68th Annual Meeting
of the
MGH Scientific Advisory Committee

SAC 2015

April 1 and 2, 2015
Simches Auditorium
185 Cambridge Street, 3rd Floor

Poster Session Abstracts

RESEARCH MANAGEMENT | *Mainstay of MGH Innovation*

Executive Committee on RESEARCH | *Fostering Innovation at MGH*

Contents

SAC 2015

Wednesday, April 1, 2015

Annual Celebration of Science at MGH

11:00 am–1:45 pm | Simches, Floors 2 & 3

SAC 2015 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)

2015 MGH Research Scholars

Dr. Kingston

2:15–2:45 pm

2015 Martin Prize for Basic Research

Targeting Cancer Metastasis through Circulating Tumor Cells
Shyamala Maheswaran, PhD

2:45–3:15 pm

2015 Martin Prize for Clinical Research

Leveraging human 'knockouts' to understand wellness and disease
Sekar Kathiresan, MD

3:15–3:45 pm

2015 Goodman Award

Regulation of starvation survival and fat storage by thrifty metabolic pathways
Alexander A. Soukas, MD, PhD

3:45–4:00 pm

Break

4:00–5:00 pm

Keynote Address

Reflections on research training
Constance L. Cepko, PhD, Professor, Genetics and Ophthalmology, Harvard Medical School
Investigator, Howard Hughes Medical Institute

6:00–9:00 pm

Colloquium Dinner & Reception (for invited guests)

6:00–7:00 pm

Reception

7:00–9:00 pm

Dinner

After Dinner Remarks

The MGH Research Institute
Susan A. Slaugenhaupt, PhD
Scientific Director, MGH Research Institute

SAC 2015

Thursday, April 2, 2015

Annual Celebration of Science at MGH

8:00–9:00 am / Simches 3.120

Breakfast

SAC Members with ECOR leadership

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

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

Ragon Institute of MGH, MIT and Harvard
Bruce Walker, MD

10:40–10:50 am

Break

10:50–11:30 am

MGH Cancer Center, Daniel A. Haber, MD, PhD

11:30–1:00 pm / Simches, Floor 2

Lunch

SAC Members with Faculty

1:10–4:15 pm / Simches 3.110

Research Training: The Roads Ahead

1:10–1:20 pm

Opening Summary/Overview of Issue
Robert E. Kingston, PhD

Discussion Items

Roadblocks and Speedbumps
The current challenges facing trainees
Destinations
The opportunities & possible solutions
Bridges & Highways
The resources required to reach solutions

1:20–2:00 pm

Graduate Students

Moderator: Thilo Deckersbach, PhD
Panel: David Fisher, MD, PhD; David Langenau, PhD;
Andrea McClatchey, PhD

2:00–2:40 pm

Basic Scientist Fellows

Moderator: Dennis Brown, PhD
Panel: Bradley E. Bernstein, MD, PhD;
Sylvie Breton, PhD; Robert E. Kingston, PhD

2:40–3:00 pm

Break

3:00–3:40 pm

Physician Scientist Fellows

Moderator: Andrew A. Nierenberg, MD
Panel: Marcia Goldberg, MD; Jay Rajagopal, MD;
Ravi Thadhani, MD, MPH

3:40–4:15 pm

Open discussion:
"How does MGH capitalize in times of disruption?"

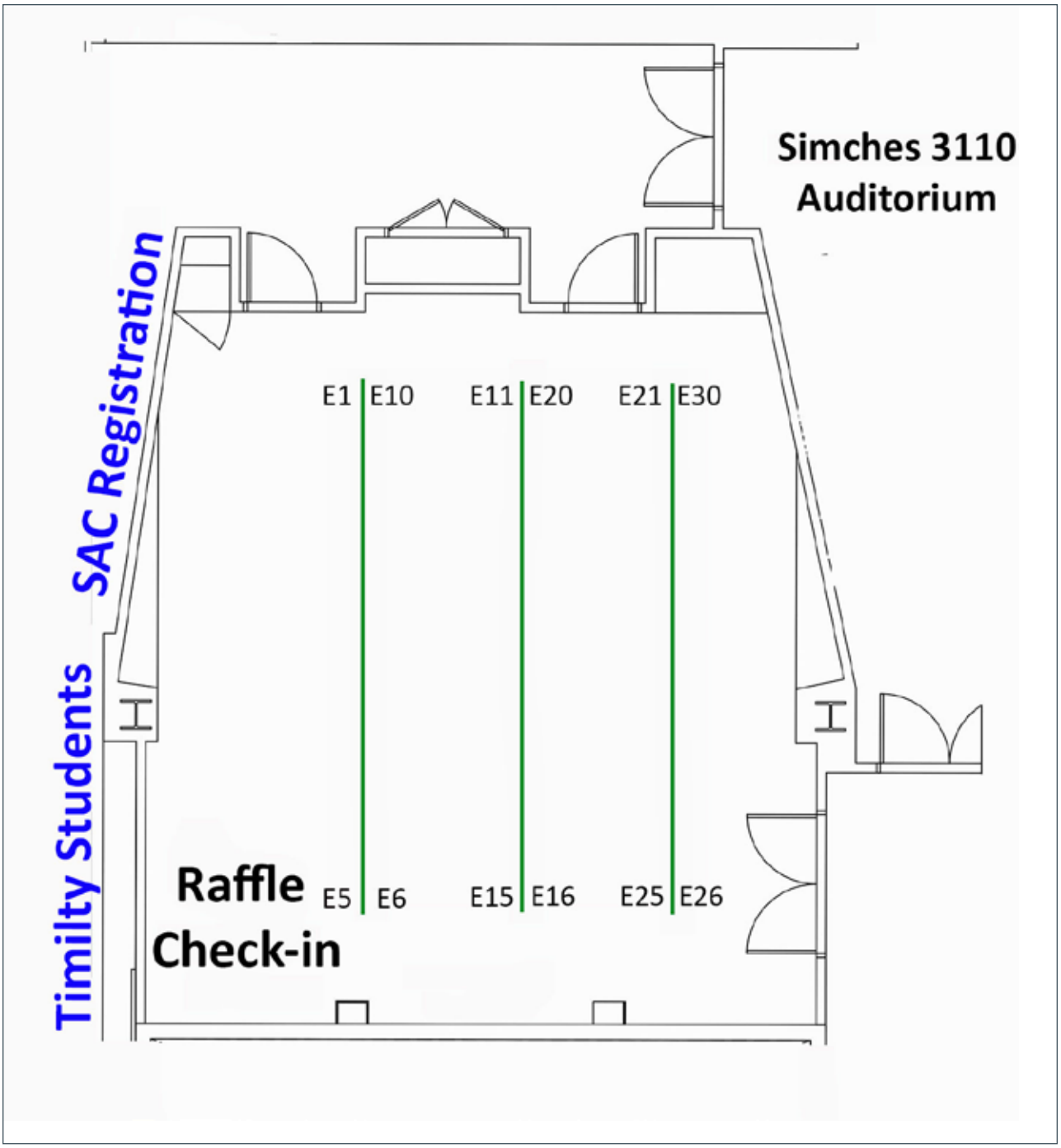
4:15–4:45 pm / Simches 3.120

Executive Session (SAC members only)

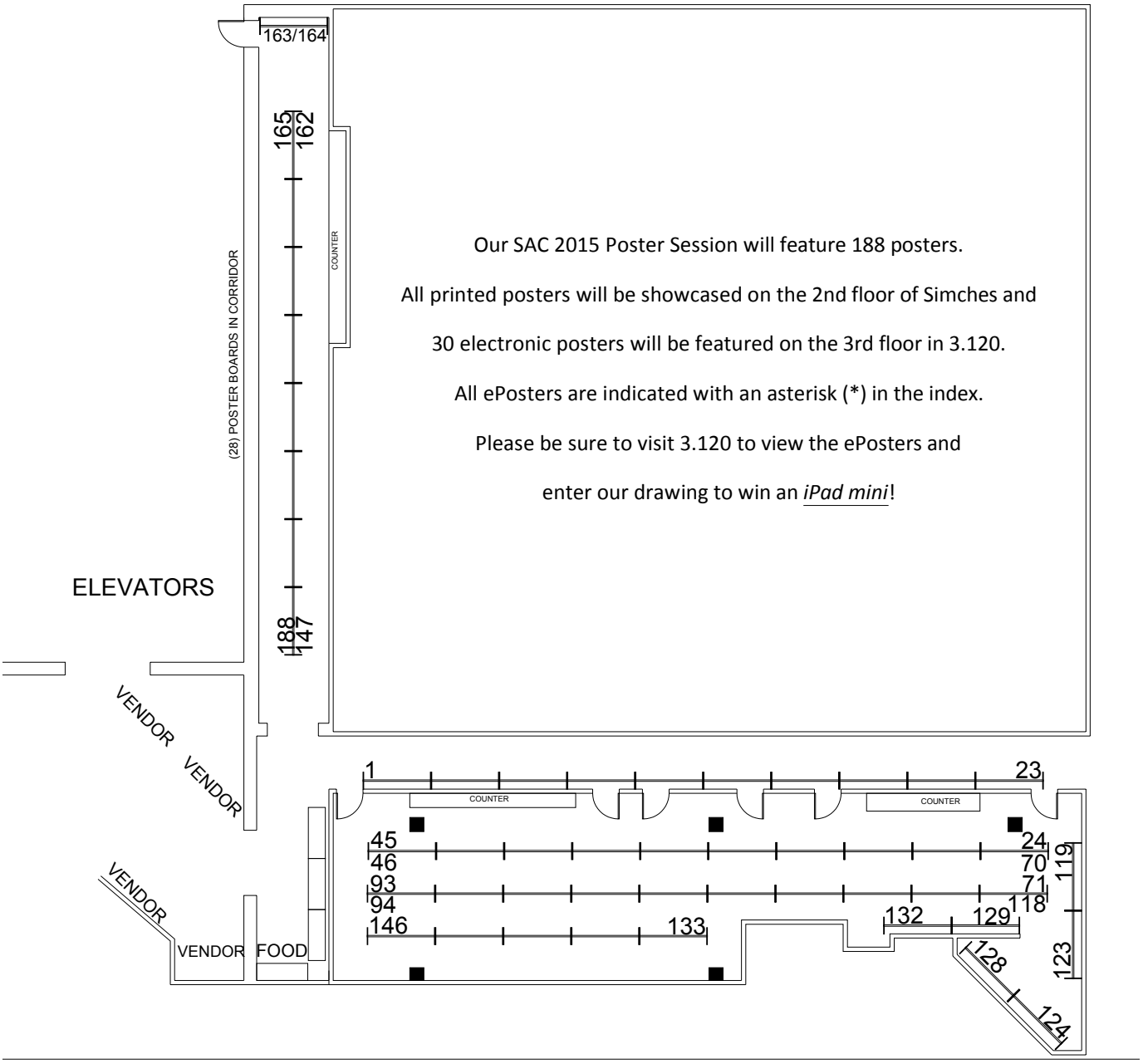
4:45–5:15 pm / Simches 3.120

Debriefing (SAC members and MGH Leadership)

Session Floor Plan
Simches Research Building, Room 3.120



Session Floor Plan
Simches Research Building, 2nd Floor



The James P. Timilty Middle 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). Students are matched with an MGH Volunteer mentor and meet at MGH every other Friday morning, over the course of 4 months, to complete projects. Below is a list of the participating students; their MGH mentors are shown in parenthesis.

7th Grade Students

- **Cynthia Hydes**—*Effects of cell phone use on driver behavior*
  (Lori Rizzo, MGH Operating Room Administration)
- **Marangela James**—*What is the effect of different toothpastes on teeth whitening?*
  (Irma Vlasac, MGH Saxena Lab)
- **Melissa Mendez**—*Weather & Human Emotio*n
  (Erin McGivney, MGH Executive Committee on Research)
- **Manyeuris Soriano**—*The effects of different beverages on our teeth*
  (Christine Elliot, MGH Center for Immunology and Inflammatory Diseases (CIID))

8th Grade Students

- **Peace Idahor**—*What is the effect of weather on student attendance and patient scheduling?*
  (Omar Lopez, Physical Therapy Services)
- **Aaron Cumberbatch**—*Kinematics: How do different surfaces affect the Kinematics of a ball rolling down a ramp?*
  (Rushil Desai & Sahil Shah, MGH Cancer Center)

  Angelys Matos—*Which locations in the home have the most bacteria?*
  (Dorothea Letner, MGH Gastrointestinal Associates)
- **Janelle Robinson**—*What area of the classroom has the most bacteria and under what conditions do the bacteria grow best?*
  (Joelle Brown & Tess Carley, MGH Gastrointestinal Associates)

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BIOENGINEERING & DEVICES

Poster Number 1

Christopher Chenelle, BA
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Comparison of the tracheal wall pressure exerted by five endotracheal tube cuffs including a novel low-volume cuff: a bench evaluation

Investigators: C. T. Chenelle, T. Itagaki, D. F. Fisher, L. Berra, R. M. Kacmarek

Background: The PneuX ETT (Venner Medical) uses a novel low-volume silicone cuff that has preliminarily shown superior leak prevention and equivalent tracheal wall pressures (TWP) to high-volume low-pressure (HVLP) cuffs when its intracuff pressure (ICP) is 80cmH2O. Our goal was to determine TWP of the PneuX and other ETTs.

Methods: The PneuX and four HVLP-cuffed ETTs (Mallinckrodt: Hi-Lo, TaperGuard, SealGuard; Kimberly-Clark: Microcuff) were tested (each n=3). TWP was determined by measuring the ability of the cuff to support a column of water (heights 20, 25, 30, 35, 40 cm) in a rigid trachea model. Each cuff was inflated to 20cmH2O above recommended ICP to establish the column, then ICP was slowly decreased while recorded in WINDAQ. The ICP where steady leak began, indicating TWP=column height, was recorded. All heights were tested with/without CPAP (10cmH2O) and lubrication (20 runs per tube).

Results: Neither CPAP nor lubrication affected TWP. For water heights 20, 25, 30, 35, and 40 cm, steady leak was recorded at ICPs 19.64±1.42, 24.66±1.41, 29.45±1.41, 34.47±1.71, and 39.88±1.88 cmH2O, respectively, for all HVLP cuffs. No difference was seen between HVLP tube types. The respective PneuX ICPs were 73.51±2.87, 78.08±2.36, 82.14±3.38, 86.41±2.76, and 91.59±3.15 cmH2O, yielding the equation: TWPAvg=1.1218*ICP-62.377, with TWP equated to column height in cm (r=0.999). Thus, TWPAvg of tested PneuX ETTs at ICP=80cmH2O was 27.37cmH2O.

Conclusions: Our findings support the manufacturer's claim that, at ICP=80cmH2O, PneuX ETTs, on average, exert TWPs within 20-30cmH2O, though some variability was seen. For HVLP cuffs, ICP≈TWP, as previously shown.

Poster Number 2

Ouri Cohen, PhD
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Vascular Repair by MR RF Coagulation

Investigators: O. Cohen, E. Nevo, R. G. Gonzalez, A. J. Yoo, J. L. Ackerman

Intracranial aneurysms occur in an estimated 1-6% of the population.. Current treatment methods consist of either i) microvascular neurosurgical clipping, ii) endovascular coiling or iii) embolization by a coagulable material. Neurosurgical clipping is highly invasive and presents less favorable outcomes compared to 'coiling' where detachable coils are deposited inside the aneurysm to induce coagulation. A disadvantage of coiling is the possible reopening of the aneurysm that may be caused by impaction of the coils. Radiofrequency ablation (RFA) is a low cost and minimally invasive method traditionally used in the treatment of primary and metastatic liver tumors. A lack of ionizing radiation and high soft tissue contrast make MRI an attractive choice for image guidedance in RFA. A novel RF ablation device was recently introduced that does not require an external RF power generator or connections to any external system, avoiding the need for a grounding pad to complete the electrical path and eliminating the possibility of accidental skin burns. By harnessing the RF energy outputted by the MR scanner using a passive conductive device (e.g. wire) localized heating is obtained with temperature increases of 50-70°C demonstrated in in-vivo porcine livers.

