by showing the effect of glycyrrhizin on the burn-induced increase in the ROS production.

Poster Number 143

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Nitric Oxide Synthase 3 Deficiency Does Not Prevent the Adverse Effects of Transfusing Stored Blood in Murine Hemorrhagic Shock

Investigators: Binglan Yu, PhD; Liu Han, MD; Patricio Leyton, MD; Kenneth D. Bloch, MD; Warren M. Zapol, MD

Background: During prolonged storage, erythrocytes undergo functional changes leading to hemolysis and release of oxyhemoglobin, a potent scavenger of nitric oxide (NO). Mice with a congenital deficiency of endothelial NO synthase (NOS3-/-) are protected from the hypertensive response to infusion of cell-free hemoglobin. We, therefore, tested whether NOS3-/- would impact the adverse effects (tissue injury, inflammation, and mortality) associated with resuscitating mice with hemorrhagic shock using fresh or stored erythrocytes.

Methods: Wild-type and NOS3-/- mice were subjected to 90 minutes of hemorrhagic shock, followed by resuscitation with leukoreduced, syngeneic erythrocytes stored for less than 24 hours (fresh erythrocytes) or stored for 2 weeks (stored erythrocytes). Survival rates, as well as markers of tissue injury and inflammation, were measured.

Results: After hemorrhagic shock, transfusion with stored erythrocytes increased the mortality rate, tissue injury, and levels of inflammatory mediators more than did transfusion with fresh erythrocytes in both genotypes. Four hours after resuscitation with stored erythrocytes, but not fresh erythrocytes, significantly increased plasma hemoglobin levels in both wild-type and NOS3-/- mice. Survival rate and markers of tissue injury and inflammation did not differ when wild-type and NOS3-/- mice were resuscitated with stored erythrocytes.

Conclusions: Our data suggest that NOS3-/- mice are not protected from the adverse effects of hemorrhagic shock resuscitation with stored erythrocytes. These findings suggest that the adverse effects of transfusing stored erythrocytes in mice with hemorrhagic shock are not exclusively attributable to scavenging of NOS3-generated NO by increased plasma hemoglobin levels.

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Poster Number 144

Kerv, A Novel Virulence Factor Of Pseudomonas aeruginosa Dampens Host Defense Response By Modulating the Expression Of the Type VI Secretion Effector Protein HcpC

Investigators: Arunava Bandyopadhaya, PhD; Yiorgos Apidianakis, PhD; Diogo de A. Meireles, PhD; Laurence G. Rahme, PhD

Pseudomonas aeruginosa is an important Gram-negative human opportunistic pathogen. This highly virulent pathogen suppresses host defense mechanisms to promote acute and chronic infections, but how the bacterium overcomes early host innate immune responses remains largely unknown. We have demonstrated earlier that P. aeruginosa (PA14) KerV mediated virulence is conserved in several human pathogens and required for full virulence against evolutionary divergent eukaryotic hosts. Our findings show that kerV controls the expression of the Type VI secretion effector, hcpC, in PA14. The infection of human airway epithelial cells, A549, by kerV and hcpC mutant leads to increased production of interleukin (IL)-8 as compared to the IL-8 expression elicited by isogenic PA14. Both kerV and hcpC mutants showed early IkB phosphorylation and consequently degradation of IkB, NF-kB phosphorylation and NF-kB translocation into the nucleus of the host cell, as well as p38 MAPK phosphorylation, whereas the PA14 significantly delayed activation of NF-kB and p38 MAPK. Moreover, Bay11-7085 and SB203580 treatment prior to infection suggesting that NF-kB and p38 MAPK activation is required for the activation of IL-8. Furthermore, the upregulation of IL-8 upon infection with kerV mutant and its reversal upon complementation with hcpC gene, indicates that kerV regulates the TypeVI secretion effector hcpC and that this effector may be responsible for dampening the host innate response following P. aeruginosa infection. The same effect on IL-8 were also observed with Yersina pseudotuberculosis kerV mutant. Our findings suggested that kerV has a novel and broad significance as a virulence factor in bacterial pathogenesis.

Poster Number 145

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Differential cross-presentation of HIV epitopes by dendritic cells and macrophages identifies variable intracellular peptide degradation rates

Investigators: J. Dinter, MSc; E. Duong, BSc; Y. Xu, BSc; N. Y. Lai, MA; T. Q. Zhu, BSc; S. Le Gall, PhD

Generation of efficient CD8+ T cell responses against HIV requires presentation of viral epitopes on MHC-I by antigen-presenting cells. During cross-presentation phagocytosed antigens are degraded in endo-lysosomes following further degradation by cytosolic proteases before presentation to CTLs. Whether proteases in these cell compartments differentially affect epitope presentation remains open. To address this question we analyzed the cross-presentation of HIV p24-derived epitopes by dendritic cells (DCs) and macrophages to CTL clones. In parallel we compared the epitope production in cross-presentation competent cell compartments by mass spectrometry.

Cross-presentation of HIV p24-protein to epitope-specific CTLs showed significant differences among CTL responses to three HLA-B57 epitopes despite comparable peptide avidities. Preincubation of DCs with protease inhibitors increased certain CTL responses, suggesting that higher peptide degradation limits their presentation. Degradation of long peptide fragments in lysosomal and cytosolic extracts of both cell-subsets analyzed by mass spectrometry showed higher production of fragments containing epitopes efficiently cross-presented. Finally, we assessed by RP-HPLC analysis the intracellular stability of epitopes, which contributes to the amount of epitope available for presentation. Despite high variability among epitope-stability in different compartments we identified a hierarchy, where the most stable epitopes corresponded to epitopes well cross-presented.

Our results show that processing of HIV-proteins by proteases located in different cell compartments affects their degradation patterns thereby contributing to the amount of epitope available for presentation. This information is critical for design of HIV-immunogens that will be delivered to various compartments of DCs where epitopes should be efficiently processed for priming of effective immune responses.

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Poster Number 146

CprA, a putative short-chain dehydrogenase/reductase, is required for antimicrobial peptide resistance of Pseudomonas aeruginosa

Investigators: Alina D. Gutu, PhD; Nicole Sgambati, BS; Jihye Park, PhD; Samuel M. Moskowitz, MD

Pseudomonas is an opportunistic pathogen that causes airway infections in people with cystic fibrosis. Clinical isolates of Pseudomonas are increasingly multi-drug resistant, including resistance to polymyxins, peptide antibiotics that are a clinical "last line of defense". Polymyxin resistance has been attributed to lipopolysaccharide modification, specifically, addition of 4-amino-L-arabinose (L-Ara4N) to lipid A. Several two-component systems (PmrAB, PhoPQ, ParRS, and CprRS) regulate polymyxin resistance. These systems are capable of physiologically inducing expression of the arn operon, encoding enzymes that catalyze L-Ara4N synthesis and addition to lipid A. Specific mutations in these regulatory systems (e.g., mutation of PhoQ) result in constitutive L-Ara4N addition and polymyxin resistance. Indeed, all polymyxin-resistant clinical strains studied to date have L-Ara4N-modified lipid A, indicating that this modification is required for resistance. However, L-Ara4N modification alone is not sufficient for resistance. For example, some polymyxin-susceptible clinical strains have L-Ara4N-modified lipid A, and deletion of the CprRS system in a phoQ mutant results in loss of Pm resistance without loss of L-Ara4N modification. This implies that other loci regulated by the PmrAB, PhoPQ, ParRS, and CprRS systems may be necessary for resistance. Indeed, we have identified one such locus, designated cprA, encoding a putative short-chain dehydrogenase/reductase. Deletion of the cprA gene in a phoQ mutant results in substantial loss of polymyxin resistance, without loss of L-Ara4N modification. These results indicate that both L-Ara4N modification of lipid A and CprA activity mediate polymyxin resistance, thus rendering CprA an attractive target for development of novel anti-pseudomonal drugs that inhibit polymyxin resistance.

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Poster Number 147

RNA interference for mammalian antiviral immunity mediated by Argonautes 2 and 4

Investigators: Kate L. Jeffrey, PhD; Megha Basavappa, BS

RNA interference (RNAi) is a potent nucleic acid sequence-specific means of gene silencing guided by short, double-stranded non-coding RNA. Plants, nematodes and arthropods use RNAi to directly combat viral infections and deletion of RNAi machinery in these organisms leads to uncontrolled virus replication. Whether RNAi can autonomously or cooperatively mediate antiviral immunity in mature mammalian cells with intact pattern recognition receptors and type I interferon (IFN) production remains undemonstrated. The key RNAi effector proteins, Argonautes (AGO)1-4 are functional in mammalian cells, being essential for the development and regulation of the host microRNA and RNAi systems. Though, any specific role for AGOs in mammalian host-virus interactions remains to be fully characterized. In addition, whether individual roles of the four mammalian AGOs exists in any physiological context remains unclear. Here we demonstrate an IFN-independent mode of antiviral immunity mediated by Argonaute (AGO)2 slicing activity and AGO4. Mouse embryonic fibroblasts (MEFs) from AGO2 catalytic inactive knock-in mice and primary macrophages from AGO4 deficient mice displayed significantly enhanced virus replication when infected with Influenza A, Vesicular Stomatitis Virus or Encephalomyocarditis virus, in the presence or absence of type I IFN production and signalling. AGO1 and AGO3 deficient macrophages however displayed no differences in virus levels. Moreover, using crosslinking immunoprecipitation following by high throughput sequencing (HITS-CLIP) analysis in Influenza A-infected macrophages we reveal AGO-bound influenza-derived RNAs with a predominant length of 20-29 nucleotides. Together, this data reveals an important role for AGO2 and AGO4 in antiviral immunity in mature mammalian cells.

Poster Number 148

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The RPF (Rise, Plateau and Fall) Curve; A Common Trend in QA Falls in Radiology

Investigators: Shima Aran, MD; Khalid W. Shaqdan, MD; Laleh Daftaribesheli, MD; Thrall H. James, MD; Hani H. Abujudeh, MD, MBA, FSIR

Purpose: Falls are preventable causes of patient harm. Although several risk factors for falls have been described, no definitive predictor of falls has been reported. Successful interventions for falls prevention are not yet widely in place. We implemented an Outpatient Falls Guideline (OFG) in 2008 in the Radiology department and we aim to describe our multi-year experience.

Methods: IRB-approved retrospective study between Apr-2006 to Sep-2013 to investigate falls. The span of the study was divided into eight periods. The incident reporting system was searched for the fall-related variables. Wilcoxon-signed-rank test was used for statistical analysis.

Results: A total of 327 falls out of 5,080,512 radiology examinations (rate:0.64/10,000 total examinations) were reported (M:F 131:196, mean age of 59.1±20.8). There was a significant increase in total number of falls reported between the period-2 (26/637965; rate:0.41) and period-3 (47/643207; rate:0.73) (p=0.02). There was a statistically significant decrease in outpatient falls between the period-6 (49/538251; rate:0.91/10,000) and period-7 (27/569739; rate:0.47/10,000) (p=0.01). The rate of falls in patients ≥60 and <60y/o was (177/2180093; rate:0.81/10,000) and (150/2900419; rate:0.52/10,000), with a significant difference (p=0.007). Although the rate of fall was higher in females, there was no significant difference between genders (p=0.18).

Conclusion: This curve, the rise the plateau and the fall (RPF) seems to make sense in QA projects. The initial rise may be due to uncovering of "under-reporting". The plateau may represent the closest value to the true incidence, and the fall may represent the effectiveness of the program, although drifting may be a causative factor.

Poster Number 149

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Altered manifestations of skin disease at sites affected by nerve damage

Investigators: Ehsan Azimi, MD; Ethan A. Lerner, MD, PhD; Sarina B. Elmariah, MD, PhD

We hypothesize that inflammation, at least in the context of skin diseases, requires neural input. This hypothesis emerges as a result of several observations. First, the concept of 'neurogenic inflammation', was proposed a century ago. Second, our laboratory demonstrated a physical connection between Langerhans cells and sensory fibers, a finding that has been extended by others to mast cells and keratinocytes. Third, thymic stromal lymphopoietin, the cytokine considered the key mediator of the atopic march from eczema to asthma, interacts directly with sensory nerves to mediate itch. Fourth, there is evidence that many inflammatory conditions, including asthma, inflammatory bowel disease, rheumatoid arthritis, and those that affect the skin, require an intact neural component for their conventional manifestations. We searched the literature and identified 23 cases of altered manifestations of skin disorders in patients with acquired central or peripheral neural damage or dysfunction. The clinical entities included psoriasis, atopic dermatitis, contact dermatitis, rosacea, bullous pemphigoid and scleroderma. In nineteen cases, near or complete resolution of skin lesions was observed in the areas innervated by the injured nerves. Skin lesions cleared or were diminished in all cases of psoriasis and scleroderma and in nine of ten patients with eczematous dermatoses. In four cases, recurrence was observed following recovery from the nerve injury. These cases, akin to conditional knockouts, highlight the importance of neural innervation and neurogenic inflammation in the development of inflammatory skin diseases. Our hypothesis suggests that targeting of sensory nerves may provide an approach to the treatment of inflammatory diseases.

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Poster Number 150

Hip Geometry and Strength are Impaired in Women with Anorexia Nervosa and Increased in Overweight/Obese Women

Investigators: Katherine Bachmann, MD; Pouneh Fazeli, MD; Elizabeth Lawson, MD, MMSc; Brian Russell, BA; Ariana Riccio, BA; Erinne Meenaghan, NP; Anu Gerweck, NP; Miriam Bredella, MD; Anne Klibanski, MD; Karen Miller, MD

Context: Anorexia nervosa is complicated by elevated fracture risk, and obesity may be associated with increased fracture risk at some skeletal sites. Traditional bone mineral density (BMD) measurements have limited ability to predict fracture at both weight extremes. Hip Structural Analysis (HSA) uses dual energy xray absorptiometry (DXA) data to assess hip geometry and model hip strength. HSA variables predict fracture risk and correlate strongly with quantitative CT, without exposure to CT radiation doses.

Methods: We performed HSA in 368 women (ages 19-45): 246 with anorexia nervosa, 69 normal-weight, and 53 overweight/obese women of comparable mean age.

Results: Hip geometry (subperiosteal width, cortical thickness; $p \leq 0.02$) and all hip strength parameters (cross-sectional area, cross-sectional moment of inertia, section modulus, buckling ratio; $p < 0.003$) were impaired at all sites (narrow neck, intertrochanteric region, femoral shaft) in anorexia nervosa compared to normal-weight women. Among anorexia nervosa women, cortical thickness and all hip strength parameters were positively associated with BMI (R 0.18 to 0.36) and lean mass (R 0.17 to 0.70), and negatively associated with duration of anorexia nervosa (R -0.18 to -0.48) and amenorrhea (R -0.14 to -0.48). Overweight/obese women had greater cortical thickness ($p < 0.002$) and strength compared to normal-weight controls at all sites ($p \leq 0.03$) except for buckling ratio at the narrow neck.

Conclusions: Women with anorexia nervosa have impaired hip geometry and strength, consistent with higher reported fracture rates, which may help explain discrepancies between BMD assessment and fracture rates. In contrast, hip strength in overweight/obese women is increased compared to normal-weight controls.

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Poster Number 151

Preoperative Erythropoietin Alpha Reduces Postoperative Transfusion in THA and TKA, But is Not Cost Effective

Investigators: Hany Bedair, MD; Judy Yang, MD; Maureen Dwyer, PhD, ATC; Joseph McCarthy, MD

Pre-operative erthropeoitin alpha (EPO) has been shown to be effective at reducing post-operative blood transfusions following total hip and knee replacement surgery; however, treatment with EPO is associated with high costs. The goal of this study was to evaluate the cost effectiveness of preoperative EPO in patients at high risk of requiring transfusion after joint replacement surgery. We showed that preoperative EPO was highly effective at reducing the need for post-operative transfusions in high risk THA and TKA patients, but was not found to be cost-effective. The transfusion rate was significantly lower in the EPO group; however, the decision tree analysis demonstrated that EPO ($380/unit) is not cost effective. The sensitivity analysis demonstrated that the cost of EPO would need to be less than $129/unit for this strategy to be cost effective. The willingness-to-pay calculation demonstrated that the desirability of avoiding a blood transfusion would have to be equal to or more than the monetary equivalent of $832 per patient in order for the EPO strategy to be cost-effective. While the high cost of EPO may necessitate consideration of other strategies, it is difficult for these types of analyses to capture intangible benefits of lower transfusion rates for patients. Given the effectiveness of preoperative EPO on reducing the need for transfusions in high risk THA or TKA patients, the high costs associated with this treatment need to be re-evaulated.

Poster Number 152

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Association between 25-hydroxyvitamin D level and pre-operative quality of life in joint replacement surgery patients

Investigators: L. Blum, BA; S.I. Young; H. Bedair, MD; A. Freiberg, MD; S.A. Quraishi, MD, MHA

Background: Vitamin D is important for optimal musculoskeletal health and may have significant implications for quality of life (QoL) in elderly patients. Since the prevalence of 25-hydroxyvitamin D (25[OH]D) levels <20 ng/mL before elective joint replacement surgery exceeds 40%, our goal was to investigate whether vitamin D status is associated with QoL in this cohort of patients.

Methods: We performed a single-center retrospective analysis of patients who underwent elective hip or knee replacement surgery from 2002–2012. To investigate the association of 25[OH]D (< or ≥20 ng/mL) with QoL (EQ-5D < or ≥75), we performed a multivariable logistic regression analysis while controlling for age, sex, race, body mass index, and American Society of Anesthesiologists physical status score.

Results: We identified 248 patients who met inclusion criteria. Mean ± standard deviation 25[OH] D level and EQ-5D score were 25±13 ng/mL and 72±21, respectively. Patients with 25[OH]D levels ≥20 ng/mL were 5 times more likely to have EQ-5D scores ≥75 [adjusted odds ratio (OR) 5.02; 95% confidence interval 1.11-12.77]. Further adjusting for "type of 25[OH]D assay" or "type of joint surgery" did not materially change this result.

Conclusion: In our cohort of hip and knee joint replacement surgery patients, 25(OH)D levels were associated with EQ-5D scores. 25(OH)D levels may represent a novel biomarker for assessing QoL in patients with musculoskeletal disorders. Prospective, randomized, clinical trials are needed to verify whether optimizing pre-operative vitamin D status may improve QoL and influence clinical decision-making in hip and knee joint replacement surgery candidates.

Poster Number 153

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Outcomes after Implementation of an Inpatient Antibiotic Prescribing Pathway for Patients with Penicillin or Cephalosporin Allergy

Investigators: Kimberly G. Blumenthal, MD; Erica S. Shenoy, MD, PhD; Christy Varughese, Pharm D; David Hooper, MD, PhD; Aleena Banerji, MD

Rationale: There are no standardized guidelines for inpatient providers on drug allergy history-taking or antibiotic prescribing for penicillin (PCN) or cephalosporin-allergic patients.

Methods: An antibiotic prescribing pathway was developed to assist providers in history-taking, antibiotic prescribing, and performance of test doses for inpatients with PCN or cephalosporin allergy. The pathway was implemented at a 947-bed tertiary care facility beginning April 2013. All test doses through the pathway were performed by the primary inpatient team unless the pathway directed Allergy/Immunology (AI) consult. A retrospective pre/post analysis compared test doses, hypersensitivity reactions (HSRs), and use of PCN skin testing quarterly in the year prior to implementation of the pathway to the first quarter following implementation.

Results: Prior to pathway implementation, an average of 11 test doses per quarter were performed, all with AI consult. In the post-period, 39 test doses were performed of which 7 (18%) were performed with AI consult. There were only 3 HSRs among all test doses with no difference in rate between the pre and post periods (p>0.5). In the post-period, 100% of test doses performed with AI appropriately consulted and required PCN skin testing, which was significantly higher than the rate of test doses that required PCN skin testing prior to pathway implementation (48%, p =0.012).

Conclusion: The implementation of an antibiotic prescribing pathway resulted in a 3.5-fold increase in the overall number of test doses to PCN and cephalosporin antibiotics. The pathway increased the efficiency of AI consultation for PCN allergy while maintaining patient safety.

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Poster Number 154

One Man's Noise is Another Man's Data

Investigators: Mia Borzello; Cat Chu, MD; A.M. Chan, PhD; Omar Ahmed, PhD; Emad Eskandar, MD; Sydney S. Cash, MD, PhD

Previous investigations of the spatiotemporal dynamics of oscillatory behavior in the brain has led to the concept that slower activity has coherence spanning a wider area than that of higher frequency activity. This is seen as one of a small number of nearly universal rules which dictates activity in cortical and subcortical structures. There is substantial evidence for this proposition in the literature but surprisingly few direct investigations, especially in human cortex. Furthermore, the majority of investigations of this issue have focused on relatively large distances. To more completely characterize the spatial characteristics of ongoing brain activity, we investigated the coherence in different brain states, awake and sleep, at different frequencies and with respect to a wide range of distances using both standard pial surface macroelectrode arrays (1 cm spacing), macroelectrode arrays which penetrate the brain parenchyma and both microgrid (1 mm spacing) and NeuroPort microelectrode arrays (400 micron spacing). As expected, we found that coherence decreases as a function of increasing interelectrode distance.For distances 1 cm and greater, the relationship was largely non-linear, and smaller gaps behave more linearly. This relationship was not strongly affected by recording location, nor was the overall coherence significantly different between awake and asleep states. The impact of frequency on spatial relationship was variable from subject to subject but showed a trend toward more rapid declines in coherence in higher frequency bands. These data are congruent with the overall notion that frequency and spatial relationships are inversely related with faster frequencies being more focal.

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Poster Number 155

The role of predictive algorithm selection on the accuracy of MRI-based prediction of tissue outcome after acute ischemic stroke

Investigators: Mark JRJ Bouts, PhD; Elissa McIntosh, BA; Raquel Bezerra, MD; Izzuddin Diwan, BS; Steven JT Mocking, MS; Priya Garg, PT; William T. Kimberly, MD, PhD; Ethem M. Arsava, MD; William A. Copen, MD; Pamela W. Schaefer, MD; Hakan Ay, MD; Aneesh B. Singhal, MD; Bruce R. Rosen, MD PhD; Rick M. Dijkhuizen, PhD; Ona Wu, PhD

Early assessment of tissue at risk of infarction is crucial for treatment decision making in acute ischemic stroke. Current clinical guidelines impose a strict time window of at most 3-4.5 hours for thrombolysis, but may unnecessarily exclude certain patients that may benefit beyond this time-window. Patient care may be strongly improved by a more individualized assessment of tissue at risk of infarction. Magnetic resonance imaging (MRI)-based prediction algorithms integrate on a voxel-wise basis multiple acute MRI parameters in a single, quantitative probabilistic index and have shown great promise as an effective tool for rapid evaluation of tissue outcome to guide patient triage. Yet the merits of certain of these algorithms over others have remained largely unclear. In this study we retrospectively analyzed and compared four different MRI-based predictive algorithms (generalized linear model (GLM), generalized additive model, adaptive boosting (ADA), and random forest) in their ability to predict tissue outcome after stroke in patients after acute ischemic stroke (n=111), that did not receive subsequent interventional revascularization treatment. We showed that all predictive algorithms were more accurate in predicting the extent of tissue infarction over single MRI parameter-based predictions. Furthermore, in contrast to experimental stroke imaging results, we found that more complex prediction algorithms (like ADA) -that assume non-linear relationships between acute imaging parameters and tissue outcome - showed significantly improved predictions of eventual tissue infarction over those that do not assume such relationships (like GLM). Therefore more complex predictive algorithms may better capture human stroke development, improving voxel-based tissue outcome predictions.

Poster Number 156

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A Multi-center Study Evaluating the Reasons for Revision of ASR Hip Implants

Investigators: Charles Bragdon, PhD; Orhun Muratoglu, PhD; Daniel Hussey, BS; Henrik Malchau, MD, PhD

Introduction: The ASR hip system was recalled due to adverse tissue reaction. The purpose of this multicenter study was to review revision rates and reasons for revision of ASR hip replacement.

Methods: Demographic and surgical data was provided to this multi-center study from 10 clinical centers in six different countries.