Here we describe a minimally invasive embolization method that requires no insertion of artifical objects (coils) or foreign materials to achieve embolization. Instead, human serum albumin, a common, nonthrombogenic and non-immunogenic protein solution is injected into the aneurysm and coagulation is achieved via MR RF heat deposition. Feasibility of the method is demonstrated by electromagnetic simulations and phantom experiments.

Poster Number 3

Lin Jin, MS
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Nonenzymatic RNA Replication Inside Giant Multilamellar Protocells

Investigators: L. Jin, J. Szostak

There is considerable interest in preparing giant multilamellar vesicles as protocell model for origin of life studies. The modern cell-like size provides more possiblities for constructing multiple dynamic activities inside, which are stablized and protected by the multi-bilayer structure from the outside perturbation. Here, we report a nonenzymatic RNA replication activity inside large monodisperse multilamellar fatty acid vesicles, prepared by extrusion-dialysis method (Zhu TF 2009). With the presence of Mg2+ chelators, we can eliminate the vesicle disruption effect of high Mg concentration, which is required for the ribozyme-catalyzed non-enzymatic RNA copying reaction (Adamala K 2013). By adding activated G monomer guanosine 5'-phosphor (2-methyl) imidazolide, we found the primer extension rate in large multilamellar protocells is faster than the small unilamellar ones, which indicates the multilamellar structure doesn't block the monomer diffusion, instead, it provides a more favorable environment for RNA replication reaction.

Poster Number 4

Adam Patenaude, BS
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Tissue Engineered Bone with Three-Dimensionally Printed β-TCP/PCL Scaffolds and Early Implantation: An in vivo Study in a Porcine Mandible Model

Investigators: S. Konopnicki, B. Sharaf, C. Resnick, A. Patenaude, T. Pogal-Sussman, K. Hwang, H. Abukawa, M. Troulis

Purpose: Deep bone penetration and angiogenesis into implanted scaffolds remains a challenge in tissue engineering. The purpose was to evaluate bone penetration depth and angiogenesis within 3Dimensionally (3D) printed β-Tricalcium phosphate (βTCP) /Polycaprolactone (PCL) scaffolds, seeded with porcine bone marrow-derived progenitor cells (pBMPC), and implanted early in vivo.

Methods: Scaffolds were 3D printed with 50% βTCP/50% PCL. pBMPC were harvested, isolated, expanded and differentiated into osteoblasts. Cells were seeded into scaffolds and incubated in a rotational oxygen-permeable bioreactor for 14 days. Six critical size defects were created per mandible (n=2 minipigs): 6 constructs (seeded scaffolds) placed within defects, 6 defects were controls (scaffodls n=3, empty n=3). Eight weeks after surgery, specimens were harvested and analyzed by H&E, DAPI and CD31 staining for bone penetration, cell count and angiogenesis respectively.

Results: All specimens (n=12) showed peripheral bone. Of 6 constructs, 4 exhibited central bone formation. Histomorphometric analysis of H&E stained sections showed an average of 23.8% central bone formation in constructs group, versus 2.6% in scaffolds ($p<.05$). The two constructs, without bone in the center, showed by DAPI massive cell penetration (2109 cells/57mm2 in center) compared to controls (1114 cells/57mm2) ($p<.05$). Central CD31 expression was higher in constructs compared to scaffolds ($p<.05$).

Conclusion: 3D printed β-TCP/PCL scaffolds, seeded with pBMPC, implanted early in critically sized mandibular defects display good bone penetration depth. Further study with larger samples and defects must precede clinical applications.

Poster Number 5

ePoster#1

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Can Simulated Activities Confirm the Need of Anatomical Articular Surface Together with ACL Preservation to Restore Normal Activity Dependent Knee Kinematics?

Investigators: T. Zumbrunn, K. Mangudi Varadarajan, H. E. Rubash, H. Malchau, G. Li, O. K. Muratoglu

In native knees anterior cruciate ligament (ACL) and asymmetric tibial articular surface with a convex lateral plateau are responsible for differential medial and lateral femoral rollback. Therefore, our hypothesis was that an asymmetric biomimetic articular surface together with ACL preservation would restore normal activity dependent knee kinematics for various activities.

In vivo kinematics of healthy knees obtained via bi-planar fluoroscopy was used to design a biomimetic bi-cruciate retaining (BCR) implant.

Kinematics of the biomimetic BCR design (asymmetric tibia with convex lateral surface), a contemporary BCR (symmetric shallow dished tibia) and CR implant (symmetric dished tibia) were analyzed using LifeModeler KneeSIM software. Femoral condyle center motions relative to tibia were tracked.

During chair-sit, the biomimetic BCR showed medial pivot motion similar to healthy knees in vivo with greater rollback of the lateral condyle (11mm vs. 5mm medial). The CR implant showed posterior femoral subluxation in extension and paradoxical anterior sliding followed by limited rollback without medial pivot rotation. The conventional BCR implant reduced the initial posterior femoral shift; however, medial pivot rotation and steady posterior rollback was not achieved.

During walking and stair-ascent the ACL sacrificing implant showed posterior subluxation in extension with paradoxical anterior motion. The biomimetic BCR design and the contemporary BCR implant showed kinematics similar to published in vivo data.

By simulating a variety of daily activities with different ranges of knee motion we were able to show that the ACL preserving biomimetic implant could restore activity dependent knee kinematics unlike

Poster Number 6

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Evaluating the therapeutic potential of CRISPR-Cas9 genome editing technology in human stem cells

Investigators: R. L. Collins, H. Brand, P. K. Mandal, A. Veres, A. Stortchevoi, C. Hanscom, A. Ragavendran, S. Erdin, D. Rossi, K. Musunuru, C. Cowan, M. E. Talkowski

Recent innovations in genome editing with targeted nucleases such as CRISPR-Cas9 have revolutionized in vivo genetic engineering. These new technologies bear tremendous potential for applications in both gene therapy and basic research. However, their efficiency and capacity to produce unintended consequences have not been extensively evaluated. Here, we assessed the on-target efficiency and off-target mutation frequency of both single- and dual-guide CRISPR-Cas9 treatment targeting three clinically relevant genes (SORT1, CCR5, B2M) in human embryonic stem cell clones and populations of hematopoietic progenitor cells using deep whole-genome sequencing (60X coverage) and exhaustive targeted sequencing (3,390X coverage). We evaluated the mutational landscape in each sample, and found on-target treatment efficiencies as high as 48.0%. Scrutiny of targeted sequencing data revealed a broad spectrum of mutations induced "on-target," including frequent chromosomal micro-rearrangements such as deletions (up to 42.13%) and inversions (up to 3.12%). Additionally, in one treatment we observed a 264bp fragment translocated from chr4 into the target site in a single CRISPR-treated clone. Further, we found minimal predictable off-target mutagenesis from both the whole-genome sequencing and targeted capture sequencing experiments. In the latter, we only observed one predictable off-target site to harbor a significant mutational burden above baseline, and that site was located in a paralog (CCR2) of the target locus CCR5 in that experiment. In summary, we find good efficiency and relatively high specificity of CRISPR-Cas9, suggesting that CRISPR represents a powerful tool in basic research and with further optimizations it could provide a technical leap forward in gene ablation therapy.

Poster Number 7

Gianluca De Novi, PhD
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Progress on Ocular and Facial Trauma Training Simulator

Investigators: G. De Novi, G. J. Loan, M. P. Ottensmeyer

An eye and face trauma training simulator is under development to fill a gap in available commercial systems for first responders and ophthalmologists, which focus on limb and torso trauma and retinal/cataract simulation, respectively. Here we present progress over the last year in the simulated anatomical components, compatibility with a commercial medical training mannequin, expansion of the instrument suite and user interface and simulation software. The system is being used in a validation study at the 2015 USUHS Ocular Trauma course.

We have fabricated new, realistic full-face silicone trauma modules, which feature lid laceration, reparable canaliculi with connections to the naso-lacrimal sac, edema and retrobulbar hemorrhage, and facial fracture/trauma. Bony components can be fixed or move relative to the skull to present palpable evidence of fracture. The face is compatible with a head/neck that can be mounted to a Laerdal SimMan Essential, integrating power and user interface components. Fourteen instruments are now available for the ophthalmologist's use, including a punctal dilator, additional needle holder, swab device and two miniature tying forceps. The user interface now detects hand gestures about the simulator's head, triggering updates in video/graphical content presented to the trainee. Software now tracks motion and zoom controls of an augmented reality binocular surgical microscope, and reads and controls mannequin physiological parameters so that the head form behaves consistently with the mannequin body, and interventions made on the head are reflected in the mannequin's behavior. It is currently in use in a comparison of training experience.

Poster Number 8

Vittorio Fontana, BS
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3D Gelatin Printer for Variable Stiffness Soft Tissue Anatomical Models

Investigators: V. Fontana, J. F. Hasson, M. R. Shayer, M. P. Ottensmeyer, G. De Novi, G. J. Loan, B. J. Fligor, M. P. Ottensmeyer

Rapid prototyping is applied to tissue engineering, but fabrication of simulated anatomy for medical training is yet a novel area of research. Here we present the development of a low-cost "3D printer" to build customized anatomical components to augment medical training scenarios. It uses non-toxic, food-grade gelatin and similar materials to produce variable stiffness tissue blocks with internal structures (e.g. vessels or tumors) that can be dissected, injected and palpated.

The rep-rap style printer's plastic extruder is replaced with a multi-element peristaltic mixing pump based on open source, downloadable designs. Concentrated gelatin is pre-heated, diluted and dispensed in the desired concentration. We characterized the melting temperature and stiffness of gelatin from saturated solution to the lowest concentration that gels. An ice-water chilled cooling plate rapidly solidifies the deposited liquid gelatin. Software tools are under development to convert CT data to instruction sets for rasterized gel deposition. Future versions will combine colorants, add fibers for texture and add saline solution to support dissection using electro-cautery.

Initial printed anatomy will be for simulators we developed with the Center for Medical Simulation for tumor dissection and with Boston Children's Hospital for IV cannulation training. The designs and code will be placed online for other sim centers to build their own printers and create custom anatomy with realistic tissue properties for their own training scenarios. In turn, we hope that this will evolve into a community that shares anatomical designs and hardware improvements to facilitate further advancements in medical training.

Poster Number 9

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Eradication of Multidrug-Resistant Pseudomonas Biofilm from Prolene Mesh with Pulsed Electric Fields

Investigators: S. I. Khan, D. Vecchio, G. Blumrosen, A. Golberg, M. C. McCormack, M. L. Yarmush, M. R. Hamblin, W. G. Austen, Jr.

Background: Biofilm formation is a significant problem, accounting for over eighty percent of microbial infections in the body. Control of biofilm persistence is problematic due to increased resistance to antibiotics and antimicrobials as compared to planktonic cells. The purpose of this study was to investigate the effect of Pulsed Electric Fields (PEF) on biofilm-infected mesh.

Study Design: Prolene mesh was infected with bioluminescent Pseudomonas and treated with PEF using a concentric electrode system to derive the critical electric field strength needed to kill bacteria. The effect of the number of pulses (with a fixed voltage, pulse length duration, and frequency) on bacterial eradication was investigated. For all experiments, biofilm formation and disruption were confirmed with bioluminescent imaging and Scanning Electron Microscopy (SEM).

Results: The critical electric field strength (Ecr) needed to eradicate bacteria was 792 V/mm. Bacterial eradication increases with number of pulses and electrical field strength. The area of complete bacterial eradication was 221.2, 281.2, and 305.9 mm2 for 100, 150, and 300 pulses, respectively ($p = 0.000056$), indicating that increased efficacy of treatment is due to the increased number of pulses delivered.

Conclusion: We have demonstrated a novel method for successful biofilm eradication using pulsed electric fields. We hypothesize that in the clinical setting, combining systemic antibacterial therapy with PEF will yield a synergistic effect leading to improved eradication of mesh infections. The results of this work indicate that high fields and increased number of pulses lead to increased bacterial eradication.

Poster Number 10

Yang Li, PhD
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Expansion of biological pathways based on evolutionary inference

Investigators: Y. Li, S. E. Calvo, R. Gutman, J. S. Liu, V. K. Mootha

Availability of genome sequences from diverse organisms provides a special opportunity to chart the evolutionary history of genes of interest. Such analyses provide insights into evolutionary pressures driving gene retention or loss and help to predict gene function based on correlated evolution. A major challenge in defining the phylogeny of a pathway, however, lies in the fact that pathway members typically do not exhibit a single, coherent ancestry, but rather, comprise a mosaic of evolutionary gene modules. We introduce a new computational method for automated detection and expansion of such modules in eukaryotes. Our method, called CLIME (clustering by inferred models of evolution), accepts as input a predefined species tree, a homology matrix, and a gene set of interest. CLIME partitions the input gene set into disjoint modules, simultaneously learning the number of modules and an evolutionary model that defines each module.CLIME scores all genes in the genome for the likelihood of having emerged under a module's inferred history, thereby expanding its membership. We applied CLIME to a tree of life consisting of 138 eukaryotic organisms. CLIME faithfully recovers known evolutionary modules within mitochondrial complex I, the calcium uniporter, and cilia while yielding new predictions. We have also applied it systematically to over 1000 classically defined human pathways, as well as the entire proteomes of yeast, red algae, and malaria. The results reveal unanticipated evolutionary modularity and novel, co-evolving components within many well-studied pathways. CLIME should become increasingly useful with the growing wealth of genome sequences from highly diverse organisms.

Poster Number 11

ePoster#2

Audrey Nebergall, BA
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Soft-Tissue Impingement in Dual Mobility Components: A Proposed Mechanism of Intraprosthetic Dislocation using Cadaver Models and Retrievals

Investigators: A.K. Nebergall, A. A. Freiberg, M. E. Greene, H. Malchau, O. Muratoglu, S. Rowell, T. Zumbrunn, K. Mangudi Varadarajan

Introduction: The large diameter mobile liner of dual mobility implant provides increased resistance to dislocation. However, intraprosthetic dislocation (IPD) of the small diameter head out of the liner is a problem specific to the dual mobility system.

Questions/purposes: We hypothesized that impingement of the liner with the surrounding soft-tissue inhibits liner motion, thereby facilitating IPD by transferring load from the femoral neck to the liner. This mechanism of soft-tissue impingement was evaluated via cadaver experiments; and retrievals were used to assess prevalence of polyethylene rim damage.

Methods: Dual mobility components with embedded metal wires were implanted in 10 cadaver hips. The interaction of the liner with the soft-tissue was studied using fluoroscopy and visual observation. Fifteen surgically-retrieved liners (for reasons other than IPD) were assessed for rim edge and rim chamfer damage (average 31.4 months in vivo).

Results: The cadaver experiments showed liner impingement with the iliopsoas tendon and hip capsule in low flexion angles, which impeded liner motion. All retrievals showed damage on the rim and the chamfer surface. The most common damage seen was scratching/pitting

Conclusions: The cadaver studies showed that liner motion can be impeded by impingement with the iliopsoas and hip capsule. All retrieved liners showed damage despite their limited time in vivo and despite being retrieved for reasons other than IPD. This suggests that soft-tissue impingement may inhibit liner motion routinely in vivo, resulting in load transfer from the femoral neck to the rim of the liner.

Poster Number 12

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Training Simulator for 3D Optical Ear Canal Scanner

Investigators: G. DeNovi, G. J. Loan, B. J. Fligor, M. P. Ottensmeyer

Hearing aid, ear phone and ear plug fitting requires accurate ear canal 3D geometry. Injecting two-part liquid silicone to form a rubber mold can be uncomfortable, and for patients with tortuous ear canals, difficult to complete. Processing from mold to completed device can be slow. Optical scanners under development can quickly capture 3D shape and jaw position-dependent variations, and export shape to a CAD program, shortening time to device delivery. Training for successful scanning teaches use of lateral (cartilage-supported) canal wall regions for stabilization while avoiding painful optical probe tip contact with the medial (bone-supported) ear canal or tympanic membrane (TM).

Here we present a simulation-based trainer with a realistic silicone head, interchangeable, CT-derived ear modules with varying difficulty, and contact force and scanner position sensors. For each ear, lateral, medial and TM force is measured using two nested x/y/z- and one TM-force sensing platforms and an Arduino Mega microprocessor. A TrackIR V aimed at either the left or right sides of the head tracks the scanner. A GUI displays forces relative to expert-defined pain thresholds and guidance on relative orientations of head and scanner for proper manipulation to acquire full 3D canal geometry.

Quantitative feedback will accelerate skill acquisition without risk to patients' or volunteers' own ear canals. Simulator-based training will result in more capable audiologists, less patient discomfort in the initial procedures performed by a new user of the scanner, and fewer contacts between scanner probe tip and the medial ear canal walls and tympanic membrane.

Poster Number 13
ePoster#3

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Tracking the Sleep Onset Process: An Empirical Model of Behavioral and Physiological Dynamics

Investigators: M. J. Prerau, K. E. Hartnack, G. Obregon-Henao, A. Sampson, M. Merlino, K. Gannon, M. T. Bianchi, J. M. Ellenbogen, P. L. Purdon

The sleep onset process (SOP) is a dynamic process correlated with a multitude of behavioral and physiological markers. A principled analysis of the SOP can serve as a foundation for answering questions of fundamental importance in basic neuroscience and sleep medicine. Unfortunately, current methods for analyzing the SOP fail to account for the overwhelming evidence that the wake/sleep transition is governed by continuous, dynamic physiological processes. Instead, current practices coarsely discretize sleep both in terms of state, where it is viewed as a binary (wake or sleep) process, and in time, where it is viewed as a single time point derived from subjectively scored stages in 30-second epochs, effectively eliminating SOP dynamics from the analysis. These methods also fail to integrate information from both behavioral and physiological data. It is thus imperative to resolve the mismatch between the physiological evidence and analysis methodologies. In this paper, we develop a statistically and physiologically principled dynamic framework and empirical SOP model, combining simultaneously-recorded physiological measurements with behavioral data from a novel breathing task requiring no arousing external sensory stimuli. Our model significantly outperformed the instantaneous transition models implicit in clinical definitions of sleep onset. Our framework also provides a principled means for cross-subject data alignment as a function of wake probability, allowing us to characterize and compare SOP dynamics across different populations. This analysis enabled us to quantitatively compare the EEG of subjects showing reduced alpha power with the remaining subjects at identical response probabilities.

Poster Number 14

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Determination of the allele specific huntingtin protein level using parallel reaction monitoring mass spectrometry

Investigators: B. Shin, A. Shin, J. Shin, K. Kim, T. Gillis, S. Kwak, M. E. MacDonald, J. F. Gusella, J. Lee, I. Seong

Huntington's Disease is a dominantly inherited neurodegenerative disorder caused by an expanded CAG repeat tract in the huntingtin gene. The ability to selectively measure endogenous mutant huntingtin level is useful; for example, recent therapeutic approaches strive to selectively reduce mutant huntingtin level. There is a delta 2642 glutamic acid deletion polymorphism (resulting in 5E instead of normal 6E) that is the defining characteristic for the most frequently found HTT haplotype in HD patients. We used this single amino acid difference to discern mutant alleles from normal to produce an allele-specific huntingtin protein quantification method using parallel reaction monitoring mass spectrometry (PRM-MS). We confirmed the ability of this assay to differentially measure mutant and normal huntingtin protein level using 5E/5E, 6E/6E and 5E/6E lymphoblastoid cells. The linearity and sensitivity of this assay were validated using different concentrations of 5E/6E lysate or multiple mixtures composed of different ratios of homozygous 5E/5E and 6E/6E lysates, respectively. Since there have been discrepancies regarding whether or not HTT mRNA transcription levels were CAG-length dependent, we decided to examine this postulated relationship using our PRM-MS method. We analyzed allele-specific huntingtin protein expression levels in 28 HD patient-derived 5E/6E lymphoblastoid cells representing the CAG-repeat range of adult onset using PRM-MS. The results proved that the lengths of CAG repeats were not significantly associated with HTT gene expression levels; thus, refuting previous arguments claiming that CAG repeat lengths influenced HTT transcription. This development is a significant forward of accurate evaluation of various HD models and gene silencing approaches.

Poster Number 15

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Engineering of Human iPS Cells to contain Copy Number Variants Associated with the Recurrent 16p11.2 Genomic Disorder

Investigators: D. J. Tai, A. Ragavendran, A. Stortchevoi, C. M. Seabra, P. Manavalan, I. Blumenthal, J. F. Gusella, M. E. Talkowski

Recurrent genomic disorders are caused by copy number variation (CNV) mediated by highly homologous segmental duplications flanking genic segments. Mispairing of the flanking regions leads to loss (deletion) or reciprocal gain (duplication) of the intervening genomic segment by non-allelic homologous recombination (NAHR). Collectively, NAHR-mediated recurrent CNVs are among the most frequent causes of autism spectrum disorder (ASD) schizophrenia, and intellectual disability. Unfortunately, each individual recurrent CNV is relatively rare, and accurate modeling of their impact in model systems represents a major challenge. Here, we developed a CRISPR/Cas9-mediated genome engineering method to create the 16p11.2 CNV which underlies one of the most common reciprocal CNV syndromes in humans. Using a dual-guide CRISPR/Cas9 approach in which the segmental duplications remain intact, we generated reliable microdeletion of all unique genes within the recurrent CNV segment (~545kb) in human iPS cells. To more closely model what occurs in vivo, we also generated a larger 740 kb microdeletion by designing a single guide RNA that uniquely binds to the 16p11.2 flanking duplications but not elsewhere in the genome. This resulted in deletions and duplications of the full CNV segment while ablating a single copy of each gene within the flanking segmental duplications in deletions. Using RNA sequencing as a readout, we obtained convincing evidence that our approach accurately generates reciprocal dosage changes of the region and thus accurately models the effects of the microdeletion syndrome lesions. We believe that this genome engineering approach will provide tremendous value in modeling of all recurrent genomic disorders.

Poster Number 16

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Mapping the Single Cell Non-Coding Landscape

Investigators: V. Thapar, M. Aryee, D. T. Ting

Functional noncoding DNA sequences have important biological functions in genome organization and transcriptional and translational regulation of coding sequences. Noncoding DNA comprises structural elements including centromeres, telomeres and scaffold attachment regions (SARs), and give rise to a range of functional RNA products. As part of our efforts to survey the landscape of cancer-associated noncoding RNA, we have created a bioinformatics pipeline to identify and annotate these regions in single cells. We define transcriptional units by classifying regions in which all base pairs are present in at least two samples or more and have two reads or more in each sample. We then go on to annotate these regions with features such as GC content, proximity to nearest gene etc. before we take clusters of single cells to find novel differentially expressed regions between these groups. Our current pipeline has been designed and annotated for the mouse genome and a similar pipeline for human is currently being developed.

Poster Number 17

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Site specific modification of proteins by ExoT-mediated ADP-ribosylation

Investigators: C-L. Tsai, B. Seed, S. Sassi

Proteins bearing specific sequence tags (ExoTags) derived from the ExoT substrate Crk (CT10 regulator of kinase) can be specifically modified via an ADP-ribosylation reaction catalyzed by the ADP-ribosyltransferase of Pseudomonas ExoT using NAD or NAD analogs. The ExoTag can be placed at either the N- or the C-terminus of the protein of interest or internally. The minimal ExoTag length is a 4 amino acid motif Arg-Leu-Ser-Arg (RLSR). Tandem copies of the minimal ExoTag greatly enhance the efficiency of modification and allow modification at multiple sites within the ExoTag. Several biologically active molecules such as biotin, fluorophores and RNA, can be attached to target proteins via ExoT mediated reactions. This system combines the selectivity of enzymatic labeling and the versatility of chemical labeling, allowing chemically diverse functionalities to be conjugated to substrate proteins in a site specific manner. The technology can be used in the development of protein based sensors and therapeutic protein-small molecule conjugates.

Poster Number 18

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Connectomic Biomarkers of Alzheimer's Disease Observed in Multi-Synaptic Pathways

Investigators: I. Aganj, G. Prasad, P. Srinivasan, A. Yendiki, P. Thompson, B. Fischl

The human brain consists of a set of complex structural and functional networks. Network-based analysis of brain white matter connections has proved promising in revealing the structural basis of cognitive dysfunction in Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI), and discovery of diagnostically and therapeutically important biomarkers. Standard approaches to computing structural connectivity often define the connection strength between two brain regions based on the tractography streamline between them. Such a direct fiber bundle is expected to be the major signal carrier between the two brain areas; however, multi-synaptic neural pathways—those relayed through other regions—also provide connectivity.

In this work, we develop and validate a novel mathematical model to account for indirect multi-synaptic neural pathways, thereby improving understanding of the brain network, and deriving more accurate connectomic AD biomarkers through augmenting the information offered by measures of direct brain connectivity. We exploit the mathematical convenience provided by Kirchhoff's circuit laws to account for indirect pathways. We model the multiple pathways connecting two regions by analogizing individual connectivity of each fiber bundle to the electrical conductance of a resistor, making two basic cases of connectivity similar to parallel and series circuits. We then use the Kirchhoff's circuit laws and graph Laplacian methods to compute the total connectivity of pairs of brain regions.

We validate our conductance model on a dataset of 200 subjects from the Alzheimer's Disease Neuroimaging Initiative, and show improved classification among Normal, early MCI, and late MCI groups by using the proposed technique.

Poster Number 19

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A novel electrocorticography grid using conductive nanoparticles in a polymer thick film on an organic substrate improves CT and MR imaging

Investigators: E. Ahmadi, L. Daftari Besheli, M. Y. Villeneuve, M. H. Lev, A. J. Golby, R. Gupta, G. Bonmassar

Purpose: Electrocorticography (ECoG) grids are routinely implanted over brain cortex for cortical mapping prior to epilepsy surgery. Such grids produce extensive metal artifacts at CT and MR imaging. This study compares CT and MR artifacts from conventional ECoG grids with those from a grid developed by deposition of conductive nanoparticles in polymer thick film on an organic substrate (PTFOS).

Methods: A 64-contact ECoG grid was developed in a PTFOS construct via deposition of silver nanoparticles on denaturized collagen. We compared the susceptibility artifacts at MR imaging, both in the field strengths of 3T and 7T, between the PTFOS grid and a platinum grid. We also compared metal streak artifacts at CT imaging between the PTFOS grid and a stainless steel grid. Platinum and stainless steel grids were used in MR and CT, respectively, as these materials tend to produce fewer artifacts in these respective modalities. All imaging was performed on a cadaveric human head specimen that was repeatedly imaged without and with different ECoG grids both in CT and MR scanners.

Results: The PTFOS grid produced essentially no artifacts in MR and CT images; the quality of the images with the PTFOS grid was comparable to images without any grid. Platinum and stainless steel grids, on the other hand, produced extensive and expected artifacts in the vicinity of the grid electrodes that severely degraded the quality of MR and CT images.

Conclusion: PTFOS technology can be used to suppress imaging artifacts from ECoG grids in both CT and MR imaging.

Poster Number 20

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Regional Aeration and Inflammation in Healthy Volunteers and Subjects with ARDS and Heart Failure

Investigators: K. A. Hibbert, T. W. Winkler, V. J. Kelly, K. Cosgrove, C. Holland, M. F. Vidal Mello, R. S. Harris, E. K. Bajwa

The acute respiratory distress syndrome (ARDS) is a highly prevalent and morbid condition in which the diagnosis relies on clinical criteria, which do not reliably discriminate between ARDS from causes of hypoxemic respiratory failure. We hypothesized that inflammation, measured by [18F]-fluorodeoxyglucose(FDG) PET, would be greater in patients with ARDS compared to those with heart failure(HF), and that both groups would have more inflammation than healthy volunteers(HV).

Three patients in each group underwent CT to assess aeration by gas fraction (FGas) and [18-F]-FDG PET to assess inflammation. Patlak analysis of [18F]-FDG kinetics was used to determine the Ki (uptake rate)—in the absence of malignancy, Ki is a measure of neutrophil activity. 18F-FDG kinetics and aeration were then analyzed in three isogravitational regions of interest.

FDG uptake by Ki was greater in ARDS patients than in HF and HV subjects ($p< 0.001$). There was a trend toward higher Ki in HF patients compared to HV subjects ($p=0.05$). In ROIs with similar aeration and aeration heterogeneity (non-dependent and middle regions), ARDS patients had significantly higher regional Ki than both HF and HV subjects ($p<0.05$).

Here we show that ARDS patients had significantly higher Ki than HF and HV patients. This was true even in regions with normal aeration. There was also a trend toward greater inflammation in the well-aerated regions of heart failure patients compared with healthy controls. These data demonstrate differences in metabolic activity among ARDS, heart failure and healthy subjects that are likely due to variations in neutrophilic inflammation.

Poster Number 21

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Novel tools for label-free, microscopic, in vivo imaging of vascular and cellular dynamics for physiology and pathology animal model studies

Investigators: J. Lee, D. A. Boas

Imaging technologies with unprecedented resolution have always led to a better understanding of physiological phenomena and pathological mechanisms. Here, this poster will outline our novel technologies for label-free, microscopic, in vivo imaging of vascular and cellular dynamics that are targeted for animal model studies. The technologies are based on optical coherence tomography (OCT). While OCT has mainly been used for in situ imaging of tissue structures at micrometer scale, we extended its utility into in vivo imaging of tissue dynamics at micrometer scale spatially and down to millisecond scale in temporal resolution.

In this poster, we will introduce four techniques:

(1) Quantification of capillary perfusion level through rapid volumetric angiogram imaging; (2) Quantitative measurements of the red blood cell speed and flux over many capillaries at the same time, leading to capillary network maps of the speed and flux; (3) High-speed imaging of the red blood cell flux over a capillary network with 1-s temporal resolution, for monitoring how the capillary network flow pattern changes in time; and (4) Single-cell resolution imaging of intracellular organelle movement over individual neurons, which can represent viability of each cell.

We are applying these technologies for physiological and pathological animal model studies through collaboration; for instance, we used the technique (1) for monitoring how the capillary perfusion level changes before and after ischemic stroke and reperfusion. As we seek further collaboration opportunities, this poster will help us and other investigators discuss what we can do together.

Poster Number 22

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18F-Labeling based on iodonium ylide: Validation of a radiopharmaceutical for human use

Investigators: S. H. Liang, B. H. Rotstein, N. Stephenson, L. Wang, L. Collier, N. Vasdev

Several promising new methodologies for labeling non-activated and/or sterically hindered aromatic molecules with fluorine-18 have emerged; however, translation for human use remains a challenge. We have recently reported a radiofluorination method for non-activated arenes using spirocyclic iodonium ylide (SCIDY) precursors. The goal of the present study was to expand the substrate scope of such reactions with new hypervalent iodine (III) ylides to include well-functionalized heterocycles and tertiary amines and demonstrate their suitability for routine clinical research studies.