Results: The overall revision rate for the entire cohort of 2918 hips was 18% with an overall revision rate for ALTR of 8%. Of the revision cases, 45% were for ALTR or had the presence of ALTR observed at the time of surgery. Of the non-ALTAR revisions, the overall revision rate for cup loosening was the highest at 7.1%. Sepsis and stem loosening each accounted for 1% of the overall revision rate. The average Co and Cr blood ion levels of the revised patients were higher at each center than the average for the non-revised patients, though there was a significant overlap between the two groups.

There was considerable variation in the overall revision rate between centers, 5-30%. The revision rate for ALTR was similarly broad, 0-32%. The overall revision rate of ASR XL hips was 23% while that of ASR hips was 8%. A multi-variant analysis of this data in currently being performed.

Discussion: There was considerable variation between centers in the overall revision rate of this particular MOM implant system and the incidence of ALTR was similarly broad. Interestingly, the overall revision rate of the ASR XL hips was significantly higher than the ASR resurfacing hips.

Poster Number 157

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Effects of growth hormone administration for 6 months on bone turnover and bone marrow fat in obese premenopausal women

Investigators: Miriam A. Bredella, MD; Anu V. Gerweck, NP; Lauren A. Barber, BS; Martin Torriani, MD; Karen K. Miller, MD

Abdominal adiposity is associated with low bone mineral density (BMD) and decreased growth hormone (GH) secretion, an important regulator of bone homeostasis. The purpose of our study was to determine the effects of GH administration for 6 months on markers of bone turnover and bone marrow fat in premenopausal women with abdominal obesity. We hypothesized that GH administration for 6 months would increase bone formation and increase bone marrow fat, and that increased bone formation is mediated by an improvement in body composition (decrease in abdominal fat and increase in muscle mass), an increase in circulating vitamin D, and a reduction in inflammatory cytokines and lipoproteins. In a 6-month, randomized, double-blind, placebo-controlled trial we studied 79 abdominally obese premenopausal women. Here we show that short-term (6 months) GH administration increases markers of bone formation and bone marrow fat. The increase in bone formation is associated with a decrease in abdominal fat, inflammation and lipoproteins and an increase in IGF-1 levels, vitamin D and muscle mass, suggesting that increases in bone formation by GH may be at least in part mediated by increases in circulating IGF-1 levels, changes in body composition, increases in circulating vitamin D levels and decreases in systemic inflammation and Apo B. The increase in bone marrow fat may reflect changes in energy demand from increased bone turnover. The effects of a longer course of GH therapy in obese individuals, such as has been shown to increase BMD in hypopituitary populations, are unknown and warrant further investigation.

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Poster Number 158

Prepackaged Noodles in Styrofoam Microwavable Container Result in Increased Scald Burns

Investigators: M. Donovan, RN, PNP; J.A. Fabbri, RN, PNP; R.L. Sheridan, MD; P. Chang, MD

Introduction: Over the last few years prepackaged noodle soups have been reported as a causative agent in soup scald burns. The purpose of this study was to determine if burns related to prepackaged noodle soup follow a predictable pattern.

Methods: Information on patients < 18 years of age, treated for scald burns from prepackaged noddle soup during the years 2011 through 2013 was gathered from a data registry bank maintained by the outpatient department of a tertiary burn care facility.

Results: Thirty children, aged 11 months to 17 years, were seen at our facility. The majority of burns occurred in school aged children (53%). Soup packaged in polystyrene cups represented the highest incidence of these scalds (81%) vs. square noodle packs in bowl (19 %). The most frequently reported cooking method was microwave heating (85%) vs. stove (15%). The most frequent burn site was the anterior thighs and the perineum (60%). Burn injury most commonly occurred with the child as the preparer (60%), during removal and transport (48%), seated on the couch (30%) and reaching and spilling onto self (22%).

Conclusion: The risk for scald injury is high in school age children who prepare soup in microwave polystyrene cups. As providers of anticipatory guidance we must take an active role in educating parents and consumers on the safety issues of prepackaged noodle soup.

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Poster Number 159

Assessment, Screening, and Quality Control Efforts in an Outpatient Clinic Serving OEF/OIF/OND Veterans and Families

Investigators: Yang Chen, BS; Eric Bui, MD, PhD; Rebecca Weintraub Brendel, MD; Bonnie Ohye, PhD; Elizabeth Goetter, PhD; Naomi Simon, MD, MSc

Post-traumatic stress disorder (PTSD) and traumatic brain injury (TBI) have become signature injuries of Iraq and Afghanistan veterans. Nonetheless, many veterans report a variety of other mental health problems that may impede treatment for PTSD and TBI. Implementing standardized screening and assessment procedures can help better identify conditions needing attention for the veteran and distressed family members, and ultimately improve clinical care. The Red Sox Foundation/Massachusetts General Hospital Home Base Program was developed in 2009 to increase care options for veterans. We integrated evidence-based screening procedures to promote comprehensive clinical care. Data were examined from a data repository for 277 patients (mean age = 34.0 [SD=9.1], 23% women, 83% veterans, 17% family members) who participated in the evaluation assessments since April 2012. Patients were systematically assessed for PTSD symptoms, alcohol use disorder, loss and complicated grief, pain, stress, anxiety, and depressive symptoms, couples satisfaction, child functioning, and parenting competence. Over 85% of patients completed at least one screening measure. Results revealed that among veterans, 51% scored above standard cut points for problematic alcohol use, 90% scored above the cutoff for problematic anger, 35% screened positive for severe depression, and 24% had complicated grief. Among patients in a relationship, 54% indicated significant relationship distress. Among those with children, 35% reported problems with parenting and 24% reported emotional problems in their children. Standardized screening is useful in identifying comorbid conditions that may interfere with successful course of treatment in veterans and their family members. Such screening allows for comprehensive and individually-tailored care.

Poster Number 160

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Short-Term Succinylcholine Infusion During General Anesthesia May Not Result In Clinically Significant Phase II Block

Investigators: Christopher T. Chenelle, BA; Demet Sulemanji, MD; Geng Li, MD; Jingping Wang, MD, PhD; Mazen Maktabi, MBBCh; Robert M. Kacmarek, RRT, PhD; Yandong Jiang, MD, PhD

Succinylcholine has many advantages as a muscle relaxant including quick onset and reversal and profound muscle relaxation. Although it is used frequently as a single bolus for endotracheal tube placement, continuous succinylcholine infusion is not commonly used due to anesthesiologists' fear of side effects including prolonged neuromuscular block due to deficiencies of plasma cholinesterase.

Our aim was to determine if succinylcholine infusions during short-duration surgery produced clinically significant prolonged block or increased the need for post-extubation ventilatory support.

Electronic anesthesia records of all laparoscopic cholecystectomy cases performed by a single surgeon over a seven-year period at our institution were retrospectively reviewed. Cases were allocated into 2 groups: Group S, patients who received succinylcholine infusion only (n=531), and Group N, patients who received non-depolarizing neuromuscular agents only (n=95). The time from the end of surgery to extubation and the rate of post-extubation ventilatory support were calculated.

The average duration of succinylcholine infusion was 26.3±9.3 minutes, delivering a total dose of 3.9 (3.3, 4.6) mg/kg. Time to extubation was shorter in group S than group N (N: 9 (5-15), S: 5 minutes (3-9), $p<0.001$). The requirement for post-extubation ventilatory support did not differ between groups, with 3 of 531 patients (0.56%) requiring additional support in group S, and 1 of 95 patients (1.05%) in group N.

Our results demonstrate that short-term succinylcholine infusions did not cause clinically significant prolonged neuromuscular block or alter the rate of postoperative ventilatory support. A prospective study is warranted to validate these findings.

Poster Number 161

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Is the Massachusetts Graduated Driver Licensing Program Effective in Preventing Fatal Motor Vehicle Crashes in Teenage Drivers?

Investigators: Catrina Cropano, BSc; Yuchiao Chang, PhD; Jarone Lee, MD, MPH; Haytham Kaafarani, MD, MPH; Toby Raybould, MS; Alice Gervasini, PhD, RN; Laurie Petrovick, CPHQ, MSc; Chris DePesa, RN, MS; Peter Masiakos, MD

Introduction: Comprehensive Graduated Driver Licensing (GDL) programs restricting and phasing in driving privileges for teenage drivers intend to reduce traffic incidents and fatalities in novice drivers. However, the effect of GDL laws is controversial. Here, we evaluate the effect of the 2007 Massachusetts Junior Operating Law to determine whether it has reduced the rate of fatal MVCs in young drivers.

Methods: The Fatality Analysis and Reporting System database was queried for all fatal MVCs between 2002 and 2011. Three driver age groups were compared: 16-17, 18-20, and 25-29. The latter group served as a control group that the law does not affect. We compared the rates of fatal MVCs (per population) for each age group in the pre-law (2002–2006) and post-law (2007–2011) periods. As a sensitivity analysis, we then compared the rates of fatal MVCs using the number of issued licenses as the denominator. Population data was obtained via the Missouri Census Data Center.

Results: Following the enactment of the MA GDL, the rate of fatal MVCs has decreased for the 16-17 age cohort, (14.0, 8.6, $p=0.0006$) and 18-20 age cohort, (21.2, 13.7, $p<0.0001$). The rate of fatal MVCs in the 25-29 age group (1.4, 1.8, $p=0.58$) have remained unchanged. Similar results were obtained when the numbers of licenses were used as denominators.

Conclusions: The 2007 GDL was effective in decreasing the rate of fatal MVCs in the Massachusetts teenage driver population. Similar GDLs should be considered in other states to decrease the rate of fatal MVCs in this population.

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Poster Number 162

Real Time fMRI Neurofeedback: Effect on Food and Cigarette Cue Reactivity

Investigators: V. Calderon, BA; M. T. Curran, BS; S. Ghosh, PhD; A. Keshavan, BS; J.P. Stern, BA; S. Whitfield-Gabrieli, PhD; J. Gabrieli, PhD; A. E. Evins, MD, MPH; L. E. Stoeckel, PhD

Drugs of abuse and highly palatable foods activate similar neural circuits, and abnormalities in these brain systems can lead to addiction and, in the case of food, ingestive behaviors that resemble addiction. Real time fMRI (rtfMRI) neurofeedback is one novel tool that may help people self-regulate their regional brain activity to improve control of these disrupted behaviors.

In this study, we investigated whether rtfMRI neurofeedback could be used to enhance the effectiveness of cognitive reappraisal to help smokers and otherwise healthy controls improve self-regulation of cue-induced cravings for cigarettes (smokers) and highly palatable foods (controls). 16 otherwise healthy smokers and 16 controls were trained to develop cognitive regulation strategies that they would use while viewing smoking or food cues in an fMRI scanner during two study visits.

Our results showed that smokers were less effective than controls at regulating the following reward and emotion-related brain regions: medial orbitofrontal cortex (mOFC), right ventral striatum (rVS), and right amygdala (rAMYG). Both groups reported decreased craving in down- vs. up-regulation trials, but this was not associated with fMRI-based brain activity levels.

Compared to non-smokers, smokers appear to be less effective at regulating reward- and emotion-related brain activity, which may contribute to the maintenance of harmful smoking behavior. Future research will explore the relationship between participants' emotional regulation style as measured by the Emotion Regulation Questionnaire and their ability to self-regulate disrupted reward and emotion-related brain activity to determine whether this information could be used to optimize the development of brain-based emotion regulation therapies.

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Poster Number 163

Dog Attacks On Children: An Increasing Cause Of Major Pediatric Trauma

Investigators: Michael M. Fuenfer, MD; Robert Sheridan, MD; Curtis L. Cetrulo, MD; Natan Noviski, MD; Peter T. Masiakos, MD

Purpose: The incidence of dog attacks on children is increasing and may result in devastating multisystem injuries. A well-coordinated, multidisciplinary approach involving both pediatric and adult specialists may be beneficial for optimal management of these injuries. The purpose of this study was to define the epidemiology of serious dog attacks on children and to report our institutional approach to the treatment of these injuries.

Methods: After Institutional Review Board approval, records of children admitted from 2007–2010 after dog attacks were reviewed. The American College of Surgeons National Trauma Data Base (NTDB) was queried to define the epidemiology of these injuries.

Results: Between 2007 and 2010, the number of serious injuries as a result of dog attacks in the pediatric population has nearly doubled (841 to 1672). Younger children were most likely victims and the head, neck, face, and extremities were the most common injuries. Because these injuries involve multiple regions and may include massive soft tissue loss, and vascular compromise, we used an early multidisciplinary treatment approach at our institution. After initial resuscitation, a thorough assessment of the extent of injury was performed by a team which included trauma, vascular, and facial plastic surgeons and an intensivist.

Conclusions: The incidence of traumatic injuries resulting from dog attacks on children is increasing. A controlled, well coordinated, and multidisciplinary approach to these complex injuries has resulted in excellent cosmetic and functional outcomes in our patients.

Poster Number 164

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Neuropsychological Function and Predictors of Cognitive Impairment in Geriatric Mood Disorders

Investigators: Jennifer R. Gatchel, MD, PhD; Brittany Jordan-Authur, BA; Kathryn E. Lewandowski, PhD; Cara F. McCabe, BA; David Harper, PhD; Brent Forester, MD

Older adults with major depressive disorder (MDD) and bipolar disorder (BPAD) experience cognitive deficits in memory, attention, executive functioning and processing speed. However, findings regarding the nature of cognitive deficits between these diagnostic groups remain inconsistent. We examined 119 older adults with mood disorders (n=47 MDD and n=72 BPAD (n=61 males and 52 females; mean age 67.4 years)) and 45 healthy controls (n= 28 males and 16 females, mean age 65.4 years). Mood was evaluated using the Montgomery-Asberg Depression Rating Scale, Hamilton Depression Rating Scale 17 item, and the Geriatric Depression Scale-Short Form. Cognitive performance was evaluated using scales from the Consortium to Establish a Registry for Alzheimer's Disease Clinical and Neuropsychology (CERAD), the Wisconsin Card Sorting Test (WCST), Trails A and B and the Stroop Color and Word Task. The Clinical Illness Rating Scale (Geriatric) (CIRS(G)) was used to assess medical co-morbidity and the Wechsler Abbreviated Scale of Intelligence (WASI) to measure intellectual functioning. Older adults with mood disorders performed worse than controls on most measures, including Trials A and B, WCST, and the CERAD, and also differed based on diagnosis. Executive functioning and verbal memory deficits remained significantly different between patients and controls after adjusting for IQ. Older adults with MDD and BPAD have significant decline in cognitive function across mulitple domains that is distinct between disorders. Ongoing work examining the effects of gender and medical comorbidity on cognition will provide insight into the psychopathology and has implications for clinical management of older adults with mood disorders.

Poster Number 165

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Early Follow-up of a Long-term Global Multicenter study on New Materials in Total Hip Replacement

Investigators: Meridith E. Greene, BA; Audrey K. Nebergall, BA; James I. Huddleston, MD; Roger Emerson, MD; Eduardo Garcia Cimbrelo, MD; Peter Gebuhr, MD; Anders Troelsen, MD; Henrik Malchau, MD, PhD

Introduction: Preclinical studies of vitamin E diffused highly cross-linked polyethylene (VEPE) show improved fatigue strength and enhanced mechanical properties that are less prone to wear. Both early and long-term clinical outcomes are important to document to ensure no detrimental effects of new materials and assess their performance for clinical use.

Methods: 977 patients from 17 centers in North America and Europe are enrolled into a prospective 10-year outcome study. Patients received either a VEPE liner or medium cross-linked (XLPE) liner. At each follow-up interval, 3 radiographs are obtained and 5 patient reported outcome measures (PROMs) completed (Harris hip score, case mix indicator, UCLA, SF-36, EQ-5D). Radiographs were measured for cup and stem position and femoral head penetration into the liners. Postoperative complications and revisions are also collected.

Results: Mean age at surgery was 62±9 years, 51% were male, and 90% were white. At 3-year follow-up there were 15 dislocations in 11 patients and 13 revisions. Five patients died due to unrelated causes. Wear analysis from postop to 3-years showed a penetration rate at 0.01 mm/year for XLPE and a penetration rate of 0.003 mm/year for VEPE with no significant difference ($p=0.43$). Improvement was seen in all PROMs pre-op to 3-years postop ($p<0.0001$).

Conclusion: Early follow-up of VEPE liners provide encouraging clinical and radiographic results with few intra- and post-operative complications. PROMs indicate improvement in functionality and quality of life across the centers after hip replacement. We have not observed any early adverse effects from diffusing the liners with vitamin E.

Poster Number 166

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Clinical implementation of 3D quality assurance protocol for Intensity Modulated Radiation Therapy of prostate, Head and Neck, and thoracic malignancies

Investigators: Gueorgui Gueorguiev, MS; Christopher Cotter, MS; Meredith Reynolds, BS; Julie Catherine Turcotte, MS; Bruce Crawford, MS; Gregory Sharp, PhD; Mufeed Mah'D, PhD

Purpose: Design novel 3D Intensity Modulated Radiation Therapy quality assurance protocol that will test patient's radiation treatment plan viability.

Methods: For this study, measurements were performed on 13 prostate, 25 Head and Neck and 25 thoracic patients. All patient plans passed traditional quality assurance methods, including single ion chamber measurement and 2D gamma analysis. Total of 102 measurements were performed on selected patients using COMPASS system for 3D quality assurance. Based on the results we determined set of treatment plan structures, statistical parameters that will be investigated during quality assurance process and tolerance levels for those parameters that determine if quality assurance passes. The following statistical parameters were evaluated: difference between Treatment Planning System computed and measured average radiation doses, 3D gamma test, structure volume for which the Treatment Planning System computed and measured dose difference is greater than 6%.

Results: Based on the measurements performed, we established the following tolerance levels determining if quality assurance passes for: maximum allowed computed and measured average dose difference is 6%; maximum 4% of the structure volume may fail 3D gamma test; maximum 4% of structure volume may have computed and measured absolute dose difference greater that 6%.

Conclusion: 3D Intensity Modulated Radiation Therapy quality assurance protocol is a powerful and versatile tool for pre-treatment plan verification, with ability to perform variety of statistical tests. We showed that it is superior to current quality assurance methods and allows us to make much more informed decision if patient's radiation treatment plan is clinically viable.

Poster Number 167

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Quantification in Imaging Reimagined: Evidence-based Approach in Exploring Imaging Biomarkers

Investigators: Amir Imanzadeh, MD; Anand K. Singh, MD; Garry Choy, MD; Gordon J. Harris, PhD

Purpose: Tumor response to systemic therapy on imaging is assessed by Response Evaluation Criteria in Solid Tumors (RECIST) along with 3D estimation of tumor volumes at selected centers; however, some chemotherapeutic agents may show false positive enlargement of the total tumor size. Thus an imaging-based biomarker method where quantification of necrotic and viable fractions in tumor can be done may be a more reliable guide. In this study we compared RECIST criteria, interval change in total tumor volume (TTV) criteria and proposed non-necrotic volume (NNV) criteria for prediction of therapy response in hepatocellular carcinoma (HCC).

Methodology: Pre-treatment and post-treatment CT datasets of 39 consecutive patients with pathologically proven & treated HCC were included in this study. Non-necrotic tumor volume derived after subtraction of necrosis from TTV. Inter-method agreement (kappa) and Kaplan Meier curves for overall survival by each method were calculated. Univariate and step-wise logistic regression analysis used to derive the most optimal fitting method model.

Results: Kappa for agreement between RECIST criteria and other methods was low (κ <0.1). Response assessment by the proposed NNV criteria had a better relation to the overall outcome [Odds ratio (OR) =0.0171; p<0.0001] compared to TTV (OR=0.1154; p=0.0185) and RECIST (OR=0.3697; p=0.1688) with stepwise logistic regression analysis revealing NNV criteria as the most optimal method in the study model.

Conclusion: Based on our preliminary findings interval change in CT-based, non-necrotic liver tumor volume can be successfully estimated and is a better predictor of therapy response than existing RECIST and total tumor volume criteria's.

Poster Number 168

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Advanced Cross-sectional Imaging for Congenital Heart Disease: a Comparison of the Radiation and Time Expense of Cardiovascular Computed Tomography and Magnetic Resonance Imaging at a Tertiary Medical Center

Investigators: Phillip Kim, MS; Harshna Vadvala, MD; Ashley Lee, BS; Ami Bhatt, MD; Doreen Defaria Yeh, MD; Richard Liberthson, MD; Thomas Macgillivray, MD; Ignacio Inglessis, MD; Godtfred Holmvang, MD; Sanjeev Francis, MD; Quynh Truong, MD; Rajiv Gupta, MD; Mannudeep Kalra, MD; Udo Hoffmann, MD; Suhny Abbara, MD; Brian Ghoshhajra, MD

Background: Cardiac Magnetic Resonance Imaging (MRI) and cardiac Computed Tomography (CT) offer distinct yet overlapping information in congenital heart disease patients. While the decision of whether to image and which modality to use is complex, tradeoffs between the two modalities include radiation exposure and acquisition time.

Objective: We aimed to analyze the radiation and time expense of congenital heart disease imaging by CT versus MRI.

Methods: We recorded radiation exposure, scan times, methods, and indications for all congenital heart disease patients whom underwent CT with 128-slice dual source CT at a tertiary care facility between May 2011 and July 2013. An indication-matched cohort of MRI scans was identified and the same information recorded. Age-adjusted effective dose (ED) was stratified according to scan acquisition (anatomy, function, or delayed imaging), and body-mass index (BMI).

Results: 89 CT patients, and 55 indication-matched MRI scans were identified (coronary evaluation was not compared). The median ED for CT was 3.9 milliSieverts (mSv) (all patients, total), with median 2.2 mSv for structural, 2.1 mSv for functional, and 1.3 mSv for delayed enhancement acquisitions, respectively. Radiation expense varied with BMI, from 1.0 mSv for delayed imaging at BMI<25, to 5.2 mSv for functional acquisition at BMI >35. CT exam time was 13 ± 8 minutes versus 93 ± 25 minutes for MRI.

Conclusion: The incremental radiation expense of each CT acquisition, and the time expense of MRI, should be considered when selecting the appropriate test for each individual patient.

Poster Number 169

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Opening Pandora's Box: Understanding the Nature, Patterns, and 30-day Outcomes of Intraoperative Adverse Events

Investigators: Michael N. Mavros, MD; George C. Velmahos, MD, PhD; Andreas Larentzakis, MD, PhD; LeilyNaraghi, MD; Dante Yeh, MD; Peter Fagenholz, MD; Marc DeMoya, MD; David R. King, MD; Jarone Lee, MD, MPH; Haytham M.A. Kaafarani, MD, MPH

Background: Little evidence exists about the characteristics of intraoperative adverse events (iAEs).

Design: The ICD-9-CM based Patient Safety Indicator "Accidental Puncture/Laceration" was used to screen the National Surgical Quality Improvement Program (ACS-NSQIP) database for iAEs. All iAEs were confirmed with a systematic review of operative reports. Standard peri-operative and intraoperative data were supplemented with additional variables including type/organ of injury, phase of operation iAE occurred, adhesions, type of repair, and need for intraoperative consultation.

Results: Of 9292 patients, 227 iAEs in 187 patients were confirmed. The most common injuries were enterotomies in intestinal surgery and vessel injuries in hepatopancreaticobiliary surgery. Injuries occurred most often during the dissection and resection/reconstruction operative phases. Adhesions were present in 120 patients (64%); 108 iAEs (48%) specifically occurred during adhesiolysis, more commonly in intestinal vs. other abdominal surgery (73% vs. 35%, $p<0.001$). A third of the iAEs required organ/tissue resection or complex reconstruction (24% and 13%, respectively), while the rest needed simple suture repair (50%) or vessel ligation/cauterization (13%). Due to iAEs, 20 intraoperative consults (11%) were requested and 9 of 66 (16%) laparoscopic cases were converted to open. Thirty-day mortality and morbidity were as high as 6% and 58%, respectively. The most common 30-day complications were perioperative transfusions (36%), surgical site infection (19%), systemic sepsis (13%), and failure to wean off the ventilator (12%).

Conclusions: iAEs commonly occur in re-operative cases requiring lysis of adhesions, and possibly lead to increased patient morbidity. Understanding iAEs is essential to prevent their occurrence and mitigate their consequences.

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Poster Number 170

Kinematics of Knee is not Restored 3-Years after ACL Reconstruction

Investigators: Jing-Sheng Li, MS; Ali Hosseini, PhD; Chunbao Li, MS; Felix Yang; Amit Chawla; Thomas J. Gill, MD; Guoan Li, PhD

Anterior cruciate ligament (ACL) is the most commonly injured ligament of the knee joint. However, a majority of those patients will suffer post-operative osteoarthritis development. In order to understand the biomechanical mechanisms that may be related to the post-operative degeneration, this study was designed to investigate the in-vivo 6 degree of freedom (DOF) kinematics of ACL injured knees in step-up motion pre-operatively and 3-years post-operatively, and compared the kinematics data with those of intact contralateral knee.

Ten unilateral ACL-injured patients were included in this study with IRB approval. 3D computer model and anatomic joint coordinate system was created for each knee for calculation of knee joint kinematics. Then dual fluoroscopic analyses were performed to invest the 6DOF kinematics.

In general, the 3YR knees had more extension at the end stage of the step-up. Both the PRE and 3YR knees had significantly larger femoral medial translation. Further, the 3YR knees had significantly lower values in proximal translation which meant the distance between tibial and femoral joint centers was significantly shortened 3 years after ACL reconstruction. These kinematics variations after ACL reconstruction might imply a precursor of early knee joint degeneration. The changes in medial-lateral translation may cause shifts of the tibiofemoral contact locations and cause impingement of the tibiofemoral contact at the tibial spine. The increased inferior femoral translation might indicate larger contact deformation at the tibiofemoral joint after the surgery. These data may indicate an altered knee joint kinematics after ACL reconstruction that could lead to post-operative knee joint degeneration.

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Poster Number 171

Saving cost for hospitalized patients by improving pre-colonoscopy bowel preparation

Investigators: Syed Kashif Mahmood, MBBS, MPH; Emily J. Campbell, MPH; James M. Richter, MD

Background/Rationale: Of the 14.2 million colonoscopies annually in the United States, approximately 15% were performed on inpatients (Survey of Endoscopic Capacity, Centers for Disease Control). Hospitalized patients undergoing colonoscopy, differ from outpatients in terms of comorbidities, age and procedural indications. Suboptimal bowel preparation leads to to poorer colonoscopy outcomes and adds to direct gastroenterology cost due to need for repeat procedure. Goal of this study was to identify statistically significant variables predicting quality of bowel preparation for hospitalized patients and to identify resultant cost saving.

Methods: We collected data on 377 inpatients undergoing colonoscopy at Massachusetts General Hospital during 2012. Outcome was bowel preparation quality and covariates included patient demographics, narcotic use, diet, adjunct laxative use, bowel preparation timing & procedure time. We also identified direct Gastroenterology cost related to repeat procedure. Constructed logistic regression models and calculated Odds ratios.

Conclusions: Here we showed that for inpatients, gastroenterology cost rises significantly if a colonoscopy needs to be repeated (potential cost increase of 60-257%). Poor bowel preparation is an important cause of aborted exams (51% of patients had suboptimal bowel preparation) and approximately 4% of these patients required repeat colonoscopy during same hospitalization. For inpatients, statistically significant ($p < 0.05$) variables affecting inpatient bowel preparation quality include time interval between bowel preparation & colonoscopy, gender (females are better at clearing their bowels) and indication for colonoscopy.

Poster Number 172

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Use of Aminocaproic Acid in Patients with Hematologic Malignancy: A Case Series from the Massachusetts General Hospital

Investigators: Ariela Marshall, MD; Adrienne Drucker, PharmD; Walter Dzik, MD

Background: Small observational studies have suggested that the antifibrinolytic aminocaproic acid may be useful for prevention or treatment of bleeding due in patients with hematologic disorders, but use has not been rigorously evaluated.

Methods: We performed a retrospective review of patients with hematologic malignancies who received aminocaproic acid between January 1, 2011 and December 31, 2012. Inclusion criteria included age 18 or older, diagnosis of hematologic malignancy, inpatient oncology admission, and use of aminocaproic acid for at least 24 hours.

Results: 29 patients met inclusion criteria. Median age was 62.3 years (range 19.9-77.0). Indications for use of aminocaproic acid included 15 uses (52.7%) for refractory thrombocytopenia with bleeding, 9 uses (31.0%) for refractory thrombocytopenia without bleeding, and 5 uses (17.2%) for bleeding alone. HLA panel reactive antibody (PRA) were recorded in 10 patients (34.5%); 8 of 10 (80%) were positive. One patient (3.4%) developed deep venous thrombosis (thought to be related to underlying disease). Median platelet count at first aminocaproic acid administration was 9,000/uL (range 2,000-248,000) and was recorded in all 29 patients; median fibrinogen at first administration was 465.1 mg/dL and was recorded in 10 patients (34.5%).

Conclusions: 29 patients with a variety of hematologic malignancies received aminocaproic acid during inpatient admission to MGH between 2011 and 2012. Indications for use included both refractory thrombocytopenia without bleeding and bleeding with or without thrombocytopenia. Further investigation, including prospective clinical trials, may be useful to further characterize the safety and utility of antifibrinolytic use in the setting of hematologic malignancy.

Poster Number 173

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Improvements in functioning following treatment with cognitive therapy for obsessive compulsive disorder

Investigators: Natalie Matheny, BA; Jessica Rasmussen, PhD; Rachel Schwartz, BA; Irina Kasarskis, MPH; Sabine Wilhelm, PhD; Gail Steketee, PhD

Obsessive-compulsive disorder (OCD) is a disorder characterized by repeated obsessive thoughts as well as potentially debilitating compulsions. Research suggests that individuals with OCD oftentimes experience significant deficits in functioning. The World Health Organization characterized OCD as one of the top ten leading causes of disability worldwide. While studies have shown that patients with OCD experience a significant decrease in symptoms following treatment, changes in functioning after treatment have not been extensively examined. The present study sought to examine differences in disability scores from pre-treatment to post-treatment in adults with OCD undergoing cognitive therapy (CT).

The study sample consisted of 28 participants who met DSM-IV diagnostic criteria for OCD, and completed a treatment outcome study of CT for OCD. The sample was approximately half female (57.1%), primarily Caucasian (92.9%) and the mean age of participants was 32.9 (SD = 10.9). Participants completed a battery of questionnaires including the Disability Inventory, a self-report questionnaire used to assess disability. Paired samples t-tests showed significant differences in work ($p < .001$), social/leisure ($p < .001$), family/home ($p = .001$) and overall disability ($p = .002$) scores from pre-treatment to post-treatment. Our results suggest that improvements in disability ratings for individuals with OCD occur after receiving CT. Here we show that assessment of symptom reduction alone may not fully capture improvement for individuals with OCD following CT. Secondary analyses will examine differences in disability scores between treatment responders and non-responders, as well as potential moderators such as age, gender, or symptom severity.

Poster Number 174

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Prophylactic Mastectomies: Implications of Occult Histology and Lifetime Cost of Surveillance vs. Surgery

Investigators: David Mattos, AB; Richard G. Reish, MD; Curtis Cetrulo, MD; Amy S. Colwell, MD; Jonathan M. Winograd, MD; Michael J. Yaremchuk, MD; William G. Austen Jr., MD; Eric C. Liao, MD, PhD

Purpose: During the last decade, our institution saw a 260% increase in bilateral breast reconstructions and an age drop of 6 years in prophylactic mastectomy patients. There are limited data on the rate of histological abnormalities in this population or the trend's costs. This study measures the prevalence of occult histology in prophylactic mastectomy specimens and compare the lifetime costs of pursuing prophylactic mastectomy with reconstruction vs. surveillance in high-risk patients.

Methods: Prophylactic mastectomies from 2004–2011 were reviewed at our institution. Patient risk factors and pathology reports were collected. Predictive factors were identified with multivariate analysis. Lifetime treatment costs were estimated with Medicare billing codes. Costs were estimated for two groups: patients undergoing contralateral or bilateral prophylactic mastectomies versus surveillance. Cancer conversion rates were used to predict the percentage of surveillance patients that would require mastectomy.

Results: 495 prophylactic mastectomy specimens were identified. 2.0% of the specimens revealed breast cancer, 4.4% had ductal carcinoma in situ, and 10.9% had lobular carcinoma in situ. Age (p-value <0.001) and prior bilateral salpingo-oophorectomy (p-value = 0.042) were identified as independent predictive factors. Lifetime costs were lower in patients pursuing contralateral or bilateral prophylactic mastectomies, using implant or abdominal perforator free flap (DIEP) reconstruction, compared to surveillance.

Conclusions: Prophylactic mastectomy patients have a significant rate of histological findings, increasing with age and decreasing with prior of bilateral salpingo-oophorectomy. Lifetime cost estimates suggest a cost-saving role in prophylactic mastectomies. Ultimately, such critical decisions need to be made individually, but should not be hindered by cost.

Poster Number 175

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Combined evaluation using noncontrast CT ASPECTS and CT angiography collaterals improves clinical detection of large DWI infarcts

Investigators: Farhad Mehrkhani, MD; Shervin Kamalian, MD; Livia T. Morais, MD; Michael H. Lev, MD; Albert J. Yoo, MD

Background: DWI is the most accurate technique for delineating acute infarct core. Large DWI infarct volume (>70cc) is associated with poor outcome despite treatment, and has been used as a treatment exclusion criterion. CT is more widely available than MRI, but suffers from poor sensitivity for infarct detection. We sought to determine whether a combined approach using noncontrast CT (NCCT) and CT angiography (CTA) improves prediction of large DWI infarcts.

Methods: In a single-center, retrospective study, we identified consecutive acute ischemic stroke patients with anterior circulation proximal artery occlusions who underwent both CT and MRI. Patients were categorized based on DWI lesion volume (≤ vs. >70 cc. We utilized thresholds previously reported to have high (>95%) specificity for DWI infarct volume >70cc for NCCT ASPECTS (scores 0-4) and CTA collateral evaluation (malignant collateral profile: absent collaterals in >50% of MCA M2 division territory).

Results: Fifty-five patients satisfied study criteria. Median NIHSS was 14 (IQR 6-18), and mean age was 66.9 ± 15.2 years. Fifteen (27.2%) patients had a DWI infarct core volume >70cc. NCCT ASPECTS 0-4 had 100% specificity but only 53.3% sensitivity for identifying a large infarct on DWI. Similarly, the CTA malignant collateral profile had 97.5% specificity but only 53.3% sensitivity. In a combined approach (i.e., satisfaction of either threshold), the sensitivity was improved to 73.3% while maintaining a high specificity (97.5%).

Conclusions: Combining NCCT and CTA collateral evaluation improves the sensitivity for identifying patients with large DWI infarcts while preserving the high specificity required for treatment exclusion.

Poster Number 176

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Medically refractory epilepsy: What's next when palliation is the objective?

Investigators: Lidia M.V.R. Moura, MD; Andrew J. Cole, MD; Daniel B. Hoch, MD, PhD

Background: Palliative procedures for drug resistant epilepsy can be helpful, however, selecting the best approach for an individual remains a challenge. We compared the long-term outcomes of palliative disconnection procedures and vagal nerve stimulation (VNS) at a large academic epilepsy center.

Methods: Subjects were patients that underwent disconnection procedures (corpus callosotomy or multiple subpial transections (CC/MST)) or implantation of a VNS treated at the MGH Epilepsy Service between 1993 and 2013. Demographic data and the pertinent medical information including assessment of seizure outcome (Engel classification system) at 1, 3 and 5 years were gathered from the medical record.

Results: 31 patients were identified (CC/MST: 10, VNS: 21). Groups were comparable with regard to age, gender, seizure type and frequency, epilepsy etiology, disease duration, age at the procedure, mean length of follow-up, number and type of comorbidities. At one, three and five years, disconnection surgery was superior to stimulation device implantation with respect to seizure reduction ($p = 0.03$ at 1 year, $p = 0.04$ at 3 years and $p = 0.02$ at 5 years). Post-surgical complications were present in 30% of disconnection surgery patients (20% after subpial transections and 10% after callostomy). There were no complications in the VNS group.

Conclusions: We found that seizure control was better after surgical disconnection than after implantation of a VNS for 5 years. Disconnection procedures carry a higher complication rate. Research of the influence of pre-surgical variables will help us understand what outcomes patients value the most and how best to assess outcomes.

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Poster Number 177

Efficacy of an Established Algorithm to Reduce Constipation in Critically ill Pediatric Burn Patients

Investigators: C. Flynn, RN; K. Prelack, PhD, RD; D. Hursey, PharmD; W. Nolan, RPh; R.L. Sheridan, MD

Introduction: Constipation is a common problem for intensive care units, however most hospitals do not have protocols for prevention. We developed an algorithm to reduce constipation. The purpose of our study was to measure its effectiveness in reducing constipation in pediatric burn patients.

Methods: A retrospective review of all patients > 30% total body surface area (TBSA) in which the algorithm was utilized (AG) was conducted. Their data was compared to 9 controls (CG) who received our previous standard of practice (practitioner specific, symptom managed intervention). Data was collected on general demographics, number of days without bowel movement, and days to goal tube feeding. Differences between the two groups were compared using Student's t test.

Results: Seventeen patients with a mean burn size of 47.3 ± 13.3 (range: 32-72) % TBSA were studied. The average number of days without bowel movement in the AG vs. CG was 2.3 ± 0.7 vs. 8.7 ± 2.9 ($p<0.05$). Goal tube feeding rate was reached on an average of 7.0 ± 2.9 vs. 13.9 ±9.2 days in the AG vs. CG respectively ($p<0.05$).

Conclusion: Use of an established algorithm resulted in improved bowel function in critically ill burned children as evidenced by decreased constipation and significantly improved achievement of adequate nutrition per enteral tube feeding. This algorithm should be utilized among ICU patients deemed at risk for constipation upon admission.

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Poster Number 178

Microstructural white matter alterations correlate with cerebral amyloid angiopathy burden and cognitive measures

Investigators: Yael D. Reijmer, PhD; Panagiotis Fotiadis, BA; Sergi Martinez-Ramirez, MD; Aaron Schultz, MA; Anne K. Reed, BA; Alison M. Ayres, BA; Kristin Schwab, BA; Jonathan Rosand, MD, PhD; Anand Viswanathan, MD, PhD; Keith A. Johnson, MD, PhD; Steven M. Greenberg MD, PhD; M. Edip Gurol, MD, PhD

Background: In order to elucidate the structural mechanism linking cerebral amyloid angiopathy (CAA) to cognitive impairment, we tested the following hypotheses: 1) white matter (WM) connectivity alterations are more extensive in CAA patients than controls and they correlate with high vascular amyloid load; 2) The extent of WM connectivity alterations correlate with reduced cognitive functioning in patients with CAA.

Methods: Twenty patients with probable CAA without intracerebral hemorrhage and 25 age-matched non-demented control participants underwent multimodal imaging and cognitive testing. Microstructural alteration within WM fiber tracts were assessed with diffusion tensor imaging (DTI) MRI. Vascular amyloid deposition was quantified with PiB PET imaging. DTI parameters: fractional anisotropy (FA) and mean diffusivity (MD) were averaged across all tracts of each lobe. Correlations were adjusted for age, sex, and education level.

Results: Patients with CAA showed more severe microstructural WM alterations compared to controls, indicated by a lower FA and higher MD ($p<0.05$). Between-group differences were most pronounced in WM tracts projecting on the occipital lobe (effect size FA:-1.23±0.24, MD: 0.91±0.27, $p<0.002$), in line with the known posterior predominance of CAA pathology. Lower occipital-to-global FA ratio in patients was correlated with higher occipital PiB retention ($r=-0.62$, $p<0.03$) and with lower scores on executive functioning ($r=0.56$, $p=0.01$).

Conclusions: Patients with CAA show microstructural WM abnormalities compared to controls. The associations between WM alterations, amyloid load, and cognition strengthen the view that vascular amyloid can contribute to cognitive impairment through microstructural WM alterations even in patients without intracerebral hemorrhage.

Poster Number 179

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The Clinical Role and Cost-effectiveness of Long-acting Antiretroviral Formulations

Investigators: Eric L. Ross, AB; Milton C. Weinstein, PhD; Bruce R. Schackman, PhD; Paul E. Sax, MD; A. David Paltiel, PhD; Rochelle P. Walensky, MD, MPH; Kenneth A. Freedberg, MD, MSc; Elena Losina, PhD

Background: Long-acting antiretroviral formulations (LA-ART), currently in development, aim to achieve monthly or quarterly ART dosing; this could improve the health benefits of ART for HIV patients with difficulty maintaining daily adherence. We sought to identify the clinical and economic circumstances under which LA-ART would be cost-effective in the US.

Methods: We used a microsimulation of HIV treatment (CEPAC-US) to simulate three potential roles of LA-ART (versus daily ART only): 1) initial therapy for ART-naïve patients, 2) 2nd-line therapy for those failing 1st-line, and 3) use for patients with multiple prior failures. Model outcomes included quality-adjusted life-years (QALYs), lifetime cost, and incremental cost-effectiveness ratio (ICER); strategies with ICER<$100,000/QALY are designated "cost-effective". We simulated a cohort with mean adherence of 89% (SD=22%). Depending on adherence, HIV suppression on daily ART ranged from 0 to 91%. We assumed LA-ART's efficacy was 91% regardless of adherence to daily ART, and that LA-ART would cost $53,000/patient-year (vs. $28,000 for daily PI-based regimens).

Results: In the base case, LA-ART increased overall life expectancy compared to daily ART by 0.5-0.7 years, depending on clinical role; only LA-ART for patients with multiple failures was cost-effective ($90,000/QALY). For LA-ART to be cost-effective as 2nd-line therapy, its cost had to fall to $27,000-$34,000/patient-year.

Conclusion: LA-ART could improve survival of US HIV patients, especially those with barriers to adherence on daily ART. With a high cost, it will be a good value for use in patients with multiple prior failures; a cost close to current regimens would support broader use.

Poster Number 180

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The relationship between experiential avoidance and thought-action fusion in an undergraduate sample

Investigators: Rachel Schwartz, BA; Noah Berman, PhD; Natalie Matheny, BA; Sabine Wilhelm, PhD

Experiential avoidance (EA) is a maladaptive psychological process, in which one is unwilling to endure uncomfortable bodily sensations, thoughts, emotions, memories, or urges (i.e., psychological inflexibility). While much research has demonstrated that EA positively predicts the presence and severity of psychiatric symptoms, specifically OCD, little to no research has examined its relationship with information processing biases—a distinct psychological diathesis shown to contribute to the pathogenesis of OCD. Therefore, the current study examines the relationship between EA and a particular OCD-related information processing bias—thought action fusion (TAF). TAF comprises two subtypes—the likelihood bias (thinking about an event increases its likelihood of occurrence) and moral bias (thinking immoral thoughts is the equivalent of engaging in the behavior). Elucidating the relationship between EA and TAF can offer insight into the development of OCD and can inform innovative intervention models. Given past research, it is hypothesized that higher levels of EA will be positively associated with the severity of TAF beliefs.

Ninety-one undergraduates completed validated self-report measures to measure EA (Acceptance and Action Questionnaire-II; AAQ-II) and TAF (Thought-Action Fusion Scale). Results indicated that EA was significantly and positively associated with Likelihood ($r = .39$, $p<.001$), but not Moral TAF ($r = .17$, $p = .11$). Results partially support our hypothesis; psychological inflexibility contributes to beliefs regarding the exaggerated likelihood of external events occurring, but not the conflation of immoral thoughts and actions. Explanations for divergent TAF findings, as well as implications for treatment, will be discussed.

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Poster Number 181

Pediatric Burn Injuries in San Pedro Sula, Honduras: Prevention strategies

Investigators: M. Metivier, RN; K. Prelack, PhD, RD; R.L. Sheridan, MD

Introduction: Pediatric burn injuries are common in San Pedro Sula, Honduras. However the prevalence of burn injuries is not known. Knowledge of the timing and etiology of burn injuries in this region will provide a foundation for effective burn prevention strategies.

Purpose: The primary objective of this study was to evaluate burn data from a pediatric burn hospital in San Pedro Sula, Honduras and identify focus areas for burn prevention efforts.

Methods: Medical records of all patients treated between December 2011 and April 2013 at the pediatric burn hospital in San Pedro Sula, Honduras were retrospectively reviewed.

Results: Throughout the 5 month study period, 160 patients with an average age 7 ± 5 years were admitted to the pediatric burn hospital in San Pedro Sula. Etiology of injury was predominantly scald (57%) or flame (23%) burns. The majority of burn injuries were ≤10% total body surface area (60%), occurred during the months of December through March (61%), and involved children ≤ 5 years of age (57%). Length of stay averaged 10 ± 6 days and one mortality was reported.

Conclusions: These data highlight the specific demographics of pediatric burn injuries in San Pedro Sula, Honduras. Specifically, there is an increased frequency of scald burns among young children during the winter months, a time period when the climate is cooler and cooking increases for the holiday season. These finding provide a foundation for burn prevention strategies in this northwest region of Honduras.

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Poster Number 182

Examining the Association Between Posttraumatic Stress Disorder (PTSD) and Attention-Deficit Hyperactivity Disorder (ADHD): A Systematic Review and Meta-Analysis

Investigators: Andrea Spencer, MD; Olivia Bogucki; Amanda Pope; Mai Uchida, MD; Mohammad Milad, PhD; Thomas Spencer, MD; Yvonne Woodworth; Stephen Faraone, PhD; Joseph Biederman, MD

Posttraumatic Stress Disorder (PTSD) is a prevalent and morbid disorder for which there are few known effective treatments. Only a minority of adults and children who experience a traumatic event develop PTSD, suggesting that some individuals may have predisposing risk factors. Data from clinical and epidemiological samples document a significant association between Attention Deficit Hyperactivity Disorder (ADHD) and PTSD, suggesting that ADHD could be a risk factor. ADHD is a compelling antecedent risk factor for PTSD since it onsets in the preschool years and can be effectively treated. The aim of this study was to examine the available evidence linking ADHD to PTSD. We conducted a systematic review of the existing literature that examined the bidirectional relationship between ADHD and PTSD. We performed a literature search through PubMed and PsycINFO and mined references of relevant articles. We included only original studies in English that specifically evaluated the relationship between PTSD and ADHD. Out of 394 articles, 30 met inclusion criteria. 23 included enough information to be used in a meta-analysis. 14 of 16 studies that compared ADHD and PTSD in children and 12 of 14 studies that compared these disorders in adults showed a positive association between the two disorders. All articles that reported symptom correlations found positive correlations between symptom severities of both disorders. Our results indicate that there was a robust and bidirectional association between ADHD and PTSD in both adults and children and suggest that there is a correlation between symptom severities where the two disorders exist.

Poster Number 183

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Novel and specific small molecule HIF2a inhibitors for treatment of VHL Disease in animal models

Investigators: Laura Suarez, MD; Shannon V. Moore, BA; Scott R. Beach, MD; Carol A. Mastromauro, LICSW; Christopher M. Celano, MD; Oriana Vesga-Lopez, MD; Christina M. DuBois, BA; Jeff C. Huffman, MD

Introduction: The Patient Health Questionnaire-9 (PHQ-9) is a nine-item tool for screening depression in medically-ill patients. Item 9 assesses whether subjects have had thoughts of being dead or of self-harm over the past two weeks. We conducted an exploratory analysis of a depression and anxiety care management study for patients admitted to cardiology. The goals of the analysis were: (1) to determine whether a positive response on Item 9 was indicative of true suicidality (plan/intent requiring intervention) and (2) whether Item 9 status at baseline assessment was linked to outcomes at 6 months.

Methods: We classified participants (N=183) according to baseline Item 9 response to analyze its association with adherence to cardiac health behaviors, health-related quality of life [HRQoL], and depression severity (mean scores of remaining items ['PHQ-8']) at 6 months. Participants answering affirmatively to Item 9 underwent a detailed suicide evaluation.

Results: 24% of all enrolled subjects (anxiety and/or depression) and 27% of depression-only subjects answered affirmatively to Item 9 at baseline. All Item-9-positive participants successfully completed additional suicide evaluation; no subjects had true suicidality. Item 9 responses at each follow-up were associated with concurrent PHQ-8 mean score ($p<0.05$). Item 9 responses at baseline were not associated with 6-month depression severity, adherence, or HRQoL.

Conclusions: A suicide assessment protocol linked to the PHQ-9 was feasible but time-intensive. At baseline, positive response to Item 9 was common among depressed and anxious participants, though no subjects had true suicidality. Baseline Item 9 responses did not independently predict adverse 6-month outcomes.