Twenty substrates with focus on nitrogen-containing drug molecules and radiopharmaceuticals were labeled in 15-80% conversions. Representative heterocycles include 4-, 5- and 6-[18F]fluoroindoles (10-36%), 4- and 6-[18F]fluoroisoquinolines (73-80%), and a tertiary amine, 4-[18F]fluorobenzyl morpholine (44%). In order to demonstrate suitability of this method for human PET imaging studies, 3-[18F]fluoro-5-[(pyridin-3-yl)ethynyl] benzonitrile ([18F]FPEB; radiotracer for imaging mGluR5 in human) was synthesized in 20% (n = 3) uncorrected radiochemical yields with specific activities of 18 ± 1.4 Ci/μmol (666 ± 51.8 GBq/μmol), which featured a greater than ten-fold increase in the radiochemical yield and a greater than two-fold increase in specific activity than that of literature method. [18F]FPEB prepared by this method was validated for human use. In summary, we have demonstrated new applications of SCIDY on fully-functionalized nitrogen-containing aromatics and a one-step, regioselective, high-yielding, metal-free 18F-labeling method that is validated to prepare radiopharmaceuticals, including [18F]FPEB.

Poster Number 23

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White Matter Integrity Changes Preceding Alzheimer's Disease in Mild Cognitive Impairment

Investigators: E. R. Lindemer, D. H. Salat, B. Fischl, D. N. Greve

White matter damage is common in the brains of older adults, and its presence is elevated in Alzheimer's disease. Vascular dysfunction is the primary mechanism of these lesions, and this damage likely contributes to cognitive impairment and dementia. Little is known about the developmental trajectory of these lesions, and there is conflicting evidence about whether differences in lesions exist between individuals with mild cognitive impairment who will eventually convert to Alzheimer's and those who will not. The goal of this study was to assess quantitative and qualitative aspects of the longitudinal changes in white matter damage in individuals with mild cognitive impairment and to determine whether they contain information that may be useful in enhancing our knowledge of the conversion to Alzheimer's disease. Here we show that qualitative changes in the appearance of white matter lesions predate quantitative changes during the conversion from mild cognitive impairment to Alzheimer's disease. Tissue properties within lesions, as measured by several types of MRI, begin 18 months prior to Alzheimer's conversion, and are timed similarly to changes in hippocampal volume, a known structural indicator of Alzheimer's disease. Volumetric changes in lesions occur gradually after Alzheimer's onset, and are less robust for differentiating between individuals who will and who will not convert. This suggests that white matter lesions are important to the Alzheimer's disease conversion process, and may be an important component of the disease pathology. Furthermore, they may indicate a biological process involving cerebrovasculature that is critical to our understanding of Alzheimer's disease.

BIOMEDICAL IMAGING

Poster Number 24

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The Assessment of Atherosclerotic Plaque Classification in Spectral CT using Novel Numerical Observer

Investigators: A. Lorsakul, Q. Li, G. El Fakhri

The presence of atherosclerotic plaque is an independent predictor of risk for ischemic stroke and myocardial infarction. Spectral computed tomography (SCT) imaging is used to characterize the various structures and features important to plaque imaging. SCT (e.g. dual-energy CT (DECT) and multi-energy CT (MECT) based on photon-counting detectors) improves material differentiation by combining CT techniques with measurement of the energy-dependent attenuation properties of the examined tissues. Hence, SCT generates better image quality than conventional CT. However, literature evaluating the performance of SCT based on objective image assessment is very limited. In this work, we developed a novel numerical-observer method and used it to assess performance on discrimination vulnerable-plaque features, and compared the performance among MECT, DECT, and CT methods. Our method overcomes the main limitation of the current quantitative metrics for evaluating SCT, whose data domain is complex and multidimension. The proposed observer was designed as: first, accommodating the spatial attenuation information in each energy bin and second, integrating all spatial-attenuation information to finally assess the material characteristics acquired from multiple energy windows of SCT. The proposed methods were evaluated using simulated data based on binary classification tasks. Here we show the improvement of plaque-classification tasks, which were measured by discrimination figures-of-merit (SNR and AUC), using MECT in the range of 46.8% - 67.7% (all $p < 0.01$) for filtered backprojection images when compared to DECT and CT systems at equivalent dose. These methods can be further extended to other clinical tasks such as kidney or urinary stone identification applications.

Poster Number 25

ePoster#4

Srivalleesha Mallidi, PhD
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Utility of 3D ultrasound and photoacoustic imaging in subject stratification and treatment prediction.

Investigators: S. Mallidi, K. Watanabe, D. Timerman, T. Hasan

Prediction of response and tumor recurrence following a given therapy is necessary for effective treatment. In this talk, we demonstrate an approach towards this goal with an example of photodynamic therapy (PDT) as the treatment modality and photoacoustic imaging (PAI) as a non-invasive, response and disease recurrence monitor in a murine model of glioblastoma (GBM). PDT is a photochemistry-based, clinically used technique that consumes oxygen to generate cytotoxic species thus causing changes in blood oxygen saturation (StO2). We hypothesize that this change in StO2 can be a surrogate marker for predicting treatment efficacy and tumor recurrence. PAI is a technique that can provide a 3D atlas of tumor StO2 by measuring oxygenated and deoxygenated hemoglobin. We demonstrate that tumors responding to PDT undergo approximately 85% change in StO2 by 24-hrs post-therapy while there is no significant change in StO2 values in the non-responding group. Furthermore, the 3D tumor StO2 maps predicted if a tumor was likely to regrow at a later time point post-therapy. Information on the likelihood of tumor regrowth that normally would have been available only upon actual regrowth (10-30 days post treatment) in xenograft tumor model, was available within 24-hrs of treatment using PAI, thus making early intervention a possibility. Given the advances and push towards availability of PAI in the clinical settings, the results of this study encourage applicability of PAI as an important step, to guide and monitor therapies (e.g. PDT, radiation, anti-angiogenic) involving a change in StO2.

Poster Number 26

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Co-operative binding of mGlu4 allosteric modulators and orthosteric glutamate—Towards personalized CNS treatment?

Investigators: P. K. Poutiainen, K. E. Kil, Z. Zhang, D. Kuruppu, B. Tannous, A. L. Brownell

The interest in the role of metabotropic glutamate receptor 4 (mGlu4) in CNS related disorders have increased a need for methods to investigate the binding of allosteric drug candidates. Our results suggest that mGlu4 positive allosteric modulators have characteristic co-operative binding with orthosteric glutamate, which offers a notable insight to the further development of mGlu4 targeted therapies.

The endogenous glutamate binding activates the mGlu4 and reduces transmission at both striatopallidal synapses and synapses between subthalamic nucleus and the substantia nigra compacta, both of which are overacting for example in Parkinson's disease (PD). By modulating the dopamine system focused therapeutic approaches in the treatment of PD by allosteric modulators of mGlu4 are supposed to produce fewer side effects by elimination of dyskinesia. However, allosteric modulation involves complexity of pharmacological effects, mechanism of action, and ligand binding characteristics, which could explain why promising drug candidates often fail in the clinical trials.

In our experiments the orthosteric-allosteric co-operative binding mode was detected in the association assays between allosteric drug candidate (ML128, N-(4-chloro-3-methoxyphenyl)-2-picolinamide) and mGlu4, and the results indicate strong positive co-operativity function ($\alpha > 1$) between these co-binders. The results suggest that individual mGlu4 positive allosteric modulators have a unique co-operative binding with orhosteric ligand, which should be carefully considered when drug screening assays are conducted, data interpreted, new drug scaffolds designed and drug doses selected. Moreover, it is suggested that imaging studies should be included to optimize the drug dose for individual patients in further trials.

Poster Number 27

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Common Path Side Viewing Monolithic Ball Lens Probe for Optical Coherence Tomography

Investigators: K. Singh, D. Yamada, G. Tearney

Common path probes are highly desirable for optical coherence tomography as they reduce system complexity and cost by eliminating the need of dispersion compensation and polarization controlling optics. In this work, we demonstrate a monolithic ball lens based, common path, side viewing probe that is suitable for Fourier domain optical coherence tomography. The probe design parameters were simulated in Zemax modeling software and the simulated performance parameters were compared with experimental results. We characterized the performance of the probe by measuring its collection efficiency, resolution, and sensitivity. Our results demonstrated that with a source input power of 25 mW, we could achieve sensitivity of 100.5 dB, which is within 7 dB of the maximum possible sensitivity that could be achieved using a separate reference arm. The axial resolution of the system was found to be 15.6 μm in air and the lateral resolution (full width half maximum) was approximately 49 μm. The probe optics was assembled in a 500 micron diameter hypotube. Images of finger skin acquired using this probe demonstrated clear visualization of the stratum corneum, epidermis, and papillary dermis, along with sweat ducts.

Poster Number 28

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Organ Culture Model and Imaging Method for Studying Epithelialization Processes in Wound Healing of Skin

Investigators: Y. Wang, E. Gutierrez-Herrera, A. Ortega-Martinez, B. A. Farinelli, A. G. Doukas, R. R. Anderson, W. Franco

Background and Objectives: A wide-field UV fluorescence excitation imaging system, developed to image highly-proliferating cellular processes (295/340 nm excitation/emission wavelengths) and collagen cross-links (335/390 nm), was used to study wound epithelialization in skin organ culture. The main objective is to evaluate the feasibility of using UV fluorescence excitation imaging to monitor skin re-epithelialization.

Study Design: Circular dermal wounds of 3, 4 and 5 mm diameter were created in ex vivo human skin and cultured in different media. Fluorescence images, standard color images, histology (H&E staining) and immunohistology (pan-keratin) were used to assess epithelialization. We also measured the expression of the nuclear protein Ki67 to confirm the correlation between proliferation of cells and endogenous fluorescence.

Results: Proliferating keratinocytes forming new epidermis expressed higher endogenous fluorescence upon excitation of light at 295 nm wavelength. Fluorescence images at 295 nm were complementary to images at 335 nm, confirming the extent of the re-epithelialization area. Keratinocytes proliferation was highest in DMEM with EGF as compared to other media. Wounds of 3 mm diameter closed completely at day 12 in culture. Wounds of 4 and 5 mm diameter had closure extents of 91.1% ± 11.7 and 79.9% ± 6.7 at day 20. H&E and immunohistology (pan-keratin, Ki67) show that endogenous fluorescence excitation at 295 nm wavelength correlates with newly formed epidermis.

Conclusions: We have established an organ culture model and imaging method for studying epithelialization processes in wound healing of skin, our approach provides a non-destructive, objective, direct method for quantitative measurements in wound healing.

Poster Number 29

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In vivo studies of inflammatory response, and expression of mGlu5and dopamine D2 receptors in Shn-2 knock-out mice (schizophrenia-like model)

Investigators: J. Choi, A. Zhu, S. Hattori, K. Kil, T. Takagi, S. Ishii, T. Miyakawa, A. Brownell

Background: For development and evaluation of therapeutic approaches to schizophrenia with heterogeneous symptoms, translational models encompassing multifaceted symptoms are needed. Since deficiency of schnurri-2 (Shn-2) was reported to induce mild chronic inflammation in brain and confers molecular, neuronal and behavioral phenotypes to schizophrenia, Shn-2 knock-out (KO) mouse model was used as a schizophrenia phenotype. In this project positron emission tomography (PET) imaging was performed to evaluate expressions of metabotropic glutamate receptor-5 (mGlu5), dopamine D2 receptor and inflammatatory responses in Shn-2 KO mice.

Methods: In vivo PET imaging was performed in six male Shn-2-KO and eight control mice using ligands of mGlu5([18F]FPEB), translocator protein (TSPO) (18kDa)([11C]PBR28), and dopamine D2 ([11C] Raclopride). Locomotor activity was measured with amphetamine challenge.

Results: A significant increase of mGluR5 expression was detected in the cortex and hippocampus of the Shn-2 KO mice compared to controls. Also a significantly increased accumulation of [11C]PBR28 was found in the cortex, striatum, hippocampus and olfactory bulb of Shn-2 KO mice. [11C]Raclopride studies did not show significant difference of expression of dopamine D2 receptors between the Shn-2 KO and control mice. In the open field locomotor test the observed baseline hyperactivity of Shn-2 KO was diminished after amphetamine administration.

Conclusions: Similarly increased mGlu5 expression and TSPO uptake as observed in Shn-2 KO mice have been reported also in in vitro human schizophrenic brains. This study concludes that the Shn-2 KO-mouse model exhibited several behavioral and pathological markers resembling human schizophrenia, and the KO-mice might be a translational model for the disease.

Poster Number 30

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Targeting CXCR4 for MPNST Therapy

Investigators: S. Antoszczyk, S. D. Rabkin

Malignant peripheral nerve sheath-tumors (MPNST) are rare highly aggressive soft-tissue sarcomas arising in peripheral nerves, with very poor prognosis and resistance to traditional chemotherapy or radiation. The CXCR4/CXCL12 autocrine loop is upregulated in NF1- MPNST and promotes tumor growth. In this study we targeted CXCR4 activity with AMD3100 in orthotopic sciatic nerve MPNST models using implanted human S462 MPNST stem-like cells (MSLCs) into immunedeficient mice and mouse M2 MPNST cells, derived from a spontaneously-arising tumor in Nf1/Trp53 heterozygous mice, into syngeneic mice.

In vitro, MSLCs were quite sensitive to AMD3100, while mouse M2 MPNST cells were not. Treatment of human MSLCs with AMD3100 resulted in decreased levels of phospho-GSK3-beta, phospho-AKT and phospho-p42/44 MAPK. To evaluate in vivo efficacy of AMD3100, mouse MPNST M2 cells and human MSLCs were implanted into the sciatic nerves of immunocompetent and athymic mice, respectively. Mice bearing tumors had increased levels of serum CXCL12. Mice displaying neurologic deficits, when tumors were established, were treated with AMD3100. In both the human and mouse tumors, AMD3100 significantly inhibited tumor growth, decreased neurological deficits and extended survival in comparison to control mice. We also observed that AMD3100 treatment of mice bearing MSLC tumors led to decreased levels of phospho-GSK3-betaSer9 and phospho-AKTSer473 suggesting that CXCR4 promotes MPNST growth by activating the PI3K/ AKT and the GSK3-beta signaling pathways.

These studies demonstrate the value of sciatic nerve MPNST models for testing novel therapeutics and suggest that inhibition of the CXCR4/CXCL12 pathway warrants consideration for clinical testing in patients with MPNST.

Poster Number 31

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A specialized mechanism of miRNA mediated translation activation in cellular Quiescence

Investigators: S. I. Bukhari, S. S. Truesdell, S. Lee, A. Classon, W. Haas, S. Vasudevan

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, 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 mediated translation.

Poster Number 32

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A mechanism that produces slowly proliferating cancer cells

Investigators: I. Dey-Guha, C. P. Alves, A. C. Yeh, X. Sole, R. Darp, S. Ramaswamy

All cancers contain an admixture of rapidly and slowly proliferating cancer cells. This proliferative heterogeneity complicates the diagnosis and treatment of cancer patients because slow proliferators are hard to eradicate, can be difficult to detect, and may cause disease relapse sometimes years after apparently curative treatment. While clonal selection theory explains the presence of rapid proliferators within cancer cell populations, we do not understand how slow proliferators are produced. Here, we discover a β1-integrin / FAK / mTORC2 / AKT1-associated signaling pathway that can be triggered when rapidly proliferating cancer cells divide to produce slowly proliferating AKT1low daughter cells in vitro. These findings suggest that the proliferative heterogeneity within cancer cell populations may, in part, be produced through a targetable signaling mechanism, with potential implications for understanding cancer progression, dormancy, and therapeutic resistance.