Poster Number 184

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Preoperative cerebrospinal fluid beta-Amyloid/Tau ratio and postoperative delirium

Investigators: Zhongcong Xie, MD, PhD; Celeste A. Swain, BA; Sarah A. P. Ward, BA; Hui Zheng, PhD; Yuanlin Dong, MD; Neelakantan Sunder, MD; Dennis W. Burke, MD; Yiying Zhang, MD; Edward R. Marcantonio, MD

Introduction: Postoperative delirium is a common postoperative complication in elderly patients, yet the neuropathogenesis of postoperative delirium remains unknown. Low cerebrospinal fluid ß-amyloid protein (Aß)/Tau ratio is associated with Alzheimer's disease. We investigated whether lower preoperative cerebrospinal fluid Aß/Tau ratio was associated with a higher incidence and greater severity of postoperative delirium in elderly patients.

Methods: One hundred and fifty three participants (71±5 years, 53% males) having total hip/knee replacement under spinal anesthesia were enrolled. Cerebrospinal fluid was obtained during the administration of spinal anesthesia. On postoperative day 1 and 2, the incidence and severity of postoperative delirium were determined using the Confusion Assessment Method (CAM) and Memorial Delirium Assessment Scale (MDAS). Aß40, Aß42, and Tau levels in the cerebrospinal fluid were measured by enzyme-linked immunosorbent assay.

Results: Participants in the lowest quartile of preoperative cerebrospinal fluid Aß40/Tau and Aß42/Tau ratio had a higher incidence of delirium (32% versus 17%, P=0.0482, for both Aß40/Tau and Aß42/Tau) and greater severity (median MDAS score: 4 versus 3, P=0.034 for Aß40/Tau; median MDAS score: 4 versus 3, P=0.062 for Aß42/Tau) as compared to those in the highest 3 quartiles. After adjusting for age and gender, lower preoperative cerebrospinal fluid Aß40/Tau or Aß42/Tau ratio was associated with the median of the highest MDAS score (Aß40/Tau ratio:-0.12+0.05, P=0.018; Aß42/Tau ratio: -0.62+0.27, P=0.022).

Conclusion: We demonstrated that lower cerebrospinal fluid Aß/Tau ratio is associated with postoperative delirium in elderly patients, suggesting a role of Aß and/or tau protein in its neuropathogenesis.

Poster Number 185

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Mortality and Comorbidities of Adult Congenital Heart Disease Patients with Single Ventricle Anatomy Undergoing Transplant

Investigators: Sara Tabtabai, MD; Ada Stefanescu, MD; Michelle Keyes; Doreen DeFaria Yeh, MD; Robert Yeh, MD, MPH; Ami Bhatt, MD

Background: Congenital heart disease (CHD) outcomes post heart transplant (HT) can be promising, though single ventricle anatomy (1V) has historically worse post HT outcomes compared to other CHD patients. Little is known about post transplant mortality and associated comorbidities in the modern cohort of 1V patients.

Methods: The Nationwide Inpatient Sample (NIS) was used to identify CHD patients in the United States aged 14 years or older from 2000–2011. Diagnostic and procedural ICD9 codes were used to identify 1V individuals having undergone HT during index hospitalization.

Results: Between the years 2000 and 2011 there were 14,447 admissions for 1V individuals ≥14 years of age, 145 underwent HT. The median age was 22.5 years (range 14 to 56). Similar numbers of females and males were transplanted (p=0.76). Caucasian and Hispanic were the most commonly transplanted races (67% Caucasian and 15% Hispanic). All transplants occurred in urban centers. Eighty-one percent of transplants were done in the southern and western regions of the United States compared to the Midwest and northeast. In-hospital mortality in 1V patients undergoing HT was 23.3%. Renal and liver disease were the most common comorbidities. Renal disease was significantly associated with increased mortality (p=0.008), liver disease was not (p=0.66). There was no coronary artery disease, hypertension, or diabetes in this cohort.

Conclusions: In the modern day HT cohort, the rate of in-hospital mortality for adolescents and adults with 1V continues to be high at 23.3%. Renal disease is associated with increased mortality.

Poster Number 186

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Responsiveness of the corresponding author: restricting medicine

Investigators: Teun Teunis, MD; Sjoerd P.F.T. Nota, MD; Joseph H. Schwab, MD, MS

Background: Published research does not consistently report sufficient information warranting reproducibility or clinical implementation. Corresponding authors are designated to answer the resultant questions and their willingness to collaborate is crucial to data access. This study aims to establish author's response rate when requested for additional data and decay rate of corresponding email addresses.

Methods: On the 22nd of October 2013 we sent out a covert survey mimicking a request for additional data to 450 corresponding authors involved in clinical or basic/translational research. Authors were randomly selected from 45 journals (impact factor 52 to 0.29) from May 2003 to May 2013. The undeliverable and response rate were recorded, in addition to author characteristics.

Results: Forty-three percent responded to our request, 20% could not be reached and 37% did not reply. Response rate increased over time (21% to 58%), while email decay decreased (49% to 0%). Clinical researchers were morel likely to reply than basic/translational scientists (50% versus 34%). Impact factor and other author characteristics did not differ. Logistic regression analysis on response rate showed an odds ratio of 2.0 (95% confidence interval 1.3-2.9) for clinical research and 0.85 (95% confidence interval 0.79-0.91) for every elapsed year since publication.

Conclusion: Our results suggest that getting hold of the corresponding author is problematic throughout the whole field of academia. This problem seems based on both increasing email decay rates and a concerning low reply rate among authors. We propose several solutions to improve communication between clinicians and scientists after research publication.

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Poster Number 187

Cortical hypometabolism associated with developmental venous anomalies

Investigators: Dmitriy Timerman, BS; Jasmine A. Thum, MS; Mykol Larvie, MD, PhD

Developmental venous anomalies (DVAs) are the most common intracranial vascular malformation. They are commonly identified as incidental findings on neuroimaging examinations and are typically regarded as having no clinical significance. In this study, we evaluated metabolic activity in the cortex adjacent to DVAs as assessed by fluorodeoxyglucose (FDG) positron emission tomography (PET). Metabolic activity was correlated with patient characteristics including age, gender, diagnosis, and symptoms, and with imaging features including DVA size and presence of associated brain lesions. We analyzed a total of 24 DVAs in 22 patients who underwent MR and PET imaging. A qualitative assessment was performed for metabolic activity, size, and the presence of calcification, thrombus, hemorrhage, and atrophy within the involved brain parenchyma. Most significantly, the majority (two-thirds) of DVAs were associated with hypometabolism in the adjacent cortex. Patients with moderate and severe hypometabolism were significantly older (moderate: $P = 0.002$; severe: $P = 0.004$) than patients with DVAs that did not have abnormal metabolic activity. DVAs associated with severe hypometabolism were larger than those associated with normal ($P = 0.048$) and mildly reduced ($P = 0.005$) metabolic activity. In one case, marked hypermetabolism was associated with a DVA on a PET examination performed shortly after onset of seizure. Our results support the hypothesis that DVAs are not an element of normal brain vasculature and are frequently associated with decreased metabolic activity.

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Poster Number 188

The Effect of Hair Pulling Style on Quality of Life

Investigators: Esther Tung, BA; Matthew Tung, BA; Erin Altenburger, BA; David Pauls, PhD; Nancy Keuthen, PhD

Lowered quality of life has been repeatedly documented in chronic hair pullers. This study examined hair pulling styles (automatic versus focused) for their unique contribution to quality of life. "Automatic" hair pulling is accompanied by decreased awareness while "focused" pulling is more intentional and often associated with negative emotions or events. The Milwaukee Inventory for Subtypes of Trichotillomania has two subscales (automatic and focused) that were used to assess style of hair pulling. Previous research found that pullers with high focused scores on this measure reported more stress, anxiety, and depression than pullers with low focused scores.

We assessed quality of life using the Quality of Life Inventory (QOLI). Severity of hair pulling was assessed using the MGH Hairpulling Scale. We predicted that focused hair pullers would have lower overall QOLI and self-esteem sub-scores than automatic pullers. Exploratory analyses examining hair pulling style on the other "area of life" QOLI sub-scores were also undertaken. Our sample of 191 adults was 93.7% female with a mean age of 31.91 (SD=12.102). Our sample met DSM-IV diagnostic criteria for trichotillomania or chronic hair pulling (absence of tension and/or relief criteria).

Results indicated that automatic score was not correlated with QOLI score. In contrast, pullers with higher focused pulling scores reported lower quality of life even after controlling for hair pulling severity ($p=0.024$). High focused score pullers also had lower self-esteem scores ($p=0.004$) than low focused score pullers. Exploratory t-tests revealed differences between high and low focused pullers in play ($p=0.019$) and community ($p=0.056$).

CLINICAL–OTHER

Poster Number 189

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QTc Interval and Antidepressant Use in Youth: An Electronic Medical Record Review

Investigators: Mai Uchida, MD; Andrea Spencer, MD; Tara Kenworthy, BA; Roy Perlis, MD, MSc; Victor Castro, BS; Joseph Biederman, MD

Objective: The Food and Drug Administration announced in 2011 that the SSRI citalopram was associated with dose-related prolongation of the QTc interval of the heartbeat in adults, which could precipitate fatal arrhythmias. It is therefore essential to understand how antidepressant medications affect the QTc interval in children. Methods: An electronic medical record review was conducted of child patients (<18 yrs) in the Partners Healthcare system with at least one prescription of an antidepressant or methadone (known to prolong QTc) between February 1990 and August 2011. We extracted the QTc interval of patients who had also received an electrocardiogram (EKG) 14-90 days after antidepressant or methadone prescription (N=297). The mean QTc per medication was calculated as compared to the mean of all QTc measurements across medications. Results: Mean QTc values for each medication were in the normal range. The highest mean QTc was in patients on escitalopram (436). The mean QTc for patients on methadone was 431. Paroxetine's mean QTC was as high as methadone's at 431. The mean QTc for sertraline (416) was significantly lower than all other drugs measured. Conclusions: Compared to other antidepressants and methadone, no medication was associated with a statistically significant prolongation of QTc beyond all other study drugs. Though not statistically significant, the data suggest that escitalopram appears to have the greatest risk for prolonging QTc and sertraline has the least risk. We did not find any significant QTc prolongation by citalopram, the antidepressant that has been the object of recent concern by the FDA.

Poster Number 190

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Modeling HIV disease: the impact of HIV treatment assumptions on long-term epidemic projections

Investigators: Eric L. Ross, BA; Pamela P. Pei, PhD; Rochelle P. Walensky, MD, MPH; Elena Losina, PhD; Milton C. Weinstein, PhD; Kenneth A. Freedberg, MD, MSc

Background: Many compartmental models of HIV disease project substantial reductions in transmission with expanded HIV testing and antiretroviral therapy (ART). Often these models simulate ART as slowing, but not reversing, HIV progression. We aimed to assess the implications of incorporating a more detailed treatment simulation into epidemic projections.

Methods: We developed a dynamic transmission model compatible with both a complex HIV microsimulation (CEPAC-International), and with a compartmental model of HIV treatment. In the microsimulation, ART increases patients' CD4 counts, improving health rather than merely slowing progression. The transmission model uses trajectories of infectivity and survival derived from the treatment models to simulate an evolving epidemic; altered treatment policies change these trajectories, resulting in different epidemic projections.

We used this framework to compare two treatment policies in South Africa: standard-of-care, with HIV screening averaging once/three years and ART at CD4<350 cells/μL, and treatment-as-prevention, with HIV screening once/year, and immediate ART. Each policy was simulated in the microsimulation and in the compartmental model, and the resulting epidemic projections were compared.

Results: Comparing treatment-as-prevention to standard-of-care, the microsimulation projects 8% and 27% reductions in HIV prevalence and incidence over 20 years; the compartmental model projects 21% and 37% reductions. Results were robust to sensitivity analysis around numerous clinical and epidemic parameters.

Conclusions: Using an HIV microsimulation model, we project modest reductions in HIV incidence and prevalence with treatment-as-prevention; a compartmental treatment model produces substantially more optimistic projections. These results highlight the importance of a detailed simulation of ART in models of HIV transmission.

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Poster Number 191

How Cardinal are Cardinal Symptoms in Pediatric Bipolar Disorder?: A Familial Risk Analysis

Investigators: Janet Wozniak, MD; Laura Tarko, MA; Stephen Faraone, PhD; Giulia Serra, MD; Mariely Hernandez, MA; Katherine McDermott, BA; Olivia Bogucki, BA; Amanda Pope, BA; Joseph Biederman, MD

Background: Several Investigators have suggested that symptoms of euphoria and grandiosity are "cardinal," or fundamental symptoms of pediatric bipolar (BP)-I disorder. If euphoria and grandiosity are truly "cardinal" symptoms of pediatric BP-I disorder, children with BP-I disorder with cardinal symptoms should have more robust familial rates of BP-I disorder than BP-I disorder children without symptoms of euphoria or grandiosity.

Methods: We compared patterns of familiality of BP-I disorder in first-degree relatives of 232 children (probands) diagnosed with DSM-IV criteria for Bipolar I disorder, with and without cardinal symptoms of euphoria and grandiosity.

Results: With one exception ("decreased need for sleep 16% vs 7%, p=0.0041), patterns of familiality were indistinguishable in probands with and without individual DSM-IV symptoms of mania and both "euphoria" and "grandiosity" (vs neither), as assessed with familial risk analysis and logistic regression.

Conclusions: These familial aggregation findings further challenge the notion that euphoria and grandiosity represent cardinal symptoms of mania in children. Instead these findings indicate that all symptoms of mania as defined in the DSM-IV have similar relevance in the diagnosis of pediatric BP-I disorder. They also support the use of unmodified DSM criteria in establishing the diagnosis of mania in pediatric populations.

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Poster Number 192

Improving Self-Management and Clinical Outcomes in patients with Heart Failure: Feasibility of a novel Web-and Telephone-based Remote Monitoring Program

Investigators: Shiyi Zan, BS; Stephen O. Agboola, MD, MPH; Stephanie A. Moore, MD, FACC; Kimberly A. Parks, DO; Joseph C. Kvedar, MD; Kamal Jethwani, MD, MPH

Introduction: Intensive remote monitoring (RM) programs for heart failure (HF) have been successful in reducing readmissions, but may not be appropriate for all patients. iGetBetter (iGB), a secure web- and telephone-based (IVR) RM program for HF emphasizing self-management, offers a creative solution at lower cost. The objective of this feasibility pilot was to evaluate the effect of iGB on clinical outcomes in HF.

Methods: 21 ambulatory, adult HF patients were enrolled to use the intervention for 3 months. Outcomes assessed included engagement with iGB, hospitalizations, and health-related quality of life (HRQoL). Descriptive statistics were used to summarize data and matched controls identified from the RPDR were used as comparison for hospital admissions.

Results: 20 participants (mean age 52.6 yrs) completed the study. 95% felt more connected to their healthcare team and more confident in performing care plan activities, and 90% felt better prepared to start discussions about their health with their doctor. All participants liked RM device components but 70% did not find the IVR helpful. Over half of participants had greater than 80% engagement with iGB, though 15% relied on IVR and lacked engagement with the web-platform. Intervention participants recorded only one unplanned admission within the 30-day readmission period compared to 3 in control, though no significant differences were observed between the groups. No significant longitudinal change in HRQoL was detected.

Conclusion: This feasibility pilot demonstrated the usability of iGB and that less intensive RM programs emphasizing HF self-management could be a viable alternative for improving care and outcomes.

Poster Number 193

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Anxiety, Suicidality, and Severity of Illness in Bipolar Disorder: Results from the Bipolar CHOICE Study

Investigators: Emily E. Bernstein, BS; Leah W. Shesler, BA; Stephanie Salcedo, BA; Gustavo Kinrys, MD; Dustin J. Rabideau, MS; Thilo Deckersbach, PhD; Louisa G. Sylvia, PhD; Astrid Desrosiers, MD; Noreen A. Reilly-Harrington, PhD; David Schoenfeld, PhD; Andrew A. Nierenberg, MD

Introduction: Anxiety symptoms and disorders are often comorbid conditions in bipolar disorder, can worsen illness severity and functioning, and can increase the risk of suicide. However, the specific relationships between anxiety and individual features of bipolar disorder, including mood symptoms, suicidality, and age of illness onset, remain unclear.

Methods: We measured baseline anxiety and mood symptoms (Bipolar Inventory of Symptoms Scale; BISS), comorbid psychiatric conditions (Mini-International Neuropsychiatric Interview), illness severity (Clinical Global Impression-Bipolar Illness), and age of illness onset in an ongoing multi-site comparative effectiveness study for bipolar disorder (CHOICE; N=482). Logistic regressions were used to assess how the presence of anxiety symptoms is associated with suicidal ideation and behavior. We conducted correlation analyses between anxiety symptoms and manic symptoms, depressive symptoms, overall illness severity, and age of illness onset.

Results: More severe anxiety symptoms were associated with greater suicidality risk ($p<.001$), more suicidal thoughts and behavior ($p<.001$), more severe current depression and mania ($ps<.001$), greater overall illness severity ($p<.001$), and younger age of illness onset ($p=.05$), particularly age of first depressive episode ($p=.02$).

Discussion: With a large, heterogenous sample, this nation-wide comparative effectiveness study provides a unique opportunity to understand the clinical implications of anxiety symptoms in the presentation and burden of bipolar disorder. Results demonstrate that the presence of anxious symptoms is associated with greater clinical burden and higher risk of suicidality, and that anxiety symptoms should be considered in the assessment and treatment of bipolar disorder.

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Poster Number 194

Effectiveness and safety of flexible-dose repeated intravenous ketamine infusions as adjunct in outpatients with severe treatment-resistant Major Depressive disorder (MDD) with suicidal ideation

Investigators: Cristina Cusin, MD; Kara Pavone, BS; Kelley Durham, BA; Trina Chang, MD, MPH; Paolo Cassano, MD, PhD; Christina Dording, MD; David Mischoulon, MD, PhD; Maurizio Fava, MD

We investigated the feasibility and efficacy of open-label repeated intravenous administration of ketamine in severely depressed, treatment-resistant, suicidal outpatients.

Methods: 14 outpatients with MDD, age 18-65, Ham-Depression score ≥20, 3 or more failed treatments during the current episode, suicidal ideation. Patients received 6 infusions of ketamine over 3 weeks. Ketamine dose was 0.5 mg/kg, infused over 45 minutes, increased to to 0.75 mg/kg if the participant did not experience an improvement ≥30% in HAM-D score after infusion#3. Response was defined as 50% improvement on the HAM-D score at infusion#6; remission as HAMD ≤7. The Clinician Administered Dissociative States Scale was administered to monitor for acute dissociative symptoms. Vital signs, respiratory function, eye movements, and arousal state were recorded using frontal electroencephalography (EEG) recording (SpO2, Alice PdX monitor, Respironics).

Results: 17 subjects were screened and 14 were enrolled (11F/3M, mean age 50.0±7.8). Mean HAM-D score at screening was 28.6±4.8, average number of concomitant antidepressants 1.9±1.1, and average prior antidepressant trials 8.3±5.7. After the 6th infusion, 5/14(36%) patients met criteria for response and 3/14(17%) met criteria for remission. After the last infusion 7/14(50%) patients reported not having any suicidal ideation. The patients experienced minimal sedation and mild dissociative symptoms during the infusions. Ketamine infusions as augmentation of ongoing antidepressants were feasible and well tolerated. The overall response to ketamine in our sample seems of ketamine in our sample seems to be lower compared to published samples, which could be due to interactions with concurrent medications or higher level of treatment-resistance.

Poster Number 195

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A Prospective Open-Label Pilot Treatment Trial of Memantine Hydrochloride for Social Cognitive and Behavioral Impairments in High-Functioning Adults with Autism Spectrum Disorder

Investigators: Gagan Joshi, MD; Janet Wozniak, MD; Laura Tarko, MPH; Stephannie L. Furtak, BA; Joseph Biederman, MD

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by varying difficulties with socialization. ASD is now estimated to affect more than 2% of youth and is increasingly recognized in intellectually capable individuals significantly compromised in social functioning. No drugs have demonstrated consistent efficacy for the treatment of core ASD features including social deficits. We conducted a prospective 12-week open-label trial to assess the efficacy and tolerability of memantine hydrochloride for social impairments in high-functioning adults with ASD. ASD was diagnosed via clinical diagnostic interview based on DSM-IV-TR criteria. Assessments included Social Responsiveness Scale-Adult Research Version (SRS-A), Behavior Rating Inventory of Executive Function-Adult Self-Report (BRIEF-A), and Clinical Global Impression (CGI) scales. Tolerability was assessed with spontaneous reports of treatment-emergent adverse events, vital signs, electrocardiograms, and hematological parameters. Eighteen ASD adults (mean age=28.0±9.6; mean full-scale IQ=105.5±14.8; 78% male; 78% autistic disorder) with at least moderately severe features of ASD (baseline SRS-A raw score ≥80 and CGI-Severity ≥4) were exposed to memantine in this open-label trial (mean dose=19.7±1.2 mg/day). Here we show that memantine treatment was associated with significant improvement in social functioning, demonstrated by statistically significant change on all SRS-A subscales (Social Awareness, Social Motivation, Social Communication, Social Cognition, and Autistic Mannerisms) and SRS-A total score (standardized mean difference= 1.57 [0.81, 2.32], p<0.001). Cognitive function improved, demonstrated by significant reduction on BRIEF domains including a global index of executive dysfunction (standardized mean difference= 0.52 [-0.15, 1.18], p=0.006). Memantine treatment was well-tolerated with no serious adverse events. Future randomized-controlled trials are warranted.

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Poster Number 196

Correlates of drug use problems among substance users presenting in emergency departments: Results of a multi-site study

Investigators: Wendy Macias Konstantopoulos, MD, MPH; Jessica A Dreifuss, PhD; Katherine A. McDermott, BA; Blair A. Parry, BA; Melissa L. Howell, BA, BS; Raul N. Mandler, MD; Michael Bogenschutz, MD; Roger D. Weiss, MD

Background: Drug-related emergency department (ED) visits have steadily risen over the years, with substance users relying heavily on the ED for medical care. The present study aims to identify clinical correlates of problematic drug use that would facilitate identification of ED patients in need of substance use treatment. Using previously validated tests, 15,224 adult ED patients across 6 academic institutions were pre-screened for drug use as part of a large randomized clinical trial. Data for participants who reported using their primary drug of use in the past 30 days were included to examine the correlative value of self-reported values related to demographics, substance use, and ED visit to problematic drug use.

Conclusions: Here we identify clinical correlates of drug use problems that may assist in the identification of ED patients who would benefit from substance use treatment. Among all substance users, older age, tobacco and heavy alcohol use, primary non-cannabis drug use, resource-intense ED triage level, and perceived drug-relatedness of the ED visit were highly correlated with problematic drug use. While cannabis users report problems with their drug use the least, there are some indicators useful in identifying those for whom cannabis use is causing problems. The correlation between problematic drug use and resource-intense ED triage levels suggests that ED-based efforts to reduce the unmet need for substance use treatment among a population less likely to access primary care may also help decrease overall health care costs. Cost-effectiveness research in this regard could inform future health policy.

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Poster Number 197

Effects of GH on body composition and cardiovascular risk markers in men with visceral adiposity: a 6-month randomized, double-blind placebo controlled trial

Investigators: Miriam A. Bredella, MD; Eleanor Lin, MD; Anu V. Gerweck , NP; Melissa G. Landa, BA; Karen K. Miller, MD

Visceral obesity is associated with increased cardiometabolic risk, whereas lower body subcutaneous fat is relatively protective. Accumulation of liver fat resulting in disease, including non-alcoholic fatty liver disease, is an increasing public health problem with the increase in the prevalence of obesity. In individuals with visceral obesity, physiologic growth hormone (GH) secretion and peak stimulated GH response are impaired, and reduced GH secretion in visceral obesity is associated with increased cardiovascular (CV) risk markers. The objective of our study was to determine the effects of GH administration in viscerally obese men on body composition and cardiovascular risk markers. We hypothesized that low-dose GH administration for 6 months to obese young men with relative IGF-1 deficiency due to visceral obesity would improve body composition, -with a reduction in visceral adipose tissue (VAT) and increase in lean mass- reduce intrahepatic lipids (IHL) and inflammatory CV risk markers, and serum lipids. In a 6-month, randomized, double-blind, placebo-controlled trial we studied 62 abdominally obese men, 21-45 years. Here we show that GH administration in young men with visceral adiposity improves body composition, including a preferential reduction in VAT without a decrease in protective lower body fat, and relative improvement in IHL, compared to placebo. Moreover, GH administration exerted beneficial effects on two important cardiovascular risk markers – hsCRP and apoB/LDL. As there are few therapies that improve body fat distribution, and such an effect may have important implications for cardiometabolic health, further studies regarding the mechanisms responsible for these effects are warranted.