Poster Number 33

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Up-regulation of CD44 in the development of metastasis, recurrence and drug resistance of ovarian cancer

Investigators: Y. Gao, R. Foster, H. J. Mankin, F. J. Hornicek, M. M. Amiji, Z. Duan

Purpose: The clinical significance of Cluster of differentiation 44 (CD44) remains controversial in human ovarian cancer. The aim of this study is to evaluate the clinical significance of CD44 expression by using a unique tissue microarray, and then to determine the biological functions of CD44 in ovarian cancer.

Experimental Design: A unique ovarian cancer tissue microarray (TMA) was constructed with paired primary, metastatic, and recurrent tumor tissues from 26 individual patients. CD44 expression in TMA was assessed by immunohistochemistry. Western blotting and Immunofluorescence assay determined the expression of CD44 and/or P-glycoprotein (Pgp) in ovarian cancer cell lines and the tumor tissues of a paclitaxel treated ovarian cancer xenograft mouse model. MTT assay, wound healing assay, and matrigel invasion assay were utilized to assess the proliferation, drug sensitivity, and migration/invasion ability, respectively.

Results: Both the metastatic and recurrent ovarian cancer tissues expressed higher level of CD44 than the patient-matched primary tumor. A significant association has been shown between CD44 expression and both the disease free survival and overall survival. A strong increase of CD44 was found in the tumor recurrence of mouse model. Finally, when CD44 was knocked down, proliferation, migration/invasion activity, and spheroid formation were significantly suppressed, while drug sensitivity was enhanced.

Conclusions: Up-regulation of CD44 represents a crucial event in the development of metastasis, recurrence, and drug resistance to current treatments in ovarian cancer. Developing strategies to target CD44 may prevent metastasis, recurrence, and drug resistance in ovarian cancer.

Poster Number 34

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Increase of nuclear Survivin by XPO1 inhibition enhances radiation response in preclinical models of rectal cancer

Investigators: I. Ferreiro-Neira, N. E. Torres, L. F. Liesenfeld, Y. Landesman, W. Senapedis, S. Shacham, T. Hong, J. C. Cusack

Radiosensitizers are drugs that enhance tumor response to radiation. The combination of radiation with radiosensitizing chemotherapy improves outcomes for advanced rectal cancer. Current treatment includes 5FU-based chemoradiation prior to surgical resection, however only 15% of rectal cancer patients achieve a pathologically complete response, prompting the need to identify new radiosensitizers. XPO1 mediates the nuclear export of critical proteins required for rectal cancer proliferation and treatment resistance. We hypothesize that inhibition of XPO1 may radiosensitize cancer cells by altering the function of these critical proteins resulting in decreased radiation resistance and enhanced antitumoral effects. To test our hypothesis, we used the selective XPO1 inhibitor, Selinexor, to inhibit nuclear export in combination with radiation fractions similar to that given in clinical practice for rectal cancer: hypofractionated short-course radiation dosage of 5Gy per fraction or the conventional long-course radiation dosage of 1.2 Gy fractions. Single and combination treatments were tested in colorectal cancer (CRC) cell lines and xenograft tumor models. Combination treatment of radiation therapy and Selinexor resulted in an increase of apoptosis and decrease of proliferation compared with single treatment, which correlated with reduced tumor size. We found that the combination promoted nuclear survivin accumulation and decreased cytoplasmic survivin, which plays a critical role in its antiapoptotic function, resulting in increased apoptosis and enhanced radiation antitumoral effects. These data support the need for clinical trials with Selinexor in combination with radiation therapy for patients with rectal cancer and provide the mechanistic basis of this combination.

Poster Number 35

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EWS-FLI1 Utilizes Divergent Chromatin Remodeling Mechanisms to Directly Activate or Repress Enhancer Elements in Ewing Sarcoma

Investigators: N. Riggi, B. Knoechel, S. M. Gillespie, B. E. Bernstein, M. N. Rivera

The aberrant transcription factor EWS-FLI1 drives Ewing sarcoma, but its molecular function is not completely understood. We find that EWS-FLI1 reprograms gene regulatory circuits in Ewing sarcoma by directly inducing or repressing enhancers. At GGAA repeat elements, which lack evolutionary conservation and regulatory potential in other cell types, EWS-FLI1 multimers induce chromatin opening and create de novo enhancers that physically interact with target promoters. Conversely, EWS-FLI1 inactivates conserved enhancers containing canonical ETS motifs by displacing wild-type ETS transcription factors. These divergent chromatin-remodeling patterns repress tumor suppressors and mesenchymal lineage regulators while activating oncogenes and potential therapeutic targets, such as the kinase VRK1. Our findings demonstrate how EWS-FLI1 establishes an oncogenic regulatory program governing both tumor survival and differentiation.

Poster Number 36

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HER2 over-expressing high grade endometrial cancer expresses high levels of p95HER2 variant

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Background: Subsets of high grade endometrial cancer (EnCa) over-express HER2 (ERBB2), yet clinical trials have failed to demonstrate any anti-tumor activity utilizing trastuzumab, an approved platform for HER2 positive breast cancer (BrCa). A truncated p95HER2 variant lacking the trastuzumab binding site may confer resistance. The objective of this investigation was to characterize the expression of the p95HER2 truncated variant in EnCa.

Materials and Methods: With institutional approval, 86 high grade EnCa tumors were identified with tumor specimens from surgeries performed between 2000-2011. Clinical data were collected and all specimens underwent tumor genotyping, HER2 immunohistochemistry (IHC, HercepTest®), HER2 fluorescent in situ hybridization (FISH), along with total HER2 (H2T) and p95HER2 assessment with VeraTag® testing. Regression models were used to compare a cohort of 86 breast tumors selected for equivalent HER2 protein expression.

Results: We identified 44 high grade endometrioid and 42 uterine serous carcinomas (USC). IHC identified high HER2 expression (2+ or 3+) in 59% of the tumors. HER2 gene amplification was observed in 16 tumors (12 USC, 4 endometrioid). Both HER2 gene amplification and protein expression correlated with H2T values. High p95HER2 expression above 2.8 RF/mm2 was observed in 53% (n = 54) with significant correlation with H2T levels. When matched to a cohort of 107 breast tumors based on HercepTest HER2 expression, high grade EnCa presented with higher p95 levels ($p < 0.001$).

Conclusions: These data demonstrate that compared to BrCa, high grade EnCa expresses higher levels of p95HER2 possibly providing rationale for the trastuzumab resistance observed in EnCa.

Poster Number 37

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Establishment of a Novel Neu-positive Mammary Tumor Model for Studies of Combining Losartan with Radiotherapy to Improve Treatment for Metastatic Breast Cancer

Investigators: P. Huang, W. Li, Y. Liu, Y. Huang, R. Ramjiawan, X. Han, C. H. Leung, V. P. Chauhan, D. Fukumura, J. S. Loeffler, D. G. Duda, R. K. Jain

Improving the response of HER2/Neu-positive metastatic breast cancer to multimodal treatments is urgently needed. To this end, we established a novel Neu-positive (Neu+) metastatic mammary tumor model named MCa-M3C. We first transplanted spontaneous mammary adenocarcinoma tissue from MMTV-PyVT transgenic mouse into the mammary fat pads (MFP) of wild-type FVB mice. Subsequently, their lung metastatic tumors were collected and re-transplanted into the MFP of naïve FVB recipients. Following 3 serial in vivo selections of the breast cancer progenies spontaneously metastasized to the lungs, the fresh lung metastatic tumor tissue was harvested, cultured and successfully established a cell line in vitro. This cell line showed a stable cell doubling time (25.3±4.4 h), high platting efficiency (0.43±0.04), and resistance to irradiation in vitro, as well as high tumorigenicity and transplantability in vivo. We observed spontaneous lung metastases in about 75% of mice within 2-4 months after resection of MFP primary tumors at a size of 10 mm. These MFP tumors and their lung metastases retained adenocarcinoma histological features. Strong Neu+ expression in both the cells and tumors was confirmed by Western blot. Furthermore, we detected high collagen density in tumor stromal by Masson's Trichrome staining. We therefore combined anti-fibrotic treatment using the angiotensin receptor blocker losartan with ionizing radiotherapy in this MCa-M3C model. Following a 7–day treatment with losartan (40 mg/kg body weight gauvaged daily), a 20 Gy single dose local irradiation was given. Losartan significantly enhances irradiation local tumor control ($p=0.0018$), decreases MCa-M3C MFP-to-lung metastases ($P<0.025$), and increases host survival ($P=0.03$).

Poster Number 38

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Obesity promotes resistance to anti-VEGF therapy in Breast Cancer via pro-inflammatory and angiogenic pathways

Investigators: J. Incio, D. T. McManus, P. Suboj, N. N. Rahbari, S. M. Chin, S. Babykutty, T. Vardam-Kaur, Y. Huang, D. G. Duda, R. Soares, D. Fukumura, R. K. Jain

Background: Most breast cancer (BC) patients are overweight or obese at the time of diagnosis. Obesity is associated with increased risk, recurrence, and worse prognosis of BC. It has been shown that obesity associates with worse outcome in metastatic kidney or colon cancer treated with bevacizumab. If and how excess body weight contributes to the failure of anti-VEGF therapy in BC is unknown.

Results: Here we found that diet-induced obesity promoted resistance to anti-VEGF therapy in two syngeneic mouse breast cancer models. The effects of anti-VEGF therapy on tumor growth and metastasis, VEGF downstream signaling pathways and vessel density were significantly attenuated in obese mice. Under obesity condition, intra-tumor adipocytes increased. These adipocyte-rich regions in breast cancers were hypoxic and overexpress IL-6 or FGF-2 by adipocytes, fibroblasts, and myeloid cells. In IL-6 overexpressing obese breast cancer model (E0771), neutralization of IL-6, either genetically or pharmacologically, abrogated the obesity-induced resistance to anti-VEGF therapy seen in both primary and metastasis sites. This occurred due to a reversion of the obesity-augmented STAT3 signaling and cell proliferation, of hypoxia via vessel normalization, and of immunosuppression. In another breast cancer model (MCaIV), which overexpress FGF-2 under obesity, anti-FGF receptor antibody restored tumor sensitivity to anti-VEGF treatment in obesity.

Conclusion: Our findings indicate that obesity promotes resistance to anti-VEGF therapy in breast cancer via the production of pro-inflammatory and angiogenic factors that circumvent the loss of VEGF signaling.

Poster Number 39

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Lineage-dependent Response to Targeted BRAF Inhibition

Investigators: Z. Ji, Y. Chen, R. Kumar, M. Taylor, C. Njauw, B. Miao, D. Frederick, J. Wargo, G. Jonsson, H. Tsao

Response to targeted therapies varies significantly despite shared oncogenic mutations. Nowhere is this more apparent than in BRAF(V600E)-mutated malignancies where variable drug sensitivity- both within and between tumor types- and acquired resistance to BRAF(V600E) agents have created strong headwinds in the pursuit of durable efficacy. Both primary and secondary resistance to BRAF(V600E) inhibitors have been attributed to the upregulation and/or activation of various receptor tyrosine kinases (RTKs) though the underlying mechanisms that drive RTK activation has been largely uncharacterized. Here, we first proved that EGFR induced vemurafenib resistance was ligand dependent. We then employed whole-genome expression analysis and found that vemurafenib resistance correlates with the loss of MITF, along with its melanocyte lineage program, and with the activation of EGFR signaling. An inverse relationship between MITF, vemurafenib resistance and EGFR was then observed in patient samples of recurrent melanoma and was conserved across melanoma cell lines and patients' tumor specimens. Functional studies revealed that MITF depletion activated EGFR signaling and consequently recapitulated the vemurafenib resistance phenotype. In contrast, forced expression of MITF in melanoma and colon cancer cells inhibited EGFR and conferred sensitivity to BRAF/MEK inhibitors. These findings indicate that an "autocrine drug resistance loop" is suppressed by melanocyte lineage signal(s), such as MITF. This resistance loop modulates drug response and could explain the unique sensitivity of melanomas to BRAF inhibition.

Poster Number 40

ePoster#5

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A Survey of G0-like Cancer Cells in Human Tumors

Investigators: S. Kabraji, I. Dey-Guha, M. Bowden, M. Loda, M. Mino-Kenudson, D. Sgroi, S. Ramaswamy

Clinical evidence suggests that all human tumors have sub-populations of cancer cells that are 'slow proliferators'. These quiescent or 'sleeping' cancer cells are likely highly resistant to cancer drugs that aim to kill rapid proliferators. Strategies to target these 'sleeper' cells may provide novel insights into ways to overcome treatment resistance, dormancy and disease relapse. We have previously identified highly-proliferative cancer cells that become spontaneously quiescent in vitro and in vivo, which we term 'G0-like cancer cells'. These rare cells arise through asymmetric cancer cell division events triggered by a novel beta1-integrin-mTORC2-AKT1 signaling pathway to produce an AKT1(low) daughter cell in a 'G0-like' state. We also identified G0-like cancer cells in primary breast tumors from post-mastectomy tissues, where they appear to survive intensive exposure to neo-adjuvant combination chemotherapy. These data suggest that G0-like/AKT1(low) cancer cells are highly treatment resistant, and may be a major source of post-treatment relapse in breast and other cancers. To explore the wider clinical relevance of this finding, we undertook a survey of five human epithelial primary tumor types to determine the frequency of G0-like cancer cells. Here we show that, using immunofluorescence microscopy, we identified G0-like cancer cells in formalin-fixed paraffin embedded primary patient tumor tissues (n=20) across a range of tumor types (adenocarcinoma of breast, colon, gastro-esophageal junction, prostate and pancreas) and at a consistent frequency (1.89% ± 0.28%). These results set the stage for analysis of larger disease-specific patient cohorts to understand the role of G0-like cancer cells in resistance to cancer

Poster Number 41

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BAP1 Plays a Survival Role in Cutaneous Melanoma

Investigators: R. Kumar, M. Taylor, Z. Ji, J. Njauw, G. Jönsson, D. Frederick, H. Tsao

BAP1 in general viewed as a tumor suppressor due to its inactivation in Ocular melanoma specimen and in the BAP1 cutaneous/ocular melanoma (CM/OM), but the specific role of BAP1 gene in the malignancy of cutaneous melanoma is not completely unveiled. We subsequently set out to describe BAP1 in cutaneous melanoma and found an amazing survival impact of this protein. Tissue and cell lines investigation demonstrated that BAP1 expression was maintained, as opposed to lost, in primary melanomas contrasted with nevi and normal skin. Hereditary depletion of BAP1 in melanoma cells diminished multiplication and colony formation ability, prompted apoptosis and repressed melanoma tumor development in vivo. On the molecular level, suppression of BAP1 has driven an attending drop in the protein levels of survivin a member of anti-apoptotic proteins and a known mediator of melanoma survival. Renewal of survivin in melanoma cells somewhat saved the growth hindering impacts of BAP1 loss. In contrast to melanoma cells, stable over expression of BAP1 into immortalized however non-transformed melanocytes did suppress multiplication and decrease survivin. Taken together, these studies exhibit that BAP1 may play a growth sustaining role in melanoma cells. However, that its effect on ubiquitination underpins a complex physiology which is context and cell dependent. The study will likely impact not just on the melanoma field but be relevant to other research fields given the broad interest in BAP1 function.