Poster Number 198

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10 year follow-up of highly cross-linked polyethylene (HXLPE) using radiostereometric analysis (RSA)

Investigators: Audrey Nebergall, BA; Meridith Greene, BA; Harry Rubash, MD; Janet Dorrwachter, NP; Christoper Barr, BS; Charles Bragdon, PhD; Henrik Malchau, MD, PhD

HXLPE was introduced to decrease osteolysis secondary to the accumulation of polyethylene wear debris, thereby anticipating an increase in the long-term survivorship of THA. Previously, larger head sizes were used to improve stability and range of motion but had accelerated wear rates of conventional polyethylene compared to smaller head sizes. In vitro studies have indicated that HXPLE has the ability to maintain its dramatically reduced wear rates irrespective of head size. The objective of this study was to assess long-term in vivo head penetration and the steady state wear rate of HXLPE articulating with either 28mm or 36mm femoral heads using RSA. Twenty-nine patients received tantalum beads placed in the polyethylene liner that allowed for RSA measurement of femoral head penetration into the HXLPE over time. Each patient received a HXLPE liner, and either a 28mm (16) or 36mm (13) metal femoral head. RSA and plain radiographic follow-up was scheduled through 10 years postoperatively. Ten patients were followed at 10 years. The median± SE steady state wear for the 28 mm cohort was 0.06±0.02mm and -0.01±0.02mm for the 36mm cohort at 10 years. The RSA results indicate that patients in the two cohorts show similar low steady state wear of the HXLPE, with no statistically significant difference between these 2 groups at 10 years. These promising results suggest that the use of larger diameter femoral heads to reduce dislocation and improve range of motion is a viable option due to improved wear rates of HXLPE, regardless of head size.

Poster Number 199

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Promoting Smoking Cessation after a Hospital Stay: The Helping HAND Randomized Controlled Comparative Effectiveness Trial

Investigators: Nancy A. Rigotti, MD; Susan Regan, PhD; Sandra Japuntich, PhD; Yuchiao Chang, PhD; Douglas E. Levy, PhD; Elyse R. Park, PhD; Daniel E. Singer, MD

Background: Hospitalization is a good time to quit smoking because inpatients must abstain temporarily from smoking. Starting tobacco treatment during hospitalization is effective only if it continues after discharge. Sustaining treatment of any chronic condition, including tobacco use, in the transition from hospital to home is a challenge for health care systems who seek to improve population health outcomes.

Methods: An RCT compared the effectiveness of two programs to provide smoking cessation counseling and medication to 397 smokers admitted to MGH. All smokers received counseling as inpatients. Those who wanted to quit smoking were randomly assigned to Extended Care (EC) or Standard Care (SC) after discharge. EC provided 3 months of (1) free nicotine replacement and (2) interactive voice response (IVR) phone calls. IVR calls encouraged cessation and medication adherence and offered refills and a return call from a live counselor. SC provided medication recommendation and passive referral to free community-based telephone counseling. Smoking status was assessed 1, 3, and 6 months post-discharge.

Results: EC was superior to SC. It increased self-reported continuous abstinence post-discharge for 1, 3, and 6 mo (6 mo: 27% vs. 16%, p=.007) and biochemically validated past-week tobacco abstinence at 6 mo (26% vs 15%, p=.009).

Conclusion: A telephone-based intervention to facilitate smokers' access to tobacco treatment was superior to standard care for increasing long-term smoking cessation rates after hospital discharge. This model could be adopted by hospitals to improve population health and meet hospital quality-of-care standards.

Poster Number 200

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Multiday outpatient glycemic control in adolescents with type 1 diabetes using a bihormonal bionic pancreas: The Barton Center Summer Camp Study

Investigators: Steven J. Russell, MD, PhD; Firas H. El-Khatib, PhD; Manasi Sinha, MD, MPH; Kendra L. Magyar, MSN, NP; Katherine McKeon, ME; Laura G. Goergen, BSN, RN; Mallory A. Hillard, BS; David M. Nathan, MD; Edward R. Damiano, PhD

Background: The safety and effectiveness of automated glycemic control has not been demonstrated in multiday outpatient studies.

Methods: In a random-order cross-over study in a diabetes camp, 32 adolescents participated in five days of glycemic control with an automated bihormonal bionic pancreas and five days of control care with an insulin pump. Subjects were fully integrated into normal camp activities and meals without restrictions on diet or exercise. The bionic pancreas used data from a continuous glucose monitor (CGM) and an autonomously adaptive algorithm running on a smartphone to control subcutaneous delivery of insulin and glucagon. Plasma glucose (PG) levels measured by fingerstick and CGM levels, which were masked to subjects under usual care, were compared between the two study arms. The co-primary outcomes were difference in the average of scheduled plasma glucose (PG) measurements and percentage of these <70 mg/dl.

Results: The mean PG level was 138±18 mg/dl (range 101–185 mg/dl) on the bionic pancreas and 157±27 mg/dl (range 103–221 mg/dl) in the control period (p=0.004). The percentage of all scheduled PG measurements with values <70 mg/dl was 6.1% during the bionic pancreas period and 7.6% during the control period (p=0.23). The bionic pancreas reduced the mean frequency of treatments for hypoglycemia from once per 0.8 days to once per 1.6 days (p<0.001) and increased time in the 70-180 mg/dl range (75.9±7.9 versus 64.5±14.1, p<0.001) by CGM.

Conclusions: A wearable, automated bihormonal bionic pancreas improved glycemia without increasing hypoglycemia in a multiday outpatient study in a diabetes camp.

Poster Number 201

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Normalization of compression-induced hemodynamics in patients responding to neoadjuvant chemotherapy using dynamic tomographic optical breast imaging (TOBI)

Investigators: Amir Y. Sajjadi, PhD; Christy M. Wanyo, BS; Michelle Specht, MD; Lidia Schapira, MD; Beverly Moy, MD, MPH; Aditya Bardia, MD, MPH; Dianne M. Finkelstein, PhD; David A. Boas, PhD; Stefan A. Carp, PhD; Steven J. Isakoff, MD, PhD

We have extended diffuse optical tomography to capture hemodynamic changes driven by fractional mammographic compression for breast neoadjuvant chemotherapy (NAC) monitoring and outcome prediction. We conducted a pilot feasibility study on 12 female patients with unilateral locally advanced breast cancer undergoing standard of care NAC. The analysis was focused on pre-treatment and day 30 post-treatment dynamic TOBI scans. Breasts are compressed in turn to 4-8 lbs of force (depending on size) and optical images are acquired every 2 seconds over 2 minutes. We compute the time course of oxy (HbO), deoxy (HbR)and total (HbT) hemoglobin concentration as well as the hemoglobin oxygen saturation (SO2). At baseline, all patients (7 responders and 5 nonresponders) exhibited an initial decrease in HbT, HbO and SO2. In the tumor area, this was followed by little or no recovery as the compression plates were held in place. The normal tissue, in contrast, began recovering almost immediately. At day 30, the tumor area in the non-responders displayed similar time-course characteristics to day 0. Interestingly, however, at day 30 responders had a very similar time course in both the tumor and normal area, characterized by a slow recovery that begins soon after the compression plates stop moving. Table 1 summarizes the changes in total hemoglobin at t=90 seconds. Compression induced changes in hemoglobin at t=90 seconds during the compression is summarized below.

HbTtumor- ΔHbTnormal (μM)	Responders	Non-Responders
Day 0	-0.65 ± 0.21	-0.82 ± 0.24
Day 30	0.10 ± 0.24	-1.13 ± 0.74

Poster Number 202

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Phosphatidylserine: A potential gene modifying therapy for Familial Dysautonomia?

Investigators: M.Salani, PhD; L. Norcliffe-Kaufmann, PhD; J. Martinez, MA; E. Morini, PhD; F. Urbina; M.Nibratt, PhD; F. Axelrod MD; H. Kaufmann MD; S.A. Slaugenhaupt, PhD

Familial dysautonomia (FD) is caused by a splicing error in the IKBKAP gene that encodes human Elongator protein 1. In these patients, exon 20 is frequently skipped during mRNA splicing, but cells retain the ability to produce a low level of normal (wild-type) IKBKAP mRNA and normal IKAP protein. Phosphatidylserine (PS, Sharp-thought®), an acidic phospholipid, has been shown to raise IKAP levels in fibroblast cell lines derived from FD patients by increasing IKBKAP transcription. We conducted a preliminary study to determine if PS increases IKBKAP expression in patients with FD. We enrolled 6 patients with FD that were examined at baseline (visit 1), after 2 months of taking 300 mg/day (visit 2) and again after 2 months of taking 600 mg/day of PS (visit 3). Blood was taken at each visit and quantitative PCR was performed to measure the level of normal IKBKAP mRNA. PS was well tolerated and there were no adverse events or unexpected clinical abnormalities. No increase in IKBKAP was observed after two months of treatment with 300 mg/day, however, we did see an increase in four of six patients after an additional two months of 600mg/day PS. Our preliminary results indicate that PS might safely raise normal IKBKAP mRNA levels in blood from patients with FD, opening an exciting potential therapeutic path for the treatment of FD. A more extensive analysis using a larger number of patients and an increased dose of PS is underway.

Poster Number 203

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The Association between Psychotherapy Use and Functioning in Individuals with Bipolar Disorder

Investigators: Stephanie Salcedo, BA; Emily E. Bernstein, BS; Leah W. Shesler, BA; Louisa Sylvia, PhD; Noreen Reilly-Harrington, PhD; Dustin Rabideau, MA; David Schoenfeld, PhD; Gustavo Kinrys, MD; Astrid Desrosiers, MD, MPH; Andrew A. Nierenberg, MD; Thilo Deckersbach, PhD

Background: While functional impairment and reduced quality of life occur with bipolar disorder and adjunctive psychotherapy improves functioning, many bipolar patients do not pursue psychotherapy. This study identifies characteristics of bipolar patients who had psychotherapy versus those who did not while participating in a comparative effectiveness study.

Method: Bipolar CHOICE is a 10-site comparative effectiveness study of lithium versus quetiapine in symptomatic outpatients. At baseline, we assessed use of psychotherapy, mood, functioning, and overall health.

Results: 148/482 (30.7%) reported using psychotherapy services. Participants that reported attending psychotherapy reported significantly greater medication side effect burden. Psychotherapy users were also significantly more likely to have moderate-high suicide risk and at least one anxiety disorder. For the participants who reported being in psychotherapy, higher frequency of psychotherapy visits in the past 90 days predicted a higher medication side effect frequency, intensity, and burden. Higher frequency of psychotherapy visits also predicted a greater overall CGI score (indicative of greater illness severity), lower prevalence of metabolic syndrome, and greater prevalence of moderate-high suicide risk.

Discussion: Participants that reported attending psychotherapy at baseline appeared to show greater impairment. More frequent psychotherapy usage was associated with greater bipolar symptom severity and higher suicide risk, suggesting that individuals with greater mood symptoms attended psychotherapy more frequently. These findings highlight the importance of understanding which patients are more likely to seek psychotherapy to better understand who is underutilizing services that may improve their mood and functioning.

Poster Number 204

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Giving Patients a CHOICE (Clinical Health Outcomes Initiative in Comparative Effectiveness): Comparing a Classic Mood Stabilizer to a Second Generation Antipsychotic in Bipolar Disorder

Investigators: Andrew A. Nierenberg, MD; Leah W. Shesler, BA; Emily E. Bernstein, BS; Stephanie Salcedo, BA; Louisa G. Sylvia, PhD; Noreen A. Reilly-Harrington, PhD; Gustavo Kinrys, MD; Astrid Desrosiers, MD; Dustin Rabideau, MS; David Schoenfeld, PhD; Thilo Deckersbach, PhD

Background: Bipolar disorder (BP) is among the 10 most disabling medical conditions worldwide. While lithium (Li) has been used extensively for BP since the 1970s, second generation antipsychotics (SGAs) have supplanted Li since 1998. To date, no randomized comparative effectiveness studies have compared Li and any SGA.

Methods: Participants with BP I or II were randomized for 6 months to receive Li (n=240) or quetiapine (QTP; n=242). Li and QTP were combined with other medications for BP consistent with typical clinical practice (adjunctive personalized treatment – APT, excluding any SGA for the Li+APT group and excluding Li or any other SGA for the QTP+ATP group). Co-primary outcome measures included Clinical Global Impression-Efficacy Index (CGI-EI) and Necessary Clinical Adjustments (NCAs) which measured changes in APT. Secondary measures included a full range of symptoms, cardiovascular risk, functioning, quality of life, suicidal ideation and behavior, and adverse events.

Results: Participants improved across all measures and over 20% had a sustained response. Primary (CGI-EI; p=0.59, NCA; p=0.15), and secondary outcome changes were statistically similar. For participants with greater manic/hypomanic symptoms, CGI-EI changes were significantly more favorable with QTP+APT (p=0.02). Among those with anxiety, the Li+APT group had fewer NCAs per month (p=0.02).

Conclusions: Despite adequate power to detect clinically meaningful differences, we found mostly similar benefits with Li+APT and QTP+APT across 6 months of treatment for BP. Our findings contrast with heuristics about choosing Li or QTP based on depression, anxiety, or suicidal risk.

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Poster Number 205

HEART: Helping Emerging Adults With Congenital Heart Disease Reconcile Transition

Investigators: Olivia Hsin, MD; Ada Stefanescu, MDCM; Hayley Kamin, MD; Eric Oglesbee, MD; Brian Masek, MD; Ami Bhatt, MD, FACC

Background: There has been a rapid increase in the number of adults with congenital heart disease (ACHD) in the last few decades, and a shift towards their care in adult centers. While patients over the age of 18 have legal rights to care for their own health, not all of them have reached "adult" developmental milestones; indeed, emerging adulthood (ages 18 to 29) is now considered to be a distinct developmental period. Methods: Fifty-six participants (age 18-40, mean 29) with ACHD completed the Markers of Adulthood questionnaire, to ascertain the perception of adulthood and their achievement of traditional adult developmental milestones, as well as the Beck Depression Inventory II and the Beck Anxiety Inventory. Results: Overall, 70% of participants reported feeling they had reached adulthood unconditionally and 28% only in some ways. Significantly fewer emerging adults than those over the age of 30 reported feeling that they were adults unconditionally (54% vs 88%, p=0.03). Age, but not Bethesda classification of ACHD severity, was associated with participants' developmental achievement. No differences were seen in between age groups in self-reported depression or anxiety (respectively, 18% and 31% overall). Here we show that among ACHD patients, individuals under age 30 years may still be in the process of reaching adult developmental milestones. Patients across the spectrum of severity of disease had similar perceptions of adulthood and had reached similar milestones and by age 30 years, they had a high likelihood of becoming independently independently functioning adults.

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Poster Number 206

Cannabinoid Use Decreases ED Utilization in a Gastroenterology Clinic Population

Investigators: John Gubatan, BS; Kyle Staller, MD; Braden Kuo, MD

Purpose: Cannabinoids are used by a wide range of patients yet our understanding of their role in clinical outcomes remains limited. We sought to explore the impact of cannabinoid use on the frequency of ED visits among GI patients.

Methods: We performed a retrospective cohort analysis on GI patients with cannabinoid exposure seen in our outpatient clinic. We created effect models for number of ED visits using known and potential predictors of ED utilization for all GI patients and groups of patients based on GI diagnoses. We used these models to examine the effects of cannabis and dronabinol use on the number of ED visits via logistic regression with estimation of odds ratios and 95% confidence intervals.

Results: We included 860 patients in this analysis. For all GI patients, dronabinol use significantly decreased ED visits (OR 0.47, 95% CI 0.24-0.93), while cannabis use or double exposure had no effect on ED visits. In patients with FGID and GERD, both cannabis (OR 0.41, 95% CI 0.21-0.79) and dronabinol (OR 0.34, 95% CI 0.18-0.67) use predicted decreased ED utilization. In patients with FGID, cannabis (OR 0.17 CI 0.05-0.61) and dronabinol (OR 0.18 CI 0.05-0.69) use predicted significantly fewer ED visits. Cannabis or dronabinol use had no significant impact on the frequency of ED visits in patients with IBD, GI malignancies, or liver disease.

Conclusions: Dronabinol use decreases ED visits in GI patients. Clinicians should consider cannabinoids particularly in the management of patients with FGID as a potential means of decreasing ED utilization.

Poster Number 207

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Predictive Modeling to Identify Heart Failure Patients at Risk for 30-Day Readmission

Investigators: Uri Kartoun, PhD; Stanley Shaw, MD, PhD; Tianxi Cai, ScD; Peter Szolovits, PhD; Vishesh Kumar, MD

We address the problem of identifying attributes that have the potential to predict whether a certain patient is likely to return to the hospital for a second hospitalization within 30 days. Identifying patients at risk allows the hospital to take actions that may prevent a second hospitalization. In addition to improving the medical condition of the patient, such actions also have the potential to reduce costs. We focus on the diabetic population; we built a model that includes 5,031 MGH and BWH congestive heart failure hospitalizations, considering a cohort of 65,099 type-2 diabetic patients at 97% specificity and positive predictive value of 0.96. Additionally, we identified for each of the 5,031 hospitalizations a subsequent hospitalization not limited to a certain diagnosis (1,462 of the subsequent hospitalizations occurred within 30 days or less). We used approximately 300 attributes including socioeconomic information, labs, heart functioning parameters extracted from narrative EKG and echocardiogram notes, and comorbidities. Additionally, we used attributes that have not yet been described in the literature, such as lab slopes, i.e. lab measurement changes during a hospitalization, and driving distance and time from the patient's location to the hospital using Microsoft's Bing Maps API. By using logistic regression and random forests, we achieved prediction accuracy consistent with the literature. To improve the prediction accuracy, we proposed a model that takes into account the intermediate event information, such as outpatient visits that occur between a first discharge and a second admission. We expect the new model to yield superior prediction accuracy.

Poster Number 208

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Natural Language Processing Improves Phenotypic Accuracy in an Electronic Medical Record Cohort of Type 2 Diabetes and Cardiovascular Disease

Investigators: Vishesh Kumar, MD; Katherine P. Liao, MD, MPH; Su-Chun Cheng, ScD; Sheng Yu, PhD; Uri Kartoun, PhD; Ari Brettman, MD; Vivian Gainer, MS; Andrew Cagan, MS; Shawn Murphy, MD, PhD; Guergana Savova, PhD; Pei Chen, MS,; Peter Szolovits, PhD,; Zongqi Xia, MD, PhD; Elizabeth W. Karlson, MD; Robert M. Plenge, MD, PhD; Ashwin N. Ananthakrishnan, MD; Susanne Churchill, PhD; Tianxi Cai, ScD; Isaac Kohane, MD, PhD; Stanley Y. Shaw, MD, PhD

Background: We created an Electronic Medical Record (EMR) cohort of type 2 diabetes (T2D) patients from Partners Healthcare, to enable phenotyping of T2D patients at population scale. We hypothesize that natural language processing of narrative EMR data (e.g., physician notes) will improve phenotyping of T2D patients vs. codified data alone.

Methods: We created a data-mart of 270,068 patients based on any of the following: T2D billing code (250.xx), T2D medication or abnormal HbA1C or plasma glucose. Natural language processing (NLP) was performed on narrative notes using cTAKES. Logistic regression with LASSO penalty was used to develop T2D classification algorithms using codified data alone (e.g., ICD9 billing codes, lab data), narrative NLP data or both. Algorithm development and validation used a training and test set of 400 and 200 patients, respectively.

Results: We identified a cohort of 65,099 T2D patients at 97% specificity and positive predictive value (PPV) 0.96. The AUC was highest for an algorithm using both codified and NLP variables (0.97), compared to codified (0.95) or NLP (0.92) alone. Among 14,129 deaths, hazard ratios (HR) for death at 5 years (using lagged, race-stratified time-varying Cox models) recapitulate those in traditional cohorts, including coronary artery disease (HR 1.57, $p < 10\text{-}5$), cerebrovascular disease (HR 1.52, $p = 6\text{x}10\text{-}5$) and congestive heart failure (CHF; HR 4.57, $p < 10\text{-}5$).

Conclusion: NLP increases the accuracy and resolution of patient phenotypes in a T2D cohort. Ongoing collection of discarded blood samples will enable genotype-phenotype correlations at population scale.

Poster Number 209

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Racial Differences in False-Positive Mammogram Rates: Results from the ACRIN Digital Mammographic Imaging Screening Trial (DMIST)

Investigators: Anne Marie McCarthy, PhD; Jianing Yang, BS; Mirar Bristol, MA; Emily Conant, MD; Katrina Armstrong, MD

Introduction: Though screening mammography reduces breast cancer mortality, it carries a significant burden of false-positives, which may lead to unnecessary procedures, anxiety, and increased costs. Few studies have quantified the burden of false-positive mammograms among black women.

Methods: We compared digital mammography screening outcomes for white (N=26,446) and black (N=3176) women. False-positives were defined as BIRADS 0, 4, or 5 mammogram with no cancer diagnosed in the 15-month follow-up period. Logistic regression was used to estimate the odds of false-positive mammogram by race, breast cancer risk factors, prior films, and study site.

Results: The false-positive rate was higher among blacks (9.2%) than whites (7.8%, p=0.009). After adjusting for age, black women had 17% increased odds of false-positives compared to whites (OR=1.17, p=0.017). Breast cancer risk factors (menopause status, age at menarche, age at first birth, breastfeeding, birth control, estrogen replacement, prior biopsy, family history, breast density), prior films, and study site each attenuated the coefficient for race by more than 20%. There was no significant difference in the odds of false-positive mammogram between black and white women in the fully adjusted model (OR=1.04, p=0.561).

Conclusions: Black participants of the ACRIN DMIST trial had a 1.4% higher false-positive rate than whites. This higher burden of false-positives for black women is clinically significant, given the large numbers of women who undergo screening mammography in the U.S. The absence of prior films and study site appeared to be stronger determinants of the disparity in false-positives than patient factors.

Poster Number 210

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25-hydroxyvitamin D levels at initiation of critical care are associated with duration of mechanical ventilation in critically ill surgical patients

Investigators: Caitlin M. McCarthy, BA; Livnat Blum, BA; J. Perren Cobb, MD; Carlos A. Camargo Jr., MD, DPhil; Sadeq A. Quraishi, MD, MHA

Background: Vitamin D status may influence important outcomes in the intensive care unit (ICU), such as length of stay, readmission rate, and mortality. However, limited data exists regarding the relationship between 25-hydroxyvitamin D [25(OH)D] levels and duration of respiratory support. Therefore, our goal was to explore whether vitamin D status at ICU admission is associated with duration of mechanical ventilation (MV) in critically ill surgical patients.

Methods: We performed a secondary data analysis from a prospective cohort study involving 200 critically ill surgical patients. To explore the relationship between admission 25(OH)D levels and duration of MV, we performed a Poisson regression analysis, while controlling for clinically relevant covariates. Only patients who required ≥48 hours of MV and who survived ≥30 days from initiation of critical care were included in the analytic cohort.

Results: A total of 86 patients met inclusion criteria. Mean (± standard deviation) 25(OH)D level was 16±2 ng/mL and median (interquartile range) duration of MV was 4 (2-6) days. Poisson regression analysis, adjusted for age, sex, race, body mass index, primary surgical service, and acute physiology and chronic health evaluation II scores, demonstrated an inverse association of 25(OH)D with duration of MV (incident rate ratio 0.93; 95% confidence interval: 0.91-0.95).

Conclusion: In our cohort of critically ill surgical patients, 25(OH)D levels measured on ICU admission were associated with the duration of respiratory support. Randomized, controlled trials are needed to assess whether vitamin D supplementation can influence the duration of MV in surgical ICU patients.

Poster Number 211

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Pulmonic Valve Replacement In Adults With Tetralogy Of Fallot: Trends From A Large United States Database

Investigators: Ada Stefanescu, MD, CM; Kevin Kennedy, MSc; Sara Tabtabai, MD; Robert W. Yeh, MD, MSc, FACC; Doreen Defaria Yeh, MD, FACC; Ami B. Bhatt, MD, FACC

Background: The management of adults with congenital heart disease is rapidly evolving. Pulmonic regurgitation in individuals with repaired tetralogy of Fallot has been recognized in the last decade to carry a significant risk of heart failure and mortality. There is no current data on trends in pulmonic valve replacement incidence and outcome across our country.

Methods: We analyzed the Nationwide Inpatient Sample from 2000 to 2011 for admissions in patients over 10 years old with International Classifications of Diseases-9th revision code for tetralogy of Fallot and pulmonic valve replacement. Hospital and patient characteristics were assessed.

Results: When indexed to the population of the United States, there were 20,545 admissions for individuals with tetralogy of Fallot. The annual incidence of pulmonic valve replacement rose from 105 in 2000 to 200 in 2011 (trend p-value<0.001). Mortality during admission for pulmonic valve replacement was 0.8%, lower than in previous reports. Although 30% of pulmonic valve replacement were performed in small hospitals in 2000, there was a significant subsequent shift towards large hospitals. The proportion of pulmonic valve replacement performed in adults increased significantly, with a trend towards older age at surgery. The major co-morbidities at pulmonic valve replacement were anemia (8%), hypertension (8.1%) and pulmonary disease (7.6%).

Here we show that the incidence of pulmonic valve replacement for repaired tetralogy of Fallot has significantly increased over the past decade, particularly in adults. Further assessing resource utilization for this population will be important as their trajectory will change with surgical intervention in mid life.

Poster Number 212

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Comparison of Aspirin Use and Incident Myocardial Infarction Rates in HIV-Infected and Non-HIV-Infected Patients in a Large US Healthcare System

Investigators: Sujit Suchindran, MD, MPH; Susan Regan, PhD; James B. Meigs, MD, MPH; Steven K. Grinspoon, MD; Virginia A. Triant, MD, MPH

HIV infection has been associated with increased risk of myocardial infarction (MI), yet aspirin (ASA) use for primary prevention has not been studied in this group. We therefore investigated ASA use and risk of MI in ASA users compared to non-users, stratified by HIV infection and coronary heart disease (CHD) risk factors.

We conducted an observational cohort study of 3,698 HIV-infected and 33,348 matched non-HIV-infected patients without known baseline CHD in a large Boston healthcare system from 2000–2009. We developed an algorithm to ascertain non-episodic ASA use employing medication data and electronic health record free text search. We used Cox proportional hazard modeling to evaluate the relationship between ASA use and incident MI within the HIV and control groups, adjusting for demographics, cardiovascular risk factors and HIV-related variables when applicable. We further assessed the effect of ASA in models stratified by cardiovascular risk.

ASA use was lower among HIV-infected compared to non-HIV-infected patients for the overall group (12.4% vs. 15.3%, $p<0.001$), among patients with 0-1 CHD risk factors (5.5% vs. 6.7%, $p=0.037$) and among patients with 2 or more CHD risk factors (22.1% vs. 42.4%, $p<0.001$). ASA use was not associated with decreased MI risk (HR 0.97, 95% confidence interval [CI], 0.64-1.49, $p=0.90$) in multivariate modeling controlling for traditional risk factors among HIV-infected patients but was associated with significantly decreased risk of MI (HR 0.29, 95% CI 0.24-0.34, $p<0.001$) among non-HIV-infected patients.

Further studies are needed to investigate optimal indications and strategies for ASA use among HIV-infected patients.

HEALTH SERVICES RESEARCH

Poster Number 213

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Success of Transfer of HIV Care from a PEPFAR Site to Community-Based Clinics in South Africa

Investigators: Christie Cloete, MBChB; Susan Regan, PhD; Janet Giddy, MBChC, MFamMed; Tessa Govender, MSc; Alison Erlwanger, BA; Melanie R. Gaynes, BA; Kenneth A. Freedberg, MD, MPH; Jeffrey N. Katz, MD, MSc; Rochelle P. Walensky, MD, MPH; Elena Losina, PhD; Ingrid V. Bassett, MD, MPH

Background: PEPFAR funding changes have resulted in HIV clinic closures. We evaluated linkage to care following transfer of patients from a hospital-based HIV clinic to government-funded, community-based clinics in Durban, South Africa.

Methods: All adult (≥18y) patients were transferred from March-June 2012. Subjects were surveyed 5-10 months later to assess self-reported linkage to the target clinic. To validate self-reports, we randomly selected 8 clinics for record reviews. We estimated success of transfer as a weighted average of linkage to care for subjects reached and not reached for the survey, adjusted for results of the validation.

Results: 3,940 patients were transferred and phoned. Of the 3,384 (86%) reached patients who completed the survey, 3,376 (99.8%) reported attending a transfer clinic, though 866 (26%) reported visiting a different clinic than that assigned.

Among the 756 patients assigned to the validation clinics, 659 (87%) were reached for the survey. Among those who self-reported attending their assigned clinic, 446/531 (84%) had a visit validated in the clinic record. Of the 97 patients not reached, 59 (61%) had a validated visit at their assigned clinic. The estimated success of transfer for the cohort overall was 81%.

Conclusions: The majority of patients reported successful transfer to a community-based clinic, though a quarter attended a different clinic than assigned. Validation of attendance highlights that nearly 20% of patients may not have linked to care. Efforts to optimize transfers to community sites require accurate contact details and collaboration with receiving clinics to ensure successful linkage to care.

Poster Number 214

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Gender and Racial Differences in Perceptions of Shared Decision Making

Investigators: Lydia Chevalier; Bettina Hoeppner, PhD; Tristan Gorrindo, MD; Leigh Simmons, MD; Lauren Leavitt; Robert Birnbaum, MD, PhD

Introduction: Shared decision making elicits the opinions, goals and needs of the patient and allows them to actively participate in choosing between care options. This program sought to ascertain baseline differences in desire for and use of shared decision making.

Methods: Participants at five patient education programs completed surveys examining shared decision making experiences with their primary care provider (PCP). The survey was based on the "Six Steps to Shared Decision Making" asked participants to rate the importance of shared decision making elements and how often they observed their PCP performing these components. Participants also watched a standardized video portraying shared decision making and rated each component.

Results: Race (collapsed into white/non-white) was found to be a significant predictor for how often participants reported that their PCP used shared decision making ($t=4.26$, $p<0.01$). Post-hoc comparison of the individual items showed that Non-White participants reported experiencing all eight shared decision making actions less frequently than White participants. Women agreed more strongly than men that they saw the physician in the video enact the shared decision making actions ($t=3.36$, $p<0.01$).

Conclusions: While most participants agreed on the importance shared decision making components, discrepancies existed in how often participants of different races observed their own PCP performing them. As there were no differences in how participants of different races rated the importance of these components or in how often they recognized them in the video it suggests that PCPs may be employing shared decision making techniques more often with some groups than others.

Poster Number 215

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Cost-effectiveness of rapid HCV and HCV/HIV testing in substance abuse treatment programs

Investigators: Bruce R. Schackman, PhD; Jared A. Leff, MS; Devra M. Barter, MS; Madeline A. DiLorenzo, AB; Daniel J. Feaster, PhD; Lisa R. Metsch, PhD; Kenneth A. Freedberg, MD, MSc; Benjamin P. Linas, MD, MPH

Aims: To evaluate the cost-effectiveness of rapid Hepatitis C (HCV) and HCV/HIV testing in substance abuse treatment programs.

Design: We used a decision analytic model to compare the cost-effectiveness of off-site HCV testing referral, on-site rapid HCV testing offer, on-site rapid HCV and HIV testing offer, and no HCV testing referral or offer (no intervention). Base case inputs included 11% undetected chronic HCV, 0.4% undetected HIV, 35% HCV co-infection among HIV-infected, 53% linked to HCV care after screening positive, and 67% linked to HIV care. Disease outcomes were estimated from established computer simulation models of HCV (HEP-CE) and HIV (CEPAC).

Setting and Participants: Data on test acceptance and costs were from a national randomized trial of HIV testing strategies conducted at 12 substance abuse treatment programs.

Measurements: Lifetime costs (2011 US dollars) and quality-adjusted life years (QALYs) discounted at 3% annually; incremental cost-effectiveness ratios (ICERs)

Findings: On-site rapid HCV testing had an ICER of $18,300/QALY compared to no testing, and was more efficient than (dominated) offsite HCV testing referral. On-site rapid HCV and HIV testing had an ICER of $64,500/QALY compared to on-site rapid HCV testing alone. The ICER of on-site rapid HCV and HIV testing remained <$100,000/QALY in one and two-way sensitivity analyses, except when undetected HIV prevalence was <0.1% or we assumed frequent HIV testing elsewhere, and in approximately 90% of probabilistic sensitivity analyses.

Conclusions: On-site rapid combined HCV and HIV testing in substance abuse treatment programs has an ICER <$100,000/QALY, even with current imperfect linkage to care.

Poster Number 216

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Cost-related Changes in Antipsychotic Drug Use and Adverse Clinical Events Among Medicare Part D Beneficiaries

Investigators: Mary Price, MA; John Hsu, MD, MBA, MSCE; Vicki Fung, PhD

Background: Antipsychotics receive formulary protection under Medicare Part D drug benefits to reduce risks associated with interruptions in therapy. Antipsychotics, however, are subject to potentially high levels of cost-sharing under Part D, including a coverage gap that begins after beneficiaries reach an annual drug spending threshold ($2,400 in 2007).

Methods: We examined Medicare Advantage beneficiaries using second-generation antipsychotics (2GAs) who reached the coverage gap before October 2007 (N=4,629). We examined factors associated with changes in antipsychotic drug regimens, including discontinuing use or switching from 2GAs to first-generation antipsychotics (1GAs) using logistic regression models. We examined the associations between changes in antipsychotic use and adverse clinical events using Poisson regression models, adjusting for patient characteristics and pre-gap event rates.

Findings: After reaching the coverage gap, 24% stopped using antipsychotics and 3% switched from 2GAs to 1GAs. Those with bipolar disorder (vs. no mental health diagnoses), disabled beneficiaries <65 years old (vs. 65+ years old), and those with higher comorbidity scores were more likely to discontinue antipsychotics during the gap. Those who switched or stopped their antipsychotic use during the gap were more likely to experience ED visits or hospitalizations during the gap than those without changes (RR=1.11, 95% CI: 1.03-1.18).

Conclusions: Standard cost-sharing erodes formulary access protections for antipsychotics under Part D. Interruptions in therapy were greatest among vulnerable beneficiaries, e.g., those with bipolar disorder or greater morbidity, and these changes were associated with higher rates of adverse clinical events. Future benefit designs should align patient out-of-pocket costs with clinical goals.

Poster Number 217

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Economic Benefit to the Society at Large of TKA in the Young Patient: A Markov Analysis

Investigators: Hany Bedair, MD; Thomas D. Cha, MD, MBA; Viktor J. Hansen, MD

The economic implications of total knee arthroplasty (TKA) to society have not been assessed when considering the young, working population with knee osteoarthritis (OA). Markov analysis was used to estimate the overall average cost to society in terms of medical expenses and lost wages of delaying early TKA in favor of non-operative treatment in a working 50-year-old patient with end-stage OA, over 30 years. Earned income, lost wages, and direct medical costs related to non-operative treatment and TKA were considered. Sensitivity analysis was performed to assess the effect of variation of key model parameters on the overall outcome of the model. The model favors early TKA compared to non-operative treatment across all plausible values for most input parameters assessed during one-way sensitivity analysis. TKA was more expensive for the first 3.5 years due to higher initial cost, but over 30 years, the cost benefit was $69,800 in favor of TKA. Only when lost wages were less than 17.7 equivalent work days per year in patients treated non-operatively, or when the rate of returning to work after TKA was less than 81% did the model favor non-operative treatment. The results of this study demonstrate that the total economic cost to society for treatment of severe knee OA in a young, working patient is markedly lower with TKA compared to non-operative treatment. Increasing financial restrictions on health care providers in the United States necessitate careful consideration of the economic impact of different treatment options from the societal perspective.

Poster Number 218

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Heart Rate Variability as an Assessment of Arousal in Early Traumatic Coma: Pilot Results from the Traumatic Coma RESPONSE Study

Investigators: K. O'Connor; B.L. Edlow , MD; R.E. Hirschberg, MD; R. Gupta, MD; S.M. Greenberg , MD; J.T. Giacino, PhD; O. Wu, PhD; E. Rosenthal, MD

Introduction: The Traumatic Coma RESPONSE Study aims to identify physiological biomarkers of recovery of consciousness in patients with traumatic coma. Measures of heart rate variability (HRV) reflect the functioning of the autonomic nervous system through ascending and descending neural pathways and may serve as an early sign of recovery of brain function.

Methods: Six patients with traumatic coma were enrolled (4M, 2F; age 19-34; Glasgow Coma Scale (GCS) score 3 to 8). Patients underwent functional electroencephalography (EEG) with language, music and motor imagery stimuli. Electrocardiography (ECG) was performed during EEG. Correlations were tested between ECG data and each patient's level of arousal at the time of EEG, as defined by the arousal subscale of the Coma Recovery Scale-Revised. Artiifact software was used to calculate time and frequency domain measures of HRV.

Results: Five of six patients had an ECG performed at the time of EEG and are thus included in the analysis. Patients did not demonstrate differential heart rate RR interval during rest or stimuli ($p > 0.05$). Visual inspection of power spectra revealed greater power in high frequency bands during continuous language compared to rest. Initial GCS scores correlated with heart rate and RR intervals during rest ($r = 0.62$, [95% CI -0.84-1.00]; $r = -0.73$, [95% CI -1.00-0.77]).

Conclusion: This study provides an early assessment of HRV in traumatic coma patients. Frequency not time domain analyses demonstrated increased sympathetic activation during language stimuli. ECG may provide basic measure of recovery of arousal in traumatic coma patients.

HEALTH SERVICES RESEARCH

Poster Number 219

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Combined effects of a scout-based automated tube potential and tube current selection algorithm with breast displacement on female coronary CTA radiation dose

Investigators: Harshna Vadvala, MBBS, MD; Phillip Kim, MS; Thomas Mayrhofer, PhD; Brian Ghoshhajra, MD, MBA

Purpose: to prove the combined use of APS-AEC with breast displacement causes significant gender differences in CCTA radiation doses, with women benefitting from breast displacement out of the scan range.

Materials and Methods: We retrospectively reviewed 728 consecutive clinically indicated CCTA exams between January 2012 and July 2013 at a tertiary academic medical center. All examinations were performed using a 128-slice dual-source scanner, where all ECG-gated scans were performed with APS-AEC with breast displacement in females. Male (n=385) and female cohorts (n=343) were stratified according to standard World Health Organization Body-mass Index (BMI) ranges (<25, 25-29.9, 30-34.9 and >35). CTDIvol, total and coronary dose-length product (DLP) and Median effective dose (ED) were recorded for all scans. SSDE values was available for n= 380 scans. The results for CTDIvol and SSDE were in both cohorts were compared using regression analysis and the Wilcoxon-rank sum test.

Results: Female patients were exposed to less radiation by a difference of 2.26 coefficient median CTDIvol (p=0.004) and difference of 2.58 coefficient median SSDE (p=0.041).

The overall median CTDIvol was 12.7 (7.3-22.7) mGy for males and 8.6 (4.9-20.0) for females. The overall median effective dose (ED) was 3.6 (2.5-5.9) for males and 2.3 (1.4-5.2) for females. Linear regression analysis with log transformation of CTDIvol and SSDE showed that being male is associated with a 25.1% higher CTDIvol and 27.7% higher SSDE.

Conclusion: Here we show that combined uses of AEC-APS with breast displacement reduces radiation exposure to female patients undergoing CCTA versus their size-matched male counterparts.

Poster Number 220

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The Massachusetts Child Psychiatry Access Project (MCPAP) Care Coordination Follow-up Model

Investigators: Courtney Zulauf, BA; Jeff Bostic, MD; Betty Wang, MD; Elizabeth Pinsky, MD; Paul Hammerness, MD

Background: The Massachusetts Child Psychiatry Access Project (MCPAP) is a system of regional children's mental health consultation teams designed to help primary care providers (PCPs) meet the needs of children with psychiatric problems. The main goals of MCPAP are to improve access to treatment for children with psychiatric illness and to promote the rational utilization of scarce specialty resources for the most complex and high-risk children.

Methods: In order to follow-up on the care provided by MCPAP we have created a care coordination follow-up model designed to contact all MCPAP patients a month after services. We have gathered specific information on the resources we provided and the current status of the child. In order to provide the best care possible we have continued to communicate with the PCPs by sending them the results of our follow-up findings as well as provided additional services for children who have not been successful in finding psychiatric care. Results: This is our initial reporting on our care coordination follow-up model. As to date we have been successful in contacting majority of patients and have gathered useful information on the resources we provided and additional needs of the child.

Conclusion: We found that our care coordination follow-up model is an important tool for MCPAP and should be incorporated statewide. It has allowed us to touch base with patients while closing the loop between MCPAP and the PCP. Follow-up is important in order to provide the best care and treatment for children with psychiatric problems.

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Poster Number 221

Using transcutaneous spectrophotometry to continuously determine methemoglobin levels in Ugandan children breathing nitric oxide as an adjuvant therapy for cerebral malaria

Investigators: Ryan W. Carroll, MD, MPH; Juliet Mwanga-Amumpaire, MD; Davis Muganzi Yap Boum II, PhD; Warren M. Zapol, MD

Nitric oxide (NO) scavenging by plasma free hemoglobin contributes to the pathophysiology of cerebral malaria (CM). Phase II clinical trials examining the safety and efficacy of inhaled nitric oxide in children with severe and cerebral malaria in Uganda are underway. NO can convert hemoglobin to the oxidized ferric form, methemoglobin (metHb). For the purposes of safety, it is imperative that investigators monitor metHb levels of patients treated with inhaled NO. We used point-of-care (POC) real-time surveillance monitoring of metHb using non-invasive transcutaneous spectrophotometry. In a clinical pilot sub-study of inhaled NO (INO) at the Mbarara University of Science and Technology, 33 patients received INO and 33 received placebo (nitrogen, N2). Prior to the initiation of NO therapy, the metHb level was 2.3+/-1.2% (mean+/-SD) and 2.1+/-1.1% in NO- and N2-breathing patients, respectively (p=NS). We measured an increase in metHb levels of NO-treated patients with a mean metHb level after 2 hours of treatment of 4.9+/-1.7% and a steady plateau with mean metHb levels at 24-hours and 48-hours of 5.0+/-1.7% and 4.5+/-1.5%, respectively ($p<0.0001$ for all values when compared to baseline; $p<0.0001$ when compared to corresponding metHb in placebo patients). The mean metHb levels of N2-breathing patients at 2-hours, 24-hours, and 48-hours were unchanged from baseline. We validated the metHb levels measured by non-invasive means by comparing values with those derived from invasive blood-based spectrophotometry. The metHb (mean+/-SD) levels were 3.4+/-2.2% and 1.8+/-2.2% measured by non-invasive and invasive means, respectively. Linear regression analysis demonstrated $r2=0.717$ ($p<0.0001$).

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Poster Number 222

Ascites analysis by a microfluidic chip allows tumor-cell profiling

Investigators: Cesar M. Castro, MD; Vanessa M. Peterson, PhD; Jaehoon Chung, PhD; Nathan C. Miller; Adeeti V. Ullal, PhD; Maria D. Castano; Richard T. Penson, MD; Hakho Lee, PhD; Michael J. Birrer, MD, PhD; Ralph Weissleder, MD, PhD

Serial molecular analyses of tumor cells during treatment- and biopsy-driven clinical trials are emerging norms in oncology. Malignant ascites (cancerous fluid build up in the peritoneal cavity) is common in many advanced cancers. As such, ascites tumor cells (ATCs) represent a potentially valuable and alternative source of cells for examining tumor biology. Yet, ascites reflects a heterogeneous mixture of tumor, inflammatory cells, and blood thus challenging its reliable isolation. Here, we developed a novel microfluidic chip platform to enrich ATCs from enable molecular analyses using microliter amounts of fluid compared to liters when processed conventionally. In parallel, we evaluated 85 putative ovarian cancer protein markers and found that nearly two-thirds were either nonspecific for malignant disease or had low abundance. Using four lead markers, we prospectively studied 47 patients with ascites (33 ovarian cancer and 14 with ascites from non-malignant conditions such as cirrhosis). Here, we also show that leveraging our ATC chip with the identified marker set allows for sensitive and specific mapping of ATC counts. Through reliable ATC enrichment we demonstrate the ability to explore additional treatment-response measurements related to proliferation, protein translation, or pathway inhibition. The ability to perform longitudinal testing of ascites in a point-of-care setting using a highly portable microfluidic device could enhance drug development and clinical care.

TECHNOLOGY

Poster Number 223

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A magneto-DNA nanoparticle system for target specific bacterial identification

Investigators: Hyun Jung Chung, PhD; Cesar M. Castro, MD; Hyungsoon Im, PhD; Hakho Lee, PhD; Ralph Weissleder, MD, PhD

To date, while various diagnostic approaches for pathogen detection have been proposed, most are too expensive, lengthy or limited in specificity for clinical use. Nanoparticle systems with unique material properties, however, circumvent these problems and offer improved accuracy over current methods. Herein, we present novel magneto-DNA probes capable of rapid and specific profiling of pathogens directly in clinical samples. A nanoparticle hybridization assay, involving ubiquitous and specific probes that target bacterial 16S rRNAs, was designed to detect amplified target DNAs using a miniaturized nuclear magnetic resonance device. Ultimately, the magneto-DNA platform allowed both universal and specific detection of various clinically relevant bacterial species, with sensitivity down to single bacteria. Furthermore, the assay was robust and rapid, simultaneously diagnosing a panel of 13 bacterial species in clinical specimens within 2 hours. The generic platform described could be used to rapidly identify and phenotype pathogens for a variety of applications.

Poster Number 224

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Optical coherence tomography and near-infrared spectroscopy system and catheter for intravascular imaging

Investigators: Ali M. Fard, PhD; Joseph A. Gardecki, PhD; Robert W. Carruth, MS; Guillermo J. Tearney, MD, PhD

Advancing the understanding and diagnosis of coronary atherosclerotic plaques requires knowledge of arterial wall microstructure as well as chemical and molecular composition. Intracoronary optical coherence tomography (OCT) is a clinically available high-resolution intravascular imaging modality that can acquire microstructural images of the coronary walls in vivo. However, this technique is not capable of identifying plaque chemical/molecular contents that are associated with disease progression and rupture.

Here we describe a dual-modality catheter-based imaging system for simultaneous microstructural and compositional imaging of artery walls using co-registered OCT and diffuse near-infrared spectroscopy (NIRS). Our OCT-NIRS system integrates intravascular OCT and NIRS catheters into a single imaging device, achieving high-resolution (10-μm axial and 30-μm lateral resolution) imaging and spectroscopy over 1230-1330nm wavelengths range at an unprecedented speed of 100,000 axial scans per second. The dual-modality catheter illuminates the tissue through a single-mode fiber and collects the back-scattered OCT light with the same fiber. Another collection fiber within the catheter detects the NIRS light, 1-2 mm from the illumination location, enabling tissue spectroscopy to be performed deep into the tissue. We have used this OCT-NIRS system and catheter in preliminary studies to visualize co-incident microstructural and spectroscopic information from diseased cadaver human coronary arteries ex vivo. Our results suggest that the combination of OCT and NIRS in a single catheter enhances the ability to detect and study coronary lesions that are at the root of acute myocardial infarction.

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Poster Number 225

Web-Based Clinical Scenario Repository to Improve Patient Safety

Investigators: Julian Goldman, MD: Diego Alonso, MS; Jeffrey Plourde; David Arney; Richard Schrenker, MS

There are no publicly available tools for clinicians and the public to report and share medical device and system integration gaps that, if closed, could improve patient care. The Clinical Scenario Repository (CSR), developed by the MD PnP Research Program, is a publicly accessible database that enables users to describe in rich detail clinical and technical care-delivery challenges and hazardous situations that could be mitigated through better technology integration in clinical environments. The CSR will enable the creation of a national database to improve care delivery systems.