Poster Number 42

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Increase of radiosensitivity by a fully human cell surface GRP94-specific monoclonal antibody W9

Investigators: L. Liu, Y. Wang, N. Zhang, F. Soldano, X. Wang

Radiation therapy (RT) is one of the major therapies for cancer treatment. However, radioresisatnce posts challenges to curative-intent RT. Glucose regulated protein 94 (GRP94), which is also known as heat shock protein 90kDa beta member 1 (HSP90B1), is a chaperone protein. GRP94 is ubiquitously expressed in all nucleated cells and is located in the endoplasmic reticulum (ER). It has been shown that increased expression of GRP94 was associated with radioresistance in cervical cancer. Knockdown GRP94 protein expression by siRNA increased radiosensitivity in cervical cancer cell lines. In our laboratory, we have developed a fully human monoclonal antibody (mAb) W9, which recognizes GRP94 expressed on cell surface. Our ongoing work has demonstrated that mAbW9 stained a panel of human cancer cell lines, but however, it did not stain a normal microarray of multiple normal human organ tissues. Moreover, mAbW9 inhibited the phosphatidylinositol-3-kinase (PI3)/protein kinase B (AKT) signaling pathway, activation of which is associated with radioresistance, in human breast cancer cells. It is, therefore, our choice to use mAbW9 to selectively target GRP94 expressed on cancer cells, rather than the GRP94 inhibitor or GRP94 silencing siRNA, both of which target GRP94 in cancerous and normal cells. Our study indicates that a fully human cell surface GRP94-specific mAb W9 can selectively enhance radiosensitivity of cancer cells expressing cell surface GRP94. Moreover, mAbW9 can resensitize an acquired radioresistant cell line to radiation.

Poster Number 43

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Implications of new lumpectomy margin guidelines for breast-conserving therapy: Changes in re-excision rates and predicted rates of residual tumor

Investigators: A. L. Merrill, S. C. Coopey, R. Tang, M. McEvoy, M. C. Specht, K. S. Hughes, M. A. Gadd, B. L. Smith

2014 guidelines endorsed by SSO, ASBS and ASTRO advocate "no tumor on ink" as the new margin standard for breast conserving therapy (BCT). We used our lumpectomy margins database from 2004-2006 to predict the effect of these new guidelines on BCT. Patients with neoadjuvant therapy, pure DCIS or incomplete margin data were excluded. We applied new ("no tumor on ink") and old (≥2 mm) margin guidelines and compared rates of positive margins, re-excision and rates of residual disease found on re-excision. 437 met eligibility criteria. Median age was 55 yrs (29-91). 86% had invasive ductal carcinoma (IDC), 12% invasive lobular carcinoma (ILC) and 2% IDC and ILC. Using a ≥2mm margin standard, 36% of lumpectomies had positive margins compared with 19% using new guidelines ($p<0.0001$). 77% of patients with "tumor on ink" had residual disease found at re-excision. 50% of those re-excised for margins <2mm had residual disease ($p=0.0013$), but would not have been re-excised with new guidelines. Residual tumor was more common in re-excisions for DCIS <2mm from a margin than in those for invasive cancer (53% vs. 40%), although this was not statistically significant. Use of new lumpectomy margin guidelines would have reduced re-operation for BCT by half in our patient cohort. However, residual disease was present in many patients who would not have been re-excised with the new guidelines. Long-term follow-up of local recurrence rates is needed to determine if this increase in residual disease is clinically significant.

Poster Number 44

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SCID Zebrafish: The Next Generation Cellular Transplantation Platform

Investigators: J. C. Moore, Q. Tang, N. Torres, D. M. Langenau

Cell transplantation into immune compromised mice has revolutionized the fields of immunology, stem cell biology, regenerative medicine and serves as a critical platform for assessing therapeutic responses in vivo. However, these experiments routinely utilize small cohorts of animals, are expensive and engraftment is difficult to visualize. We have generated immune compromised strains of zebrafish by targeting genes required for immune cell function including forkhead box N1 (foxn1/nude), recombination-activating gene 2 (rag2), DNA-dependent protein kinase (prkdc), and interleukin 2 receptor gamma (il2rg) and janus kinase 3 (jak3). These mutations are known to cause severe combined immune deficient (SCID) phenotypes in both humans and mouse. Molecular and cellular characterization of these lines demonstrate variable immune competency with differential loss of T and B cell function. Importantly, individual models engraft stem cells from allogeneic donors including hematopoietic cells from whole kidney marrow and muscle satellite cells. Moreover, we demonstrate robust engraftment of primary or serial transplanted tumors including fluorescently labeled leukemias, muscle tumors, and melanomas from a wide range of zebrafish strains. This demonstrated ability to transplant non-immune matched cell types will revolutionize the types and scale of cell transplantation experiments performed in the zebrafish. We are currently optimizing protocols and genetic models for the xenotransplantation of human cells. Generation of immune compromised zebrafish that engraft human tissue has the potential to usher in a new era of personalized medicine, allowing for patient stratification into open clinical trials based on responses to therapy in the zebrafish xenograft model and for novel drug discovery.

Poster Number 45

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YAP expression and localization in pediatric liver tumors

Investigators: K. A. Mueller, M. J. LaQuaglia, J. Grijalva, A. Perez-Atayde, H. B. Kim, G. Sadri-Vakili, K. Vakili

Currently, there are no effective chemotherapies for the treatment of hepatocellular carcinoma (HCC), and both HCC and hepatoblastoma (HB) remain lethal in their metastatic forms. Therefore, understanding the molecular mechanisms of tumor survival and proliferation are critical for the development of novel therapies. Our previous findings have demonstrated that Yes-associated protein (YAP), a transcription factor, is involved in liver regeneration. Specifically, we found that increases in nuclear YAP were coupled with expression of genes important for cell proliferation. Therefore, we sought to examine whether there is increased nuclear localization, as well as increased expression, of YAP in liver tumors. Tumor tissue and normal liver tissue from 6 pediatric patients with HCC (5 males and 1 female, mean age 11.08 years), and 6 HB patients (4 males and 2 females, mean age 2.99 years) were analyzed for these studies. Reverse transcription and quantitative real-time PCR (RT-qPCR) was used to measure YAP expression. The cellular localization of YAP was measured using immunohistochemistry. While there was no change in YAP expression, there was an increase in YAP nuclear localization in liver tumors. YAP nuclear staining was significantly increased in tumor cells compared to normal liver in 5/6 of the HCC and 2/6 of the HB tumors. Increases in nuclear YAP may indicate a crucial role for YAP in tumor proliferation and survival, and suggest that targeting YAP or its upstream pathway (Hippo) may provide a novel target for therapy.

Poster Number 46

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Detection of Dual IDH1 and IDH2 Mutations by Targeted Next-Generation Sequencing in Acute Myeloid Leukemia and Myelodysplastic Syndromes

Investigators: M. Y. Platt, A. T. Fathi, D. R. Borger, A. M. Brunner, R. P. Hasserjian, D. Dias-Santagata, Z. Zheng, L. P. Le, T. A. Graubert, A. J. Iafrate, V. Nardi

Studies in myeloid neoplasms have described recurrent IDH1 and IDH2 mutations as primarily mutually exclusive. Over a six-month period of clinical testing with a targeted next-generation sequencing (NGS) assay, we evaluated 92 patients with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and chronic myelomonocytic leukemia (CMML), and identified a subset of 21 patients (23%) who harbored mutations in either IDH1 or IDH2. Intriguingly, of the 21 patients with IDH mutations, 4 (19%) were found to have single nucleotide variants (SNVs) in both IDH1 and IDH2. An additional patient included in the study was found to have two different IDH2 mutations. The mutations were typically present at different variant allelic frequencies (AFs), with one predominating over the other, consistent with the presence of multiple subclones in a single patient. In one case, the variant AFs in both IDH1 and IDH2 were equally low in the setting of a high percentage of blasts, suggesting that the IDH mutations were unlikely to be present in the founding clone. Given these data, we conclude that dual IDH1/2 mutations have likely been previously underestimated, a finding which may carry significant treatment implications.

Poster Number 47
ePoster#6

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CXCR4 inhibition in tumor microenvironment facilitates anti-PD-1 immunotherapy in sorafenib-treated hepatocellular carcinoma

Investigators: R. R. Ramjiawan, Y. Chen, T. Reiberger, R. R. Ng, T. Hato, Y. Huang, H. Ochiai, S. Kitahara, E. C. Unan, T. P. Reddy, C. Fan, P. Huang, N. Bardeesy, A. X. Zhu, R. K. Jain, D. G. Duda

Sorafenib—a broad spectrum tyrosine kinase inhibitor—is the only approved therapy for advanced hepatocellular carcinoma (HCC), but provides limited survival benefits. Recently, immunotherapy has emerged as a promising treatment strategy, but its role remains unclear in HCCs. Using orthotopic HCC models, we show that sorafenib increases hypoxia which in turn, promotes immunosuppression, characterized by increased intratumoral expression of the immune checkpoint inhibitor programmed death-ligand 1 (PD-L1) and sorafenib treatment also led to an increase of T-regulatory cells and M2-type macrophages. The recruitment of the immunosuppressive cells is mediated in part by hypoxia-induced upregulation of stromal cell-derived 1 alpha (SDF1a). Inhibition of the SDF1a; receptor (C-X-C receptor type 4 or CXCR4) using AMD3100 prevented the polarization toward an immunosuppressive microenvironment after sorafenib treatment, inhibited tumor growth, reduced lung metastasis, and improved survival. However, combination of AMD3100 and sorafenib did not increase cytotoxic CD8+ T-lymphocyte infiltration into HCC tumors and did not modify their activation status. In separate experiments, blockade of PD-1 alone showed modest anti-tumor effects in treatment-naïve tumors in orthotopic and in genetically engineered models of HCC. However, anti-PD-1 treatment had additional anti-tumor activity only when combined with sorafenib and AMD3100, and not when combined with sorafenib alone. The triple combination treatment activated CD8+ T-cells, which led to a significant increase of intratumoral infiltration and resulted into inhibition in tumor growth and lung metastases. Modulation and activation of immune responses by combining AMD3100 and anti-PD1 may be a novel approach to prevent tumor evasion from sorafenib treatment in HCC.

Poster Number 48
ePoster#7

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Targeting Tumor Heterogeneity in Bioengineered 3D Cancer Models

Investigators: I. Rizvi, E. Briars, S. Anbil, H. Gudejko, N. Xu, S. Khan, G. Cramer, J. P. Celli, T. Hasan

Tumor heterogeneity remains a major barrier to effectively treating most advanced stage cancers. Stress from physical forces and communication with stromal cells are both important determinants of tumor heterogeneity, but remain understudied. Metastatic cells overcome many obstacles as they grow and colonize distant sites, including stress from physical forces caused in part by the movement of physiological fluid and ascites. This flow-induced shear stress has been shown to cause epithelial-mesenchymal (EMT), a process by which epithelial cells trade specialized function and proliferative capabilities for a migratory and invasive phenotype. EMT is associated with increased metastatic potential and resistance in many tumors. Among the numerous heterotypic stromal partners that are worthy of investigation, tumor endothelial cells are emerging as dynamic regulators of metastatic progression and mediators of treatment resistance. Here, bioengineered in vitro 3D tumor models are described to investigate the influence of flow and tumor endothelial cells on the molecular, morphologic and genetic features of resistant disease. Current findings on the impact of EMT status and communication with tumor endothelial cells on tumor heterogeneity and variability in response to conventional and emerging therapies will be presented. A particular emphasis will be placed on identifying targeted strategies that use mechanistically-distinct treatment modalities to regionally prime stubborn tumor populations. This approach of using physiologically-inspired bioengineered models to characterize the stromal and physical drivers of treatment resistance may be critical to overcoming key barriers to effectively manage advanced stage disease in complex sites while minimizing toxicity.

Poster Number 49

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Combinatorial therapy for triple negative breast cancer using Grp94-specific monoclonal antibody and the hedgehog signalling pathway inhibitor LDE225

Investigators: M. T. Rodia, L. Yang, Y. Wang, L. Cai, X. Wang, F. Sabbatino, E. F. Brachtel, S. J. Isakoff, S. Ferrone

The need of novel effective therapies for metastatic triple negative breast cancer (TNBC) has prompted us to design a novel combinatorial strategy which targets differentiated TNBC cells and TNBC cancer initiating cells (CICs). The latter have to be eradicated in order to "cure" a malignant disease, since they are believed to be responsible for disease recurrence and metastases. The tumor antigen selected as a target is the glucose-regulated protein of 94kDa (Grp94). Grp94 regulates the activation of signaling pathways associated with cell proliferation, survival and migration. To target Grp94, we take advantage of the unique specificity of the human mAb W9 we have recently characterized. mAb W9 recognizes an extracellular Grp94 epitope selectively expressed on cancer cells, including differentiated TNBC cells and TNBC CICs, but with a restricted distribution in normal tissues. mAb W9 markedly inhibits TNBC and TNBC CICs in vitro proliferation. To enhance its anti-proliferative activity we have combined mAb W9 with LDE225, a novel inhibitor of the Sonic Hedgehog (SHH) pathway which is aberrantly activated in TNBC and especially in TNBC CICs. mAb W9 enhances the ability of LDE225 to inhibit TNBC cell in vitro growth, and to eliminate TNBC CICs by inhibiting signaling pathways involved in cell proliferation and survival of TNBC cells and TNBC CICs. Moreover, the mAb W9 and LDE225 combination is more effective than the individual agents in eradicating TNBC tumors in immunodeficient mice orthotopically grafted with the TNBC cell line SUM149. This information contributes to optimize mAb W9-based immunotherapy in TNBC patients.

Poster Number 50

ePoster#8

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Endothelial podosome rosettes regulate vascular branching in tumor angiogenesis

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The mechanism, by which angiogenic endothelial cells break the physical barrier of vascular basement membrane and consequently sprout, forming new vessels in mature tissues, is unclear. Here, we show that angiogenic endothelium is characterized by the presence of functional podosome rosettes. These extracellular matrix-degrading and adhesive structures are precursor of de novo branching points and represent a key event in the formation of new blood vessels. VEGF-A stimulation induces the formation of endothelial podosome rosettes by up-regulating integrin $\alpha 6\beta 1$; in contrast, the binding of $\alpha 6\beta 1$ integrin to vascular basement membrane laminin impairs the formation of podosome rosettes by restricting $\alpha 6\beta 1$ integrin to focal adhesions and hampering its translocation to podosomes. Using ex vivo sprouting angiogenesis assay, transgenic and knock-out mouse models and human tumor samples analysis, we provide evidences that endothelial podosome rosettes control blood vessels branching and are critical regulators of pathological angiogenesis.

Poster Number 51

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Define the early-stage of experimental bone metastases development in immunocompetent mice.

Investigators: N. Severe, N. Baryawno, D. T. Scadden

Approximately 2/3 of patients with advanced breast cancer develop bone metastases that are associated with skeletal complications and poor prognosis. Recent data generated from our lab have demonstrated that specific subsets of bone marrow stromal cells protect breast cancer cells subjected to radiation therapy in the setting of co-culture assays. Our objective is to extend this work and to study the bone metastatic niche in-vivo. We hypothesize that bone metastatic cells interact with a specific subset of bone marrow stromal cells that control the quiescence, proliferation and survival of cancer cells.

Here we recently established the first bone metastatic breast cancer cell lines with a C57Bl/6 genetic background. Bone metastatic cells have been established by injecting the parental breast cancer cells into the left ventricle of mice and harvested from long bones. This protocol has been repeated two consecutive times. These cells express the Luciferase and Tdtomato reporters that allowed us to track bone metastatic cells in-vivo and ex-vivo. We demonstrated that cancer cells are able to colonize long bones of mice by in-vivo bioluminescence imaging and ex-vivo by flow cytometry analysis. Moreover, we were able to image early-stage of experimental bone metastases development by using whole bone marrow confocal fluorescent imaging.