In contrast to typical adverse event reporting tools, the aim of this DoD-funded repository is to create a simple yet detail-rich database of events and gaps not otherwise reported. It will be populated by clinicians, patients, and other stakeholders to share with a multidisciplinary community including researchers and regulatory agencies. In contrast to reporting mechanisms typically triggered by adverse events and motivated by liability/regulatory concerns, the CSR is intended to capture observations and ideas from those who wish to improve an observed gap in technology or discuss a possible enhancement in the delivery of healthcare.

Submitted scenarios are reviewed by administrators, tagged with keywords for searching, and then made public, so the larger community can discuss the proposed solutions and related workflow implications. A "like" feature permits users to add comments or details to a previously reported scenario. We are showing the prototype CSR, recently demonstrated to multiple federal agencies and hospital personnel; their feedback is being incorporated in an updated pre-release version.

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Poster Number 226

Quantitative 3-dimensional computed tomography analyses of intra-articular fractures of the base of the middle phalanx

Investigators: Stein Janssen, MD; Dirk ter Meulen, BSc; Michiel Hageman, MD; Brandon Earp, MD; David Ring, MD, PhD

Purpose: Quantitative 3-dimensional computed tomography (3DCT) analyses can provide a more detailed understanding of fracture morphology. We hypothesized that there is no correlation between number of fracture fragments and the percentage of articular surface area involved in intra-articular fractures of the base of the middle phalanx using quantitative 3DCT analyses.

Methods: We used 13 computed tomography scans with a slice thickness of 1.25mm or less of 15 intra-articular fractures of the base of the middle phalanx to create 3-dimensional models. We resized a 3-dimensional model of a non-fractured middle phalanx to fit the fractured middle phalanx to approximate the size and shape of the fractured middle phalanx. We created a heatmap to demonstrate the location of the fractured articular surface.

Results: With the number of scans available we did not find a significant correlation between the percentage of articular surface area involved and the number of fracture fragments. The median percentage of articular surface area involved was 46% (range 21%– 90%). The heatmap demonstrated that the radio-volar side of the articular surface seems to be more involved than the ulnar-volar side in intra-articular fractures of the base of the middle phalanx.

Discussion: Quantitative 3DCT analysis of fracture fragments provides useful information that could facilitate surgery and analysis of complex fractures of the base of the middle phalanx.

Poster Number 227

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Spontaneous Neutrophil Migration as a Clinical Biomarker for Sepsis in Human Burn Patients

Investigators: Caroline N. Jones, PhD; Laurie Dimisko; Andrew Alexander; Bryan Hassell; Molly Moore, MD; Amir Ibrahim, MD; Maryelizabeth Bilodeau, RN; Ronald G. Tompkins, MD; Shawn Fagan, MD; Daniel Irimia, MD, PhD

The most important factor in improving survival rates after sepsis, the leading cause of mortality after severe burn injury, is the ability to diagnose the condition and start effective treatment promptly. However, diagnosing infection is challenging and current methods including the use of nonspecific clinical signs (leukocytosis, fever, and tachycardia) or positive blood cultures, which can take several days, too often produce false negatives. Here we show that temporal correlations exist between the changes in directional migratory function of neutrophils isolated from patients with severe burns and the subsequent infections in these patients. To measure neutrophil migratory function, we developed a microfluidic platform that establishes chemokine gradients between a central, buffer-filled channel that acts as a sink, and an array of orthogonal side channels that act as chemoattractant reservoirs. By incorporating various bifurcations and obstacles along the channels for neutrophil migration, we were able to measure, in addition to speed, various other parameters of neutrophil migration and abilities to orient in response to chemical and mechanical cues. The novel platform is robust, requires only 3 mL of blood and was successfully translated into the clinical setting. After measuring neutrophil migration in 80 blood samples from 13 burn patients with large area burns (>20% total body surface area) we found significant correlations between the occurrence of sepsis in these patients and changes in the neutrophil migration patterns (P=0.291). Further studies will test the applicability of this technology to other conditions associated with higher risks for infections and sepsis.

Poster Number 228

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Feasibility of Ultra Low Radiation Dose Chest CT: Exploring the Lowest Achievable Doses with CT

Investigators: Ranish Deedar Ali Khawaja, MD; Sarabjeet Singh, MD, MMST; Rachna Madan, MD; Amita Sharma, MD; Jo-Anne Shepard, MD; Mannudeep Kalra, MD

Background: Computed tomography (CT) radiation dose has become a top public health concern. We aimed to assess the feasbility of ultra low dose chest CT examinations reconstructed with traditional and novel iterative reconstruction techniques (IRTs).

Summary: In a prospective study, 27 patients (51-87 years; BMI 16-32 kg/m2) gave informed consent for the acquisitions of low dose (LD) series on 128-slice MDCT (iCT, Philips). Standard of care (SD, CTDIvol, 6 mGy, estimated effective dose 3.1 mSv) chest CT and three low dose series at CTDIvol 0.90 (0.5 mSv), 0.45 (0.25 mSv) and 0.20 mGy (0.1 mSv) were acquired. SD exams were reconstructed with FBP (SD-FBP) while LD images with IRTs (LD-IRT), iterative model reconstruction (IMR) and iDose. Images were independently reviewed for lesion detection, diagnostic image quality and visibility of small structures on a continuous scale (100= SD-FBP equivalent; 0= unacceptable). Objective noise was measured in thoracic aorta. On 0.1 mSv images, 34/85 lesions were missed. All lung nodules were seen equally in SD-FBP and LD-IRT images (P > .05) at all dose levels. Mediastinal lymphadenopathy was optimally assessed in 0.5 mSv (all sizes) images. LD-IMR had better visualization for mediastinal soft-tissues in 0.25 mSv and 0.1 mSv images compared to iDose. Compared to SD-FBP, objective noise was significantly lower in LD-IRT images.

Conclusions: We demonstrate that detection of lung nodules is unaffected at 0.2 mGy radiation dose. IRTs allow optimal evaluation of subtle lung findings at 0.45 mGy and mediastinal findings at 0.92 mGy in non-obese patients (<25 kg/m2).

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Poster Number 229

The Scalable Collaborative Infrastructure for a Learning Health System

Investigators: Jeffrey G. Klann, PhD; Ken Mandl, MD, MPh; Shawn N. Murphy, MD, PhD

Harvard researchers have been developing informatics infrastructure and regulatory innovation to convert the emerging electronic health record (EHR) into a research tool that could improve patient outcomes. The Patient Centered Outcome Research Institute's (PCORI's) Clinical Data Research Networks present a focused national challenge that will allow us to use these technologies to improve human health.

We present the Scalable Collaborative Infrastructure for a Learning Health System (SCILHS), which uses a common data model across 10 health systems covering more than 8 million patients, plugging universally into the point of care, generating evidence and discovery, and thereby enabling clinician and patient participation in research during the patient encounter. SCILHS uses an EHR "sidecar" solution linked together through a robust distributed network. An app-store framework will allow innovative apps to be run on data in this network in the clinic and at home. This backbone builds on and integrates four existing systems, which were developed locally, are freely available, and are used globally.

As SCILHS develops, the infrastructure will be used to generate research agendas, approve studies, identify participants from diverse populations, inform them about research, enroll them in trials, engage them, along with their families and providers, study the cohort with ongoing bidirectional communication, and return research results. Phase I addresses arthritis, pulmonary arterial hypertension and obesity, in pediatric and adult patients, at 10 diverse sites, including the major hospitals in Boston.

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Poster Number 230

A tool to utilize adverse effect profiles to identify brain-active medications for repurposing

Investigators: Thomas H. McCoy Jr., MD; Roy H. Perlis, MD, MSc

To shorten the time required to bring new treatments to the clinic, recent efforts have focused on repurposing existing FDA-approved drugs with established safety data for new indications. We hypothesized that adverse effect profile might aid in prioritizing compounds for investigation in central nervous system applications. Data were drawn from an investigation of similarity of adverse effect profiles utilizing pre- and post-marketing data. A panel of known CNS-active drugs was utilized to estimate aggregate similarity profiles for all other FDA drugs in the database. Permutation was used to test whether similarity for any given drug exceeded that expected under the null hypothesis. To estimate the performance of algorithms using such profiles, manually curated lists of known CNS-active and inactive medications were classified using logistic regression. Algorithms with and without this similarity data were compared for prediction of CNS penetrance.Models incorporating adverse effect similarity data exhibited greater discrimination of brain-penetrant and non-penetrant drugs than models without this data (area under the ROC curve 0.74 versus 0.65, p=0.02; net reclassification improvement 0.55 (95% CI 0.12-0.80)). A visualization tool was developed to allow any medication to be evaluated for adverse effect similarity to the CNS panel or a custom panel. Consideration of adverse effect profiles allows in silico prioritization of compounds for follow-up investigation for CNS indications. In concert with chemical screening approaches, this may accelerate repurposing efforts for putative CNS-active medications.

Poster Number 231

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"Normal" changes detected on post mortem CT examinations

Investigators: Sarvenaz Pourjabbar, MD; Sarabjeet Singh, MD; Ranish Khawaja, MD; Atul Padole, MD; Mannudeep Kalra, MD

Purpose: To recognize and evaluate normal postmortem findings seen on postmortem imaging.

Method: Hundred adult cadavers (61 ± 15 years, M: F 62:38) underwent postmortem CT examinations. Head to toe whole body CT scan were acquired on 128 slice MDCT (Definition FLASH, Siemens) (n= 87, mean age 61± 15years, M:F 52:35) and 64 slice MDCT (Discovery750HD, GE) (n= 13, 63 ± 15years, M:F 10:3). CT images were interpreted by experienced radiologists and report was conveyed to the autopsy team. Each case was followed up with detailed autopsy reports for every organ. These pathology and CT findings were tabulated for assessing correlation between pathology and radiology findings.

Results: Most postmortem CT examinations were performed within 24 hours (20.0 ± 28.0 hours, n=89). Loss of gray white matter was seen in all the cases. Diffuse cerebral edema was seen in 34 cases (28.6 ±28.3 hours). Hyperdense sagital sinus was seen in all patients except in 2 patients due to artifacts. Intravascular air was detected in 17 patients with time to scan range of 3.5- 172 hours. Hyper-attenuating aortic wall was seen in all the patients. Filling defect was seen in great vessels of 80/100 patients. In 48/100 patients, lungs were either collapsed or had diffuse bilateral ground glass or airspace opacities without any definitive pathology correlate, which suggested "normal" pulmonary changes following death.

Conclusion: Imaging finding in postmortem setting are different from pre-mortem; some of the pathological findings in live human are considered normal in postmortem setting.

Poster Number 232

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Tethered capsule endomicroscopy with an integrated white light camera for navigation in the upper GI tract

Investigators: Elena Quijano, BA; Michalina Gora, PhD; Robert Carruth, MS; Weina Lu, MS; Drew Carlton, BA; Amna Soomro, MBBS; Lorissa Moffit, MS; Mireille Rosenberg, PhD; Bill Puricelli, RN; Alessio Fasano, MD; Aubrey Katz, MD; Norman Nishioka, MD; Gary Tearney, MD, PhD

Celiac disease (CD) is an autoimmune disorder of the small intestine that afflicts roughly 1% of the population of the United States. Traditionally diagnosed by endoscopic biopsy, CD is often missed due to inadequate sampling. The risk of obtaining false negatives, along with the substantial cost of the procedure, makes endoscopic biopsy an imperfect tool for CD diagnosis. We have developed a swallowable tethered capsule endomicroscope (TCE) that utilizes optical frequency domain imaging (OFDI) to image the entire esophagus at the microscopic level. If properly configured to access the small intestine, this technology could diagnose CD in a minimally invasive, cost-effective, and accurate manner.

The TCE incorporates a white light camera in the distal tip of the capsule for navigation through the stomach. Small wires embedded along the tether deliver power to the camera and transmit video, which is displayed in real time together with OFDI cross-sectional images of the gastrointestinal (GI) tract. The tether is also configured to aid in maneuvering the capsule through the pylorus of the stomach to the duodenum.

We have successfully tested our TCE for imaging the upper GI tract, including the duodenum and stomach, in swine studies in vivo. We are currently preparing a pilot human study to assess the viability of our TCE device for diagnosing CD. Hopefully our new video-enabled TCE will not only prove to be a practicable means for CD diagnosis, but will also provide a useful secondary tool for diagnosing BE and other disorders of the upper GI tract.

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Poster Number 233

Differences in LV shape pattern in ischemic versus nonischemic cardiomyopathy. Insights from 3D echocardiography

Investigators: Shmuel Schwartzenberg, MD; Mark Handschumacher, MS; Xin Zeng, MD; Judy Hung, MD

Background: Left ventricle (LV) remodeling is assessed by linear dimension or LV volumes. 3D-echo of LV shape has the potential to provide quantitative morphometric assessment of LV remodeling. We aimed to assess LV shape pattern by 3D echo in patients (Pts) with ischemic (IHD) and dilated (DCM) cardiomyopathy, comparing them with normal (Ctrl).

Methods: 3D echo (Philips ie33; x5) was performed on Pts with IHD (n= 21), DCM (n=18) and Ctrl (n=23). 3D LV data sets were traced at end-systole (ES) and end-diastole (ED) in 4 planes to reconstruct LV endocardial surface area and coordinates using customized 3D software (Omni4D). A mean radius from edge of LV to a centroid line axis and fractional shortening (FS) was plotted from base to apex position normalized to body surface area (Fig), providing an LV shape pattern standardized to a central reference. Position of radii was expressed as % from base to apex (BAP). Point-by-point radii and FS were compared among all groups.

Results: The DCM group had significantly ($p<0.05$) higher ES radii than the IHD at BAP 11-12% and higher ED radii at BAP 7-12% and 31-33%. The Ctrl group had smaller radii and higher FS ($p<0.05$) than either of the 2 CMP groups at BAP 33-85%.

Conclusions: There are significant differences in LV shape pattern in patients with cardiomyopathy and normal. DCM and IHD patients also differ in LV shape in the proximal parts of the ventricle. 3D LV shape pattern may provide an important noninvasive physiological measure of LV remodeling.

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Poster Number 234

Optical thromboelastography (OTEG): a novel technology for coagulopathy assessment at point-of-care

Investigators: Markandey M. Tripathi, PhD; Zeinab Hajjarian, PhD; Elizabeth M. Van Cott, MD; Seemantini K. Nadkarni, PhD

Impaired blood coagulation is often associated with increased mortality and hospital length of stay following acute trauma, surgery, and chronic illness. Therefore, the early detection of coagulation defects at the point-of-care is critical in improving recovery and clinical outcome. In this study, we investigate a new approach termed optical thromboelastography (OTEG), to evaluate coagulation status in real-time, and demonstrate its capability to detect impaired coagulation states in patients. In OTEG, a kaolin-activated blood sample is illuminated by laser light and time-varying laser speckle patterns are captured using a high-speed CMOS sensor. During coagulation, the formation of a platelet-fibrin clot restricts the Brownian motion of light scattering particles and alters the rate of temporal speckle intensity fluctuations. To measure changes in clot viscoelasticity, the temporal speckle intensity auto-correlation function, g2(t), is measured during coagulation, the mean square displacement (MSD) of scattering particles is derived, and the clot viscoelastic modulus, G, is extracted via the generalized Stokes-Einstein relation (GSER). By monitoring changes in G during coagulation the parameters, clotting time (R), clot progression rate (α), and maximum clot strength (MA) are derived. In this study, whole blood samples from 50 patients were analyzed and OTEG coagulation parameters were compared with standard mechanical thromoboelastography (TEG). Our results showed a statistically significant correlation between OTEG and TEG measurements for R-time($R=0.81$, $p<0.001$), α ($R=0.53$, $p<0.001$), and MA ($R=0.65$, $p<0.001$). These results demonstrate the capability of OTEG for evaluating blood coagulation status and open new opportunities for detecting coagulation defects at the point-of-care.

Poster Number 235

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A Machine Learning Model Built On Florida Patient Data Positively Predicts Post-laminectomy Syndrome Among Massachusetts Patients

Investigators: M. Yelle, MD, PhD; D.A. Edwards, MD, PhD; P. Tighe, MD; A. Patel, MD; D. Hoh, MD; M. Mayo, PhD; R.W. Hurley, MD, PhD

The incidence of chronic pain after lumbar surgery (discectomy, laminectomy, and fusion) is high; estimates report between 10 and 40%. This outcome, known as post-laminectomy syndrome, can be associated with a number of factors including ethnicity, age, and weight. Machine Learning (ML) algorithms can be trained to predict post-laminectomy syndrome using data input from large, multidimensional, and imperfect databases. This study seeks to validate the trained ML model on an independent dataset from another institution.

A dataset was created from the preoperative attributes and medical history of 471 patients from the University of Florida (UF). Some attributes included patient's BMI, opioid use, past medical history, pain scores, age, sex, surgical history free text, and MRI reports. The dataset was analyzed using WEKA software, an open-source software package of machine learning algorithms. Identical input factors were collected from 200 patients treated at Massachusetts General Hospital. Trained classifiers were then tested on the MGH dataset. Classifiers' performance was evaluated using the receiver operating curve (a measure of sensitivity), cumulative lift (effectiveness), and misclassification rate.

Univariate analysis from UF and MGH datasets demonstrated significant differences in post-laminectomy syndrome according to ethnicity, age, weight at the time of surgery, weight at follow-up, and pain. Sensitivity statistics approached 80% detection of post-laminectomy syndrome on the new dataset ML algorithms offer decision support by providing likelihood-of-success predictions compared to other patients with similar features. By identifying patient attributes that determine classification, intervention can be made to optimize modifiable patient factors and deliver more informed surgical consent.

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Poster Number 236

Biomimetic Total Knee Arthroplasty with Anterior Cruciate Ligament Preservation Restores Normal Kinematics and Reduces Implant Wear

Investigators: Thomas Zumbrunn, MS; Kartik Mangudi Varadarajan, PhD; Harry E. Rubash, MD; Henrik Malchau, MD, PhD; Guoan Li, PhD; Brad Micheli, BS; Keith Wannomae, BS; Orhun K. Muratoglu, PhD

Introduction: Anterior Cruciate Ligament (ACL) retaining Total Knee Arthroplasty (TKA) provides better kinematics than ACL sacrificing (CR) TKA. In the native knee, ACL and asymmetric shape of the tibial articular surface with a convex lateral plateau are responsible for differential medial/lateral femoral rollback.

Methods: A biomimetic Bi-Cruciate Retaining (BCR) implant was designed through a novel process directly using in vivo kinematics of healthy knees. Kinematic performance of the biomimetic BCR implant (asymmetric tibia with convex lateral surface), a contemporary BCR (symmetric shallow dished tibia) and CR (symmetric dished) implant was analyzed during simulated deep knee bend using LifeModeler KneeSIM software. Wear performance of the biomimetic BCR and a contemporary CR implant were compared using knee simulator wear tests.

Results: The contemporary CR implant showed initial posterior femoral subluxation due to the absent ACL, followed by paradoxical anterior sliding until 90° flexion, and no medial pivot rotation. ACL retention in the contemporary BCR implant reduced the initial femoral posterior shift. The biomimetic BCR implant showed knee motion similar to healthy knees in vivo, with medial pivot rotation and greater, consistent rollback of the lateral femoral condyle. The biomimetic BCR wear rate was significantly less (83.2%) compared to the conventional CR implant ($p<0.0001$).

Conclusion: An ACL preserving biomimetic TKA implant could restore normal knee kinematics unlike contemporary implants, during simulated activities. The insert manufactured from a highly cross-linked vitamin-E stabilized UHMWPE showed significantly lower wear. The novel biomimetic BCR TKA provides more normal kinematics and increased implant longevity.

TRANSLATIONAL

Poster Number 237

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Stored Red Blood Cell Auto-Transfusion Causes Pulmonary Artery Hypertension In Overweight Volunteers With Endothelial Dysfunction

Investigators: Lorenzo Berra; Riccardo Pinciroli, MD; Binglan Yu, PhD; Christopher P. Stowell, MD, PhD; Kenneth D. Bloch; Warren M. Zapol, MD

Introduction: Transfusion is one of the most common medical interventions. Transfusion of packed erythrocytes stored for prolonged periods of time might increase mortality. Stored erythrocytes undergo hemolysis. Plasma cell-free hemoglobin rapidly scavenges endogenous Nitric-Oxide (NO) leading to systemic and pulmonary vasoconstriction in various species. We tested the hypothesis that stored-autologous blood transfusion would cause pulmonary vasoconstriction and an increased pulmonary artery pressure (PAP) in overweight volunteers with endothelial dysfunction (impaired endothelial production of NO). We also tested whether breathing NO gas prevents the pulmonary hypertension in response to stored blood. Methods: Fourteen overweight adults with impaired endothelial function were enrolled in a randomized cross-over study of autologous-leukoreduced stored blood transfusion. Volunteers were re-infused after a) 3-days of storage, b) 40-days of storage, or c) 40-days of storage while breathing NO. Transthoracic-echocardiography was performed before and after transfusion to estimate mean-PAP, and venous blood was sampled before and after transfusion. Results: Baseline characteristics: age: 41±13 years; BMI 33.2±4.9 Kg/m2. After autotransfusion the plasma free-hemoglobin concentrations were increased by transfusing 40-day stored blood. No increase was detected after transfusing 3-day stored blood. The estimated mean-PAP increased after transfusing 40-day stored blood (from 18 to 26mmHg, $p<0.05$), but did not change after 3-day stored blood. PAP decreased after stored blood transfusion while breathing NO (from 17mmHg to 12mmHg, $p<0.05$). Conclusions: Autologous leukoreduced stored red cell transfusion, but not fresh red cell transfusion is associated with (1) increased plasma free-hemoglobin levels and (2) pulmonary artery hypertension. Breathing 80 ppm NO prevents pulmonary hypertension.

Poster Number 238

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Indicators of Viability in the ex vivo Perfused Human Liver

Investigators: Bote G. Bruinsma, MSc; Gautham Sridharan, PhD; Heidi Yeh, MD; Pepijn Weeder, BSc; Sinan Ozer, BSc; Sharon Geerts, BSc; James Markmann, MD, PhD; Paulo Martins, MD, PhD; Maria-Lousia Izamis, PhD; Korkut Uygun, PhD

Machine perfusion of the liver as a preservation method for transplantation is rapidly gaining support. More work is showing superiority over cold storage, in particular for liver grafts injured by long warm ischemic time (WIT). One of the major advantages of machine perfusion at warm temperatures is the ability to assess the viability of the liver prior to transplantation. However, the best indicators of viability need to be identified to accurately assess the liver. To this end, we perfused 20 discarded human livers in our subnormothermic machine perfusion system, which we have tested extensively in animal transplant models. During perfusion we monitored liver function by assessment of blood gas analysis, bile production, biochemical markers of function and injury, tissue analysis and (electron-) microscopy. Perfusion parameters were correlated to characteristics of the donor and the WIT of the liver. We demonstrate here that liver function improves during perfusion, with an increase in oxygen consumption, albumin and urea output and steady bile production. Minimal injury was observed. ATP content of the liver tissue improved significantly (61.1 to 204.3 $p=0.012$). ATP content showed the highest correlation with WIT ($r2=.83$, $p=.0045$). Amongst non-invasive indicators, not the total production of bile, but whether bile production increased during perfusion correlated best with the low WIT ($p<0.006$). A positive correlation was also found between the release of alanine aminotransferase (ALT) and WIT ($r2=.58$, $p=.016$). In conclusion, ATP content, sustained bile production and ALT appear are best correlated to WIT and may be useful as indicators of liver quality.

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Poster Number 239

Anatomical Contouring of Large Diameter Heads for Soft-tissue Relief Does Not Impact Load Bearing Contact Area and Wear Performance in Ceramic on Poly Articulation

Investigators: Michael P. Duffy, MS; Kartik Mangudi Varadarajan, PhD; Keith Wannomae, BS; Brad Micheli, BS; Thomas Zumbrunn, MS; Harry E. Rubash, MD; Andrew Freiberg, MD; Henrik Malchau, MD, PhD; Orhun K. Muratoglu, PhD

Large diameter femoral heads in total hip arthroplasty provide increased range-of-motion and reduced dislocation rates compared to smaller diameter femoral heads. However, several recent studies have reported that contemporary large head prostheses can directly impinge against the local soft tissues leading to anterior hip pain. To address this we developed a novel Anatomically Contoured large diameter femoral Head . We showed that the anatomical contouring of a femoral head for soft-tissue relief can be accomplished without affecting load bearing femoroacetabular contact area or wear performance in ceramic-on-polyethylene articulation.