By using innovative technologies and recently established bone metastatic cell lines we aim to define interactions between metastatic breast cancer cells and specific subsets of bone marrow stromal cells using reporter mice. By characterizing these interactions, we hope to discover novel therapeutic target to reduce bone metastases development.

Poster Number 52

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Adeno-associated Virus 9 (AAV-9) delivered human Mullerian Inhibiting Substance (MIS) significantly inhibits growth of epithelial ovarian cancer patient derived xenografts

Investigators: A. B. Sosulski, D. Pepin, D. Wang, L. Zhang, K. Hendren, A. Yu, M. J. Birrer, G. Gao, P. K. Donahoe

Introduction: Mullerian Inhibiting Substance (MIS) has been shown to inhibit established ovarian cancer cell lines both in-vitro and in-vivo and particularly to target a putative ovarian cancer progenitor enriched population. Given the complexity of the MIS protein, we examined whether an AAV9-MIS viral vector could be used to treat a panel of primary ovarian cancer xenotransplants as a single intraperitoneal injection.

Methods: Primary ovarian cancer cell lines (n=15) were established from ascites of patients with multi-resistant, high grade serous cystadenocarcinoma. 10^6 cells from n=5 cell lines were xenografted subcutaneously into female NOD/SCID gamma mice (5/group). Tumor volumes were monitored until they reached a size 0.1cm^3 when animals were given a single intraperitoneal injection of 3E11 AAV9 viral particles carrying a modified MIS transgene or a GFP or empty vector control construct.

Results: Gene therapy using an AAV9 viral vector carrying the modified MIS resulted in sustained high blood concentrations by MIS ELISA, and significant inhibition of tumor growth in 3/5 experiments from five sets of primary patient derived xenografts ($p<0.05$).

Conclusion: AAV9-MIS successfully inhibited the growth of a panel of patient derived ovarian cancer xenografts after a single intraperitoneal injection. AAV9-MIS gene therapy is being developed as a patient friendly treatment strategy for this deadly gynecologic disease.

Poster Number 53

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Characterizing Intratumoral Heterogeneity in Human Glioma using Single Cell Transcriptome Sequencing

Investigators: A. S. Venteicher, I. Tirosh, C. Hebert, L. E. Escalante, W. T. Curry, D. Cahill, B. V. Nahed, D. N. Louis, A. Regev, M. L. Suva

Intratumoral heterogeneity within gliomas constitutes a primary barrier to treatment and a fundamental cause of recurrence. To characterize intratumoral heterogeneity, we sought to analyze primary gliomas at single-cell resolution through RNA sequencing. We isolated and dissociated fresh tumors and purified thousands of single cells using flow cytometry. Using a sensitive reverse transcriptase and amplification strategy, we generated high quality cDNA libraries from picogram amounts of RNA from each single-cell, for up to one thousand cells per tumor sample, detecting up to 8,000 transcripts per cell. Using computational methods we use RNA-seq to reconstructed genomic copy number variation and identify genetic and transcriptional subpopulations of cells within gliomas. In particular, we identify that cycling cells are restricted to a very specific transcriptional program with a distinct signature enriched for key chromatin regulators and master transcription factors. In conclusion, we identify a population of cells that propagates gliomas in humans and highlight candidate dependencies in their program.

Poster Number 54

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Myogenic Regulatory Factors and their role in embryonal rhabdomyosarcoma

Investigators: I. M. Tenente, M. Ignatius, E. Chen, M. Hayes, D. M. Langenau

Rhabdomyosarcoma (RMS) is a sarcoma of muscle. RAS pathway deregulation is the most common oncogenic event in Embryonal (ERMS) and fusion-negative Alveolar (ARMS) subtypes. We have shown in a rag2-kRASG12D-driven zebrafish ERMS model that tumor-propagating cells (TPCs) express Myf5, m-Cadherin and c-Met. MYF5 and MYOD1 are bHLH transcription factors that orchestrate skeletal muscle differentiation during development and regeneration and are sufficient to reprogram human mesenchymal cells into a myogenic fate. Given that Myf5 is up-regulated in human ERMS and the specific expression of Myf5 in TPCs in our ERMS model, we hypothesized that Myf5 regulates the specification of TPCs. Induced expression of Myf5 is sufficient to confer TPC ability to differentiated populations of ERMS cells. These “Induced TPCs” generate aggressive tumors that retain molecular markers of muscle differentiation but re-express satellite cell markers. Tumor incidence and re-growth following transplantation into rag2E450fs immune-compromised zebrafish are not impaired in myf5-/- tumors. As the identity of the cell-of-origin for RMS is controversial the redundancy between Myf5 and MyoD expression in muscle progenitors, our results suggest that the myf5-/- kRASG12D-driven zebrafish ERMS model recapitulates a distinct class of ERMS. This idea is consistent with the fact that MYF5 is expressed in only 50% of primary human ERMS and some human cell lines. Knock down experiments in human ERMS cell lines confirm independent roles of either MYF5 or MYOD1 in the maintenance of ERMS cell viability in vitro. Collectively, results support a previously unappreciated role for bHLH MRFs in ERMS cell survival and functional heterogeneity.

Poster Number 55

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Unexpected roles for KDM4A: protein synthesis and mTOR inhibitor sensitivity

Investigators: C. Van Rechem, J. C. Black, P. Greninger, Y. Zhao, C. Donado, P. d. Burrowes, B. Ladd, M. J. Aryee, S. Gräslund, D. C. Christiani, C. H. Benes, J. R. Whetstine

Single nucleotide polymorphisms (SNPs) occur within chromatin-modulating factors; however, little is known about how these variants within the coding sequence impact cancer progression or treatment. Therefore, there is a need to establish their biochemical and/or molecular contribution, their use in sub-classifying patients and their impact on therapeutic response. We demonstrate that coding SNP-A482 within the lysine tri-demethylase KDM4A/JMJD2A associates with differential outcome in non-small cell lung cancer (NSCLC) patients and promotes KDM4A protein turnover. Interestingly, homozygous SNP-482 cells have increased mTOR inhibitor sensitivity. mTOR inhibitors significantly reduce SNP-A482 protein levels, which parallels the increased drug sensitivity observed with KDM4A depletion. Furthermore, we demonstrate that KDM4A interacts with the translation initiation complex and impacts the distribution of translation initiation factors within polysome fractions. Upon KDM4A depletion, protein synthesis was reduced and there was enhanced protein synthesis suppression with mTOR inhibitors, which paralleled an increased sensitivity to these drugs. Lastly, we demonstrate that JIB-04, a JmjC demethylases inhibitor, suppresses translation initiation and enhances mTOR inhibitor sensitivity. These data highlight an unexpected role for KDM4A in regulating protein synthesis, in modulating mTOR inhibitor sensitivity and suggest potential novel therapeutic applications for this class of enzyme.

Poster Number 56

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Abnormalities in HLA expression in pancreatic ductal adenocarcinoma (PDAC): do they fulfill the need of a novel prognostic biomarker?

Investigators: V. Villani, F. Sabbatino, V. Deshpande, C. Fernández-del Castillo, B. L. Collins, K. D. Lillemoe, S. Ferrone, C. R. Ferrone

Background: The growing evidence about the role of immune surveillance in the clinical course of malignant diseases has prompted us to investigate whether PDAC patients' immune response to their tumors is not effective because neoplastic cells develop escape mechanisms. We have focused on the analysis of HLA antigens in PDAC tumors since these molecules play a crucial role in the interaction of tumor cells with the host's immune system.

Methods: Tumor specimens from 140 patients were immunohistochemically stained with HLA class I-, HLA class II-, CD8-, CD4-, FoxP3- and granzyme B-specific antibodies. HLA class I and class II expression was correlated with the immune infiltrate and the clinicopathological data.

Results: All tumors had immune infiltrates (100.0%). HLA-B,C antigens were not detected in 5 (3.7%) and were down-regulated in 92 (69.1%) patients. HLA class II antigens were expressed in 71.0% of the patients analyzed (54.0% heterogeneous and 17.0% positive samples). HLA-B,C expression positively correlated with the number of granzyme B+ T-cells (P=0.0064) and CD8+ T-cells (P=0.0082). HLA class II expression correlated with granzyme B+ T-cell infiltrate (P=0.049). Absence of HLA-B,C was associated with poor prognosis (P=0.013), and HLA class II de novo expression was associated with favorable outcome on multivariate analysis (P=0.016).

Conclusions: HLA class I antigen defects and absence of HLA class II appear to be independent predictors of poor prognosis in PDAC. Targeted therapies which modulate HLA antigens expression may have a beneficial effect on the clinical course of PDAC.

Poster Number 57

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Combinatorial therapy for triple negative breast cancer using CSPG4-specific T cells and the hedgehog signaling pathway inhibitor LDE225

Investigators: S. J. Isakoff, X. Wang, S. Ferrone

No curative therapy is available for the treatment of metastatic triple negative breast cancer (TNBC) emphasizing the urgent need to develop novel effective therapies. In response to this need, we have developed a novel combinatorial immunotherapeutic strategy. T cells genetically engineered to express a chimeric antigen receptor constructed with chondroitin sulfate proteoglycan (CSPG)4-specific monoclonal antibodies (CSPG4-specific CAR+T cells) are used as effectors in combination with the Sonic Hedgehog Homologue (SHH) pathway inhibitor LDE225. CSPG4 has been selected as a target because of its restricted distribution in normal tissues and its high expression on both differentiated TNBC cells and TNBC cancer initiating cells (CICs). CICs must be eliminated to cure TNBC since they play a major role in disease recurrence and metastatic spread, the two major causes of patients' mortality and morbidity. We found that CSPG4-specific CAR+T cells are effective in mediating the recognition and destruction of CSPG4 expressing breast cancer cells both in vitro and in vivo. Moreover, the ability of CSPG4-specific CAR+ T cells to eradicate TNBC CICs in vitro is enhanced by the SHH pathway inhibitor LDE225, since activation of this pathway decreases the sensitivity of cancer cells to T cell mediated lysis by inhibiting Fas-mediated apoptosis. In conclusion, CSPG4-specific CAR+T cells in combination with LDE225 effectively eliminate both TNBC differentiated cells and TNBC CICs in vitro. These results contribute to optimize CSPG4-specific CAR+ T cells based therapy in a clinical setting.

Poster Number 58

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Morbidity of Repeat Surgery for Recurrent Spinal Metastases following Combined Separation Surgery and Stereotactic Radiosurgery

Investigators: V. Yanamadala, K. Oh, J. H. Schwab, K. Shultz, J. H. Shin

Introduction: Complications associated with surgery after conventional radiotherapy are known and include delayed wound healing and poor functional outcomes. Stereotactic radiosurgery (SRS) is an effective post-operative adjuvant for local tumor control. Limited data is available regarding the morbidity of additional surgery following SRS and separation surgery.

Methods: All surgeries for spinal metastases treated with post-operative SRS at a single institution between 2012 and 2014 were retrospectively reviewed.

Results: Thirty seven patients were treated with SRS after separation surgery. Five patients (ages 54-77; 4 male, 1 female) required repeat surgery for local tumor recurrence (n=4) or pathological fracture (n=1). Four patients had complications related to surgery (80%). Histologies included breast, lung, renal cell, hepatocellular, and esophageal. Four patients developed local progression at the treated site (6-23 months from the time of SRS, mean 12.5 months) and one developed symptomatic fracture 12 months after SRS. With a mean post-reoperation follow-up of 5.8 months, 3 patients passed away (survival range 3 months-1 year). One patient is living with preserved function (KPS 80, ECOG 1) and the other developed recurrent cord compression 1 year after repeat surgery (KPS 50, ECOG 3). Three patients had wound defects with a mean of 2.6 months until full closure. Of these, 1 required 3 further surgeries.

Conclusions: This is the first series reporting morbidity of repeat surgery following surgery and SRS. Though there may be clinical benefit to repeat surgery, associated complications are high. Though this is a small sample, further reporting will help identify high risk patients.

Poster Number 59

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Effect of Limb Demand Ischemia on Autophagy and Mitochondrial Biogenesis in Diet Induced Obese Mice

Investigators: H. Albadawi, R. Oklu, J. D. Milner, T. P. Uong, H. J. Yoo, M. T. Watkins

Introduction: Obesity and insulin resistance are major risk factor for peripheral arterial disease, which frequently manifests as intermittent claudication due to lower limb demand ischemia. Skeletal muscle autophagy and mitochondrial biogenesis are important processes for proper oxidative capacity and energy metabolism which are compromised in diabetes. This study compares autophagy, mitochondrial biogenesis, energy metabolism and morphology in hind limb skeletal muscle subjected to demand or sedentary ischemia in obese diabetic mice.

Methods: Unilateral hind limb demand ischemia was induced in a group of diet induced obese diabetic mice after femoral artery ligation (FAL) and exercising the mice daily for 4 weeks. Another group of obese mice had unilateral FAL but remained sedentary for the same period. Hindlimb muscles were analyzed for ATP, markers of autophagy and mitochondrial biogenesis insulin sensitivity in addition to fat cells accumulation.

Results: At the end of 4-weeks of exercise, demand ischemia increased the autophagy related protein-Becline-1 but it did not alter the ratio of LC3B-II/I or markers of mitochondrial dynamic-Opa-1/Drp-1. Demand ischemia increased the level of mitochondrial protein-SDHA but not Cytochrome-C, or the molecular marker of insulin sensitivity-pS473/Akt, while significantly reduced adipocytes accumulation and mitochondrial uncoupling protein-3 without augmenting the steady state level of ATP.

Conclusions: Exercising diabetic mice decreases adipocytes accumulation in skeletal muscles demand ischemia but it did not appear to enhance autophagy, mitochondrial biogenesis, energy metabolism or insulin sensitivity. Future studies aimed at evaluating novel therapies that enhances autophagy and mitochondrial biogenesis in diabetes with demand ischemia is warranted.

Poster Number 60

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Metabolomic Prediction of Acute Kidney Injury in Patients Undergoing Transcatheter Aortic Valve Replacement

Investigators: S. Elmariah, E. P. Rhee, L. A. Farrell, M. Daher, X. Shi, M. J. Keyes, I. Inglessis, J. J. Passeri, I. F. Palacios, R. E. Gerszten

Background: Transcatheter aortic valve replacement (TAVR) is a novel alternative to surgery for high-risk patients with severe aortic stenosis that is rapidly being adopted. Acute kidney injury (AKI) occurs commonly after TAVR and is associated with markedly increased post-operative mortality. We previously identified serum metabolites predictive of chronic kidney disease, but whether metabolite profiles can identify those at risk of AKI is unknown.

Methods: We performed liquid chromatography/mass spectrometry-based metabolite profiling on plasma from patients undergoing TAVR. AKI was defined as an increase in serum creatinine ≥0.3 mg/dl or ≥1.5x baseline during the index hospitalization.

Results: Of 45 patients (mean age 81±11 yrs, 53% female) undergoing TAVR, 9 (20%) developed AKI. Of 94 metabolites, baseline xanthosine, kynurenic acid, taurine, NMMA, and 5-adenosylhomocysteine predicted peak in-hospital creatinine as well as AKI. A metabolomic risk score (MRS) based on the most predictive metabolites independently predicted AKI despite adjustment for baseline renal function (OR 1.49, 95%CI 1.08-2.04; P=0.015) or for a validated clinical risk score (OR 1.72, 95%CI 1.18-2.50; P=0.005). Compared to baseline renal function or the clinical score alone, addition of the metabolomic score significantly improved model discrimination and favorably reclassified predicted risk of AKI.

Conclusion: In an elderly population with severe aortic stenosis undergoing TAVR, metabolite profiling adds predictive value beyond baseline renal function and a widely used clinical risk model in identifying patients at higher risk for AKI. Given the multifactorial nature of AKI after TAVR, metabolite profiles may identify those patients with reduced renal reserve.

Poster Number 61
ePoster#9

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Quantitative Analysis of Cardiomyocyte Contractile Kinetics and Force Generation Using Automated Morphologic Similarity Measure

Investigators: J. Kijlstra, D. Hu, N. Mittal, P. Van der Meer, A. Garakani, I. Domian

Recent advances in stem cell biology have greatly increased the availability of stem cell-derived cardiomyocytes for studying cardiac physiology and pathophysiology in vitro. However, current techniques for functional assessment of cardiomyocytes are optimized for mature rod-like myocardial cells with distinct cell edges and highly developed sarcomeres, limiting their applicability to stem cell-derived cardiomyocytes. We propose to develop an automated methodology for the quantitative assessment of force generation and contractile kinetics of cardiomyocytes concurrently with other physiological measures such as calcium handling and action potential. Herein we perform pairwise statistical similarity measures between all frames in a video of human stem cell-derived cardiomyocytes contracting on a flexible substrate. We then generate a similarity matrix that represents a comprehensive assessment of change in cell morphology over time to compute the contraction kinetics. We validate this approach using traditional edge detection technology and adult cardiomyocytes. We further calculate the contractile force generated during each shortening period and validate this approach using established fluorescence microsphere-based traction force microscopy. With addition of the calcium indicator Fluo-4AM, we demonstrate the ability to detect subtle changes in contractility and calcium cycling in response to isoproterenol and verapamil. We combine our methodology with the FluoVolt membrane potential dye to also simultaneously assess the cardiotoxicity of dofetilide. We have developed a highly versatile novel methodology for the simultaneous quantitative analysis of contraction kinetics, force generation, calcium cycling and electrophysiology in human cardiomyocytes.

Poster Number 62

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Therapeutic silencing of leukocyte adhesion molecules in atherosclerosis and acute myocardial infarction

Investigators: H. B. Sager, P. Dutta, J. Dahlmann, G. Courties, F. Swirski, R. Weissleder, M. Nahrendorf

Introduction/Hypothesis: Leukocytes are a major component of atherosclerotic plaques, drive plaque progression and are continuously recruited from the blood. The leukocyte adhesion molecules VCAM-1, ICAM-1/2 and E-/P-Selectin are expressed on plaque endothelial cells and are the key players in this recruitment process. We here present a novel therapeutic approach that targets leukocyte recruitment to plaques.

Methods: We tested a nanoparticle system that effectively delivered short interfering RNAs targeting VCAM-1, ICAM-1/2 and E-/P-Selectin simultaneously (siCAM5) to endothelial cells of atherosclerosis prone ApoE-/- mice and C57BL/6J mice after myocardial infarction (permanent coronary ligation) and assessed leukocyte recruitment/content and plaque phenotype by flow cytometry, histology, qPCR and fluorescence molecular tomography/computed tomography imaging.

Results: Efficient degradation of VCAM-1, ICAM-1/2 and E-/P-Selectin on endothelial cells attenuated neutrophil and monocyte numbers in infarcts and atherosclerotic plaques (neutrophil numbers, siControl 10,974±1,250 vs. siCAM5 6,963±1,077, $p<0.05$; Ly6Chigh monocyte numbers, siControl 6,044±1,212 vs. siCAM5 3,770±293, $p<0.05$), reduced levels of pro-inflammatory cytokines in plaques, decreased protease activity in plaques (protease activity in pmol, siControl 49.4±2.8 vs. siCAM5 35.9±3.0, $p<0.01$), and resulted in a less inflammatory plaque phenotype (necrotic core mm2/aortic root, siControl 0.09±0.01 vs. siCAM5 0.05±0.01, $p<0.05$; fibrous cap µm/aortic root, siControl 36.7±9.5 vs. siCAM5 63.1±5.4, $p<0.05$) and lower numbers of plaque macrophages (siControl 30,313±4,414 vs. siCAM5 17,198±2,070, $p<0.01$).

Conclusions: Taken together, the described approach can successfully suppress leukocyte recruitment to atherosclerotic plaques and infarcted hearts. It may allow aggressive medical interventions in patients with inflammatory atherosclerosis.

Poster Number 63

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Androgen-sensitive hypertension associated with soluble guanylate cyclase alpha1 deficiency is mediated by 20-HETE

Investigators: S. Vandenwijngaert, A. C. Dordea, R. E. Tainsh, D. I. Nathan, M. J. Raher, A. Kirby, C. Stevens, M. J. Daly, P. Brouckaert, M. Schwartzman, K. D. Bloch, E. S. Buys

Dysregulated nitric oxide (NO) signaling contributes to the pathogenesis of hypertension. Previously, we reported gender- and strain-specific hypertension in mice deficient in the α1-subunit of the NO receptor soluble guanylate cyclase (sGCα1-/-): male mice on an Sv129/J (S6) but not a C57BL6/J (B6) background are hypertensive.

Via linkage analysis, we identified a suggestive quantitative trait locus (QTL) associated with elevated blood pressure in male sGCα1-/- S6 mice. This QTL encompasses CYP4a12a, encoding the predominant murine synthase of the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE). Renal CYP4a12a gene expression was higher in male WT and sGCα1-/- S6 mice than in female S6 mice or WT and sGCα1-/- B6 mice. Also, 20-HETE levels were higher in renal preglomerular microvessels of male sGCα1-/- S6 than of sGCα1-/- B6 mice. Treatment with the 20-HETE synthesis inhibitor HET0016 or the 20-HETE antagonist 20-HEDGE reduced mean arterial pressure (MAP) in male sGCα1-/- S6 mice. Finally, the more significant impairment of acetylcholine-induced relaxation of renal interlobar arteries in male sGCα1-/- S6 than in sGCα1-/- B6 mice, in male sGCα1-/- S6 than in WT S6 mice, and in male sham sGCα1-/- S6 mice than in orchiectomized sGCα1-/- S6 mice, was rescued by adding 20-HEDGE.

Gender- and strain-specific hypertension and vascular dysfunction in sGCα1-/- S6 mice is associated with elevated CYP4a12a gene expression and 20-HETE levels, and is abrogated by antagonizing 20-HETE. CYP4a12a represents a candidate blood pressure modifying gene in the context of deficient NO-sGC signaling. 20-HETE-generating CYP4 enzymes represent a potential therapeutic target for the treatment of hypertension.

Poster Number 64

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Genome-Wide Identification of MicroRNAs Regulating Cholesterol/Lipid Homeostasis

Investigators: A. Wagschal, A. Naar

Abnormal cholesterol/lipid homeostasis and altered energy metabolism are implicated in several cardiometabolic disorders. MicroRNAs (miRNAs), a class of short (20-24 nucleotides) regulatory RNAs that modulate mRNA translation and turnover, have recently emerged as regulators of these processes, but their full potential contribution is not yet known. Through an unbiased approach, we leveraged a Genome-Wide Association Study (GWAS) meta-analysis for plasma lipids to identify miRNAs associated with abnormal circulating cholesterol/lipid profiles in humans. We found GWAS variants in physical proximity to 69 miRNAs, and focused experimental validation on two miRNAs (miR-128-1, miR-148a) whose predicted targets include genes involved in cholesterol/lipid homeostasis, insulin signaling, and energy metabolism. We demonstrate that these miRNAs control the expression of key proteins involved in cholesterol-lipoprotein trafficking, such as the low-density lipoprotein (LDL) receptor (LDLR) and ATP-binding cassette A1 (ABCA1). Accordingly, over-expression or antisense-mediated inhibition of these miRNAs resulted in altered LDL-cholesterol uptake in human hepatic cells, and modulated cholesterol efflux in mouse macrophages. We also provide in vivo evidence highlighting a key role for miR-128-1 in the regulation of cholesterol and TAG homeostasis. Antisense targeting of miR-128-1 by a tiny seed-targeting 9-nucleotide locked nucleic acid (LNA) antimiR in apoE null mice fed a Western-type diet resulted in decreased levels of circulating total cholesterol and triglycerides, and increased the hepatic expression of the LDLR and insulin signaling components in liver. These findings suggest that miRNAs may act more broadly to control cholesterol/lipid and energy homeostasis, and may contribute to abnormal blood lipids predisposing to human cardiometabolic disorders.

Poster Number 65

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Effects of androgens and estrogens on cardiometabolic parameters in young adult men

Investigators: E. W. Yu, H. Lee, S. M. Burnett-Bowie, S. C. Hirsch, G. Abrushamian-Garcia, L. Borges, D. W. Goldstein, A. P. Taylor, K. E. Wulczyn, A. F. Moore, J. S. Finkelstein

The higher prevalence of cardiovascular disease in men than in premenopausal women suggests that testosterone (T) may promote and/or that estradiol (E) may protect against CVD. We recruited 2 cohorts of healthy men aged 20-50. All men received goserelin acetate (AstraZeneca-LP). Cohort 1 (n=198) was randomized to placebo gel or 1.25g, 2.5g, 5g, or 10g of testosterone gel (Abbvie) daily for 16 weeks. Cohort 2 (n=202) received the same regimen plus anastrozole (AstraZeneca-LP) to block T conversion to E. Intramuscular (IM) fat and labs were measured at 0 and 16 weeks. T levels ranged from prepubertal to high-normal and were similar in both cohorts. E levels increased with T dose in Cohort 1 (range 3-35 pg/mL) but remained <3 pg/mL in Cohort 2. HDL and leptin levels were inversely associated with T in both cohorts and unaltered by E suppression, indicating regulation by T alone. In contrast, fasting glucose, HOMA-IR, and IM fat increased similarly in Cohort 2 regardless of T dose group, and were significantly higher than in Cohort 1, indicating primary regulation by E. Changes in blood pressure and LDL were not significantly associated with T or E. Increased insulin resistance and body fat due to low estrogen are consistent with male predominance in CVD. HDL is regulated by testosterone, while LDL and blood pressure are not regulated by gonadal steroids. Although leptin is made by adipocytes, leptin is regulated by androgens in men. These findings further delineate the roles of gonadal steroids in gender differences in CVD.

Poster Number 66

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Fibrogenic Lung Injury Induces Non-Cell Autonomous Fibroblast Invasion

Investigators: N. Ahluwalia, B. M. Mugo, P. E. Grasberger, C. Feghali-Bostwick, A. Pardo, M. Selman, D. Lagares, A. M. Tager

Pathogenic fibroblast accumulation in idiopathic pulmonary fibrosis (IPF) depends on their acquisition of an increased ability to invade extracellular matrix. Here, we investigate whether fibroblast invasiveness is further stimulated by extrinsic mediators induced by lung injury. We isolated primary mouse lung fibroblasts and bronchoalveolar lavage fluid (BAL) after intratracheal bleomycin injection. Fibroblasts were fluorescently labeled and plated on a BioCoat Tumor Invasion System. Aliquots of BAL or defined chemoattractants were placed under the cells. We found that "cell autonomous" invasion, i.e. invasion observed in the absence of added stimuli, was increased in lung fibroblasts isolated from bleomycin-challenged versus control mice. However, we found that non-cell autonomous invasion of fibrotic lung fibroblasts induced by BAL from injured mice was dramatically greater than the cell autonomous invasion of these cells. Furthermore, BAL isolated after lipopolysaccharide challenge did not have the same effect. We also studied the effects of defined chemoattractants and found that mediators can have distinct effects on fibroblast migration and invasion. We examined the mechanisms of BAL-induced invasion, and found that it partially depends on signaling through fibroblast PDGFRβ, LPAR1, EGFR, and FGFR2. Using fibroblasts and BAL from patients with IPF and healthy controls, we found that non-cell autonomous invasion of IPF fibroblasts induced by IPF BAL was dramatically greater than cell autonomous IPF fibroblast invasion. Taken together, our data suggests that non-cell autonomous fibroblast invasion occurs in the development of pulmonary fibrosis, is induced by mediators distinct from those causing fibroblast migration, and is relevant in human IPF.

Poster Number 67

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Noncanonical activation of BMP type I receptors and SMAD1/5/8 by Activin B in hepatocytes promotes hepcidin induction by inflammation in mice

Investigators: S. Canali, K. B. Zumbrennen-Bullough, A. B. Core, A. Schneyer, A. Pietrangelo, J. L. Babitt

Induction of the iron regulatory hormone hepcidin contributes to the anemia of inflammation by restricting iron availability. Bone morphogenetic protein 6 (BMP6) signaling is a central transcriptional regulator of hepcidin. Recently, the BMP/TGF-β superfamily member Activin B was shown to be induced by lipopolysaccharide. Although Activin B typically signals via a different subset of SMAD proteins (SMAD2/3) compared with BMP6 (SMAD1/5/8), Activin B was reported to stimulate noncanonical SMAD1/5/8 signaling and hepcidin expression in hepatocytes. Here, we investigated the detailed molecular mechanism of Activin B-mediated hepcidin regulation and its functional significance in vivo. We showed that low concentrations of Activin B, but not Activin A, stimulate prolonged SMAD1/5/8 signaling and hepcidin expression in liver cells to a similar degree as canonical SMAD2/3 signaling, and with similar potency compared with BMP6. Activin B stimulates hepcidin via classical Activin type II receptors ACVR2A and ACVR2B, noncanonical BMP type I receptors ALK2 and ALK3, and SMAD5. The co-receptor hemojuvelin binds directly to Activin B and facilitates Activin B-SMAD1/5/8 signaling. Activin B-SMAD1/5/8 signaling occurs selectively in hepatocyte-derived cells and is not enabled by hemojuvelin in other cell types. Finally, the Activin inhibitor follistatin-315 has no effect on basal hepcidin expression, but blunts hepcidin induction by lipopolysaccharide in mice. Our data elucidate a novel mechanism for noncanonical SMAD activation by BMP/TGF-β superfamily members, and support a functional role for Activin B in hepcidin induction by inflammation in vivo. Targeting the Activin B-hepcidin pathway may lead to the development new therapies for anemia of inflammation.

Poster Number 68
ePoster#10

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The neuronal ceroid lipofuscinosis protein Cln3 is required for an optimal response to starvation in Dictyostelium discoideum

Investigators: R. J. Huber, S. L. Cotman

Neuronal ceroid lipofuscinosis (NCL), also known as Batten disease, is a lysosomal storage disorder that is the leading cause of neurodegeneration in children. Juvenile NCL, the most common form of the disease, is caused by mutations in the CLN3 gene. Mammalian cell models harboring mutations in CLN3 display a delayed response in a wound healing assay, however the molecular components underlying the phenotype are still unclear. The social amoeba Dictyostelium discoideum provides an extremely powerful system for studying cell-cell communication and cell movement, especially during the early stages of development. Therefore, we were interested in assessing the effect of Cln3-deficiency on this stage of the life cycle. The aggregation of cln3- cells was delayed and knockout cells formed ~30% more multicellular aggregates that were comparatively smaller than those formed by parental cells. During these early stages of development, Cln3-deficiency delayed competency towards the chemoattractant cAMP, and the initiation of the periodic pulsing of cAMP waves generated by the starving population of cells. Interestingly, treatment of cln3- cells with the calcium chelator EGTA corrected the delay in aggregation. Cln3-deficiency also altered the levels of cell-cell adhesion proteins, and the proteolytic cleavage and secretion of quorum sensing proteins, suggesting that cln3- cells were not optimally primed to enter development. Together, these results suggest that Cln3 is required for the cell to optimally respond to starvation, and when coupled with previous findings, strongly indicate that cln3- cells are compromised in their ability to respond to environmental cues.

Poster Number 69

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

Investigators: S. Lee, S. Truesdell, S. Bukhari, J. Lee, O. LeTonqueze