A finite element simulation was used to asses contact area and stresses of a conventional femoral head and the anatomically contoured femoral head against polyethylene acetabular liners. A range of implant orientations and design tolerances were considered. There was no difference in the contact area between the two implants. The wear performance of the two head designs was assessed with an AMTI 12-station hip simulator under a standardized application of gait. At 2 million cycles the two head prostheses produced no difference in the wear rate (assessed gravimetrically) of the polyethylene acetabular liner.

This study showed that an Anatomically Contoured Femoral Head designed to provide soft-tissue relief maintained the load bearing contact area and wear rate when articulating with a polyethylene liner. The novel prosthesis could mitigate the risk of soft-tissue impingement with large diameter femoral heads while providing the benefits of stability and increased range-of-motion.

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Poster Number 240

Attachment Style Moderates the Effect of Oxytocin on Pro-Social Behavior and Attentional Processing in Social Anxiety Disorder

Investigators: Angela Fang, MA; Stefan G. Hofmann, PhD

Individuals with social anxiety disorder (SAD) are often afraid of interacting with others due to fear of rejection. Given previous research demonstrating that intranasal oxytocin may promote pro-social behavior such as trust, cooperation, and emotion recognition in humans, oxytocin may have relevant clinical implications for SAD. The current investigation examined whether intranasal oxytocin impacts cooperative behavior toward a rejecting but initially cooperative confederate, and whether it modulates attentional processes toward social stimuli, while controlling for attachment style. Using a double-blind, placebo-controlled design, 54 individuals with SAD were randomly assigned to receive 24 international units of oxytocin or placebo nasal spray. Following drug administration, participants completed a computerized ball-tossing game called Cyberball, which measured social cooperation toward three other confederates, who were programmed to follow behavioral profiles reflecting different degrees of cooperative play. After Cyberball, participants completed a modified version of the Posner Task involving social stimuli. Relative to placebo, oxytocin improved cooperation for individuals with high attachment avoidance, and also reduced subjective ratings of rejection for individuals with low rejection sensitivity. Furthermore, oxytocin facilitated disengagement from all cue types regardless of emotional valence and facilitated detection of disgust and neutral faces for individuals with high attachment anxiety. These findings suggest that oxytocin may promote social cooperation, as well as a flexible attentional pattern toward social cues, but only for individuals with SAD with insecure attachment styles. These findings point to the importance of addressing individual differences in response to oxytocin, and the potential clinical utility of diagnostic subgroups.

TRANSLATIONAL

Poster Number 241

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PET/MRI evidence of neuroinflammation in migraine

Investigators: Nouchine Hadjikhani, MD, PhD; Caterina Mainero, MD, PhD; Noreen Ward, MS; Marco Loggia, PhD; Thomas Anderson, MD; Nicole Zurcher, PhD; Grae Arabasz, MS; Ciprian Catana, MD, PhD; Jacob Hooker, PhD

Despite being the 3rd most prevalent medical disorder on the planet, and representing a risk factor for both stroke and Alzheimer's disease, migraine and its pathophysiology remain incompletely understood. Neurogenic inflammation has been hypothesized as a key factor in the generation of the pain associated with migraine, and is associated with microglia activation.

Here, we show preliminary data using a novel combined PET-MRI imaging technique evaluating glial activation in migraineurs. For the first time in humans, we show that migraine induces neuroinflammation in the trigemino-vascular system. After injection of [11C]PBR28, we observed increased uptake in the entire pain-processing pathway associated with migraine, including, the trigeminal ganglion, the trigeminal nucleus (TGN)/superior salivatory nucleus (SSN), the periacqueductal gray (PAG), the thalamus and the posterior insula/S1/S2 region. Increased uptake was also observed in the meninges adjacent to the occipital pole In addition, in migraine with aura patients, we observed increased uptake in the primary visual cortex and in extrastriate visual areas. We interpret the uptake in the visual cortex as the evidence of the long-lasting and cumulative effect of astrocytic activation following CSD.

The ability to demonstrate the presence of neuroinflammation in migraine may provide a unique therapeutic monitoring marker. Most importantly, it may also open new treatment strategies for migraine.

Poster Number 242

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Sex Differences in Response to Continuously Administered Kisspeptin, a Pseudo-antagonist of the Kisspeptin Receptor

Investigators: Margaret Flynn Lippincott, MD; Yee-Ming Chan, MD, PhD; Stephanie Beth Seminara, MD

The neuropeptide kisspeptin is essential for puberty and reproduction. In rhesus monkeys, pulsatile administration of kisspeptin stimulates reproductive endocrine activity. In contrast, with continuous infusions of kisspeptin, luteinizing hormone (LH) initially increases but then drops below baseline due to desensitization of the kisspeptin receptor. When we performed similar infusion studies in the human male, LH levels also initially rose and then exhibited a partial decline, but remained above baseline throughout the infusions. We hypothesized that postmenopausal women, who lack the negative feedback effects of estrogen on hypothalamic kisspeptin secretion, are closer to the threshold of and would therefore exhibit more rapid and/or complete desensitization than men. Three healthy postmenopausal female volunteers underwent frequent blood sampling for 36 hours to evaluate their LH responses to continuous kisspeptin administration, with 6 hours of baseline sampling, continued sampling during a 24-hour infusion of kisspeptin at 12.5mcg/kg/h, and a final 6 hours of sampling after the infusion was stopped. Continuous infusion of kisspeptin appeared to partially desensitize the kisspeptin receptor, as it does in men, as LH decreased slightly. In contrast to men, the kisspeptin infusions did not initially stimulate LH secretion suggesting that in postmenopausal women endogenous stimulation of the kisspeptin receptor may be maximal or alternatively that neuroendocrine function may be in part kisspeptin-independent. Despite the sex specific differences in initial LH secretion, both men and women respond to continuous infusions of kisspeptin with partial desensitization. Thus, continuous kisspeptin may potentially serve as a pseudo-antagonist for clinical and research applications.

Poster Number 243

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Superior Donor Heart Preservation Using Hypothermic Oxygenated Perfusion

Investigators: Sebastian Michel, MD; Glenn M. LaMuraglia II, BS; Maria Lucia L. Madariaga, MD; James S. Allan, MD, MBA; James S. Titus, BS; Martin K. Selig, BA; Evan A. Farkash, MD, PhD; Lisa M. Anderson, PhD; Joren C. Madsen, MD, DPhil

Background: Hypothermic machine perfusion of donor hearts enables continuous aerobic metabolism and washout of toxic metabolic byproducts. We evaluated the effect of machine perfusion on cardiac myocyte integrity in hearts preserved for 4 hours in a novel device that provides pulsatile oxygenated hypothermic perfusion (Paragonix Sherpa Perfusion™ Cardiac Transport System).

Methods: Pig hearts were harvested and stored in Celsior® solution for 4 hours using either conventional cold storage on ice (4h CS, n=6) or the Sherpa device (4h pulsatile perfusion (PP), n=6). After cold preservation, hearts were evaluated hemodynamically using a non-working heart Langendorff system: systolic function was measured by +dp/dt, diastolic function by -dp/dt and EDP. Controls (n=3) were reperfused immediately after organ harvest. Biopsies were taken from the left ventricle before storage, after storage and after reperfusion for ultrastructural analysis using electron microscopy.

Results: 4h CS, 4h PP and control group did not show any significant differences in systolic or diastolic function (+dP/dt, -dP/dt, EDP). 4h CS hearts showed more arrhythmia compared to 4h PP and controls. Electron microscopy revealed endothelial cell rupture and damaged muscle fibers in the 4h CS group after reperfusion while the cell structures were preserved in the 4h PP group.

Conclusion: Hypothermic pulsatile perfusion of donor hearts during the storage interval is a simple technique that leads to a better preserved cell structure compared to the conventional cold storage method. This may lead to less risk of primary graft failure after orthotopic heart transplantation.

Poster Number 244

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Coronary CT Angiography and Whole Genome Sequencing to Discover Genes in Families Prone to Myocardial Infarction

Investigators: Pradeep Natarajan, MD; Nathan Stitziel, MD, PhD; Maros Ferencik, MD, PhD; Lindsey Hildebrand, BA; Namrata Gupta, PhD; Stacey Gabriel, PhD; Udo Hoffmann, MD; Sekar Kathiresan, MD

Background: Coronary artery disease (CAD) and myocardial infarction (MI) are the leading causes of death in the US and cluster in families. In some families, early CAD/MI segregates in a Mendelian pattern, implicating single causal genes. Discovering Mendelian genes has often led to new insights and therapies. Coronary computed tomography angiography (CTA) non-invasively refines CAD classification and next-generation sequencing now enables whole genome analysis for gene discovery.

Methods: We recruited a family with apparent Mendelian inheritance of early CAD/MI despite the absence of traditional risk factors. Individuals without a clinical history of CAD/MI underwent CTA. All eight living individuals underwent whole genome sequencing. We defined potential candidates as rare functional variants displaying a dominant mode of inheritance, considering affected individuals as those with symptomatic CAD, prior MI, or an epicardial coronary stenosis by CTA.

Results: The proband developed an acute coronary syndrome at age 43. His family history was enriched for premature CAD/MI unrelated to known risk factors and was consistent with autosomal dominant inheritance. One asymptomatic sibling was reclassified as affected based on a 50-70% coronary artery lesion by CTA.

Whole genome sequencing of all living family members discovered 5,255,463 variants. 255,057 variants segregated with CAD/MI in an autosomal dominant model. And five of these variants are predicted to be functional and are rare.

Conclusions: Whole genome sequencing in a single family with apparent Mendelian inheritance of CAD/MI identified multiple candidate DNA sequence variants. This study highlights the potential and challenges of this approach to discover genes for CAD/MI.

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Poster Number 245

Weight Loss Surgery Regulates Glucose Metabolism via Intestinal Metabolic Reprograming

Investigators: Nima Saeidi, PhD; Nicholas Stylopoulos, MD; Martin Yarmush, MD, PhD

The United States is at the epicenter of the obesity pandemic. In the absence of any other efficacious alternatives, bariatric surgery, particularly Roux-en-Y gastric bypass (RYGB), remain the only definitive treatment for obesity and type 2 diabetes. RYGB substantially reduces body weight and improves diabetes. Therefore, delineation of the mechanisms underlying the therapeutic effects of RYGB may offer invaluable insight into the pathophysiologic mechanisms leading to obesity, as well as to provide novel targets for therapeutic intervention.

Anatomical reconfiguration of intestine is the main premise of RYGB. Therefore, we hypothesized that small intestine plays a critical role in the resolution of diabetes post-surgery. To test it, we developed a rat model of RYGB and identified the RYGB-induced changes in the metabolism of the different intestinal sections and determined the contribution of intestine to resolution of diabetes (Saeidi et. al., Science, 2013).

Here we demonstrate that RYGB improves diabetes primary via inducing a profound intestinal metabolic remodeling. Specifically, RYGB triggers a substantial increase in the rate of intestinal glucose metabolism through induction of Glucose Transporter 1. Consequently, blocking this transporter negates the antidiabetic effects of RYGB. We also found an increased rate of intestinal cell proliferation following RYGB. Further analysis demonstrated that stimulated intestinal glucose metabolism is the consequence of a substantial intestinal reprogramming (e.g. GLUT1 and PKM2) that leads to utilization of glucose in anabolic pathways to generate the biomass required for rapidly proliferating cells. Taken together, our results identified a central mechanism responsible for resolution of diabetes following RYGB.

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Poster Number 246

Correlation of 18F-FDG Avid Volumes on Pre- and Post-RadiotherapyScans in Recurrent Lung Cancer

Investigators: Nadya Shusharina, PhD; Gregory C. Sharp, PhD; Noah C. Choi, MD

Purpose: To investigate spatial correlation between high uptake regions of pre- and post-therapy 18F-FDG PET in recurrent lung cancer.

Methods and Materials: We enrolled 106 patients with inoperable lung cancer into a prospective study whose primary objectives were to determine: (a) the earliest time point where the maximum decrease in FDG uptake representing the maximum metabolic response (MMR) is attainable, (b) the optimum cutoff value of MMR based on its predicted tumor control probability, sensitivity, and specificity. Of these, 61 completed the required 4 serial 18F-FDG PET examinations after therapy. 19 of 61 patients developed local recurrence and are subject for analysis. Volumes of interest (VOI) on pre-treatment FDG-PET were detefined using an isocontour at 50% of SUVmax (SUV ≥ 50% of SUVmax) with correction for heterogeneity. VOI on post-treatment images were defined at ≥80% of SUVmax. VOI of pre- and post-therapy FDG PET images were correlated for the extent of overlap.

Results: The size of VOI at pre-therapy images was on average 25.7% (range: 8.8 - 56.3%) of the original gross tumor volume (GTV) and their overlap fractions were 0.8 (95% CI: 0.7 - 0.9), 0.63 (95% CI: 0.49 - 0.77), and 0.38 (95% CI: 0.19 - 0.57) of VOI of post-therapy FDG PET images at 10 days, 3 months, and 6 months respectively. The residual uptake originates from the pre-treatment VOI in 15 of 17 cases.

Conclusions: VOI defined by the SUVmax-50% isocontour may be a biological target volume for escalated radiation dose.

Poster Number 247

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Unsedated And Endoscopy Free Diagnosing Of Diseases Of The Upper Gastrointesinal Tract Using Tethered Capsule Endomicroscopy

Investigators: Amna R. Soomro MBBS; Michalina Gora, PhD; William Puricelli, RN; Weina Lu; Jenny Sauk, MD; Robert Carruth, MS; Mireille Rosenberg, PhD; Norman Nishioka, MD; Guillermo Tearney; MD, PhD

We have developed a new method for screening for gastrointestinal (GI) tract diseases termed "Tethered Capsule Endomicroscopy" (TCE). This device is an easily swallowable optomechanically engineered pill that provides three-dimensional (3D) high-resolution images of subsurface tissue microstructure. TCE provides diagnosis of esophageal diseases in a quick and pain free manner, without need of endoscopy or sedation.

The device consists of a swallowable rigid capsule measuring 11 mm X 25 mm, attached to a string-like tether that allows the operator to control the capsule and navigate it as it moves along the esophagus, via peristalsis. The tether houses the optical fiber, enclosed within a rotatable driveshaft, that transmits near infrared light to the focusing optics in the capsule. Two-dimensional images of the entire circumference of the esophagus are obtained by spinning the driveshaft, optical fiber, and optics. As the capsule progresses down the esophagus, multiple cross-sectional microscopic images are displayed in real-time and recorded. Three-dimensional representations of the TCE images are generated from this data after the procedure.

We have conducted TCE in 36 subjects, including healthy volunteers, patients with GERD, Barrett's Esophagus and Eosinophilic Esophagitis (Partners IRB protocol 2011-P-002619). The capsule was swallowed by 92% (33/36) subjects and very good quality images were acquired. Each case lasted an average of 5:17 ± 1:52 minutes. The results of this study suggest that TCE has the potential to become a useful tool for screening for esophageal diseases.

Poster Number 248

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Demonstration of fast kurtosis MRI for imaging acute stroke kurtosis/diffusion lesion mismatch

Investigators: Phillip Zhe Sun, PhD; Yu Wang, MD; Emiri Mandeville, MD; W. Taylor Kimberly, MD, PhD; Aneesh B. Singhal, MD; Eng H. Lo, PhD

Background: Tissue plasminogen activator (tPA) is the only FDA-approved thrombolytic agent for treating acute stroke. However, few patients present for tPA treatment within its narrow therapeutic window. MRI, particularly, combined diffusion-weighted MRI (DWI) and perfusion-weighted MRI (PWI), has been used to guide late tPA therapy. The rationale is that DWI detects bioenergetically compromised tissue while PWI defines areas of hypoperfused ischemic tissue, and their mismatch identifies the salvageable penumbra. However, the approximation of diffusion lesion as ischemic core is controversial; ischemic injury within the diffusion lesion is heterogeneous and the percentage of diffusion lesion reversibility is independently associated with positive outcome. New methods capable of refining stroke imaging are urgently needed.

Methods: We optimized kurtosis MRI in animal stroke models and verified tissue injury within kurtosis/diffusion MRI lesions with immunohistology. Fast kurtosis imaging has been validated in normal subjects and translated to the stroke clinic.

Results: Our data confirmed that the kurtosis lesion exhibits a greater increase in single-stranded DNA immunostained cells than the diffusion lesion without kurtosis change, indicating kurtosis defines the more severely injured DWI lesion. We showed that diffusion lesions without kurtosis change respond favorably to reperfusion while lesions with both diffusion and kurtosis abnormalities show poor recovery. To facilitate patient studies, we further demonstrated a fast kurtosis imaging method that identifies kurtosis/diffusion lesion mismatch within 2 minutes.

Conclusions: Our work establishes kurtosis as a new stroke MRI index, optimized and translated fast diffusion/kurtosis imaging for the acute stroke setting, which may ultimately advance imaging-guided stroke therapy.

Poster Number 249

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Why clinical brain monitors do not work in children under general anesthesia & Novel accurate monitoring to prevent neuro-toxicity

Investigators: Jasmine A. Thum, MS; Paul G. Firth, MD; Aaron L. Sampson; Kara J. Pavone; Emery N. Brown, MD, PhD; Patrick L. Purdon, PhD

Mortality and morbidity due to general anesthesia (GA) decreased greatly with the implementation of objective clinical monitoring devices. However, because there is still no reliable way to monitor the pediatric brain during GA, neuro-toxicity remains a major concern in pediatric anesthesia.

We first determined why current clinical brain monitors do not work in pediatric patients by recording 4-lead EEG data from 28 patients (ages 0-34 years) receiving sevoflurane GA. The relationship between EEG power and age in each of the 6 canonical frequency bands was well-described ($R^2 > 0.85$ and $P < 0.001$) using a 4-parameter logistic model, and showed a decline in power across all bands between approximately 15-20 years of age. Cognitive developmental changes occurring with age parallel the observed changes in electroencephalography (EEG) total power and power distribution in the frequency spectrum. Measures of total power and ratios of power are used by current clinical brain monitors to create depth of unconsciousness indices, leading to inaccurate evaluation of pediatric patients' anesthetic state.

However, we found a stereotyped spectral "anesthetic signature" that emerged from the EEG, regardless of age. This signature has been suggested as a reliable marker for loss of consciousness in adult patients undergoing propofol GA. This formed the basis for a novel technology for brain monitoring that could be applied to pediatric populations to replace current inaccurate methods. An appropriate monitoring paradigm will increase cost effectiveness due to potentially decreasing anesthetic drug over-dosing, emergence time, OR time, and patient morbidity and mortality resulting from neurotoxicity.

Poster Number 250

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Quantitative DTI Tractography of prostate gland in prostate cancer patients

Investigators: Alexey A Tonyushkin, PhD; Sandeep S Hedgire, MD; Peter F Hahn, MD, PhD; Shahin Tabatabaei, MD; Andrew JM Kiruluta, PhD; Mukesh G Harisinghani, MD

Diffusion tensor imaging (DTI) is commonly used to perform tractography that allows visualizing neuronal fiber map in a brain. However, this technique seldom been used for other visceral organs. Recent works show that DTI tractography of the prostate is feasible in-vivo in cancer cases and well-depict congregate fibers within the prostate. We applied DTI tractography to prostate MRI and developed a quantitative approach that is able to discriminate tumor vs. normal tissue for diagnostic purposes. We carried out HIPPA compliant retrospective study of N=25 men with biopsy proven prostate cancer. All patients undergone prostate MRI with endorectal coil on a 1.5 T MRI scanner. Fiber tracts were visualized with TrackVis software. We identified multiple spherical regions of interest (ROI) over areas of pathologically proven tumor and normal peripheral zone of the gland. For quantitative analysis we introduced a tract density parameter, which is a tract number divided by the ROI volume, as a normalized measure of the number of tracts passing through the given ROI. The quantitative analysis implies differences in tract number in tumor vs. normal gland, which is in good agreement with the structural observations of the fiber tracts in the gland. Since these differences are statistically significant we can design a novel imaging tool to determine size and/or aggressiveness of tumor during diagnostic or treatment phases of imaging. We will attempt to apply our method to analyze post radiation treatment cases where other modalities fail to yield contrast.

Poster Number 251

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GNAS and KRAS Mutation Analysis of Pancreatic Cyst Fluids

Investigators: Jessica L. Wang, MD; Emily Albanese; Amanda Pawlak; Carlos Fernandez-del Castillo, MD; William Brugge, MD; Dora Dias-Santagata, PhD; Long Le, MD, PhD; A. John Iafrate, MD, PhD; Mari Mino-Kenudson, MD; Martha Bishop Pitman, MD

Introduction: GNAS mutation is purportedly unique to IPMN. We report a prospective analysis of GNAS mutation in pancreatic cyst fluids (PCF).

Methods: PCF submitted for KRAS mutation were analyzed for GNAS mutation using SNaPshot. Ancillary testing data of the PCF was gathered including CEA (elevated at 192 ng/ml (eCEA)) and cytology (high-grade atypia (HGA) or not). Clinical and imaging features were recorded. Imaging was classified as benign (B), worrisome (W) or high-risk (HR).

Results: 33 PCF were analyzed. 4 (12%) were resected, all with W imaging. 2 PCF in the same patient with HGA, eCEA and KRAS and GNAS mutations, showed a concurrent ductal adenocarcinoma (PDAC). The other 3 resected cysts were GNAS and KRAS negative and included an IPMN with necrosis and intermediate-grade dysplasia on cytology, mucinous cystic neoplasm with low-grade dysplasia, and pseudocyst (PCT). Only the PCT and the IPMN associated with a PDAC showed an eCEA.

- 7 (21%) were GNAS positive; 4/5 GNAS mutant unresected cysts had a KRAS mutation and none had eCEA.
- 26 were GNAS negative. Of the 23 non-resected cysts, 6 (26%) were KRAS mutant and 6 (26%) had eCEA.
- 12 (36%) were KRAS positive and 8 (30%) had eCEA. Clinically, 22 cysts were IPMNs: 7 (32%) were GNAS positive, 11 (50%) were KRAS positive and 4 (18%) had eCEA.
- Of the 28 non-resected PCF, none had HGA.

Conclusions: GNAS may be suggestive of IPMN, but appears to be an insensitive molecular marker in the confirmation of a clinical and imaging diagnosis of IPMN.

Poster Number 252

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Characterization of novel gamma-secretase modulators in processing of the amyloid-beta precursor protein and in the therapeutics of Alzheimer´s disease

Investigators: Martin Zhang, MD, PhD; Justin Klee, BS; Scott Schulte; Rudolph Tanzi, PhD

Alzheimer's disease (AD) is a devastating neurodegenerative disease with no cure. Considerable genetic, biochemical and molecular biological evidence support the "amyloid-hypothesis" in the pathogenesis of AD, stating that the excessive accumulation of a small peptide, amyloid-β (Aβ), is the primary pathological event leading to AD. Aβ is generated through a sequential proteolytic cleavage from the amyloid-β precursor protein (APP) via β- and γ-secretase. One class of promising drugs for AD is known as γ-secretase modulators (GSMs), a group of small molecules that modulate the cleavage activity of γ-secretase in the processing of APP and specifically lowering Aβ levels without altering cleavage of other substrates, e.g. Notch. These GSMs bind directly to γ-secretase complex, decreasing the levels of longer Aβ species (e.g. Aβ42 and Aβ40) and increasing the levels of shorter Aβ species (e.g. Aβ38 and Aβ37). Here we have developed a novel series of GSMs and characterized those with desirable safety-profile and high aqueous solubility. We showed these GSMs significantly modulated γ-secretase processing of APP and lowered both Aβ42 and Aβ40 levels. Importantly, these GSMs did not affect the processing of Notch, an essential protein involved in development. These data provide further in-depth support of the "amyloid-hypothesis" in the pathogenesis of AD and provide the mechanism-of-actions utilizing these novel GSMs to lower Aβ levels in the therapeutics of AD. Our results warrant follow-up characterization of these GSMs in animal-based neurobehavioral studies and further strongly support them as excellent candidates in clinical development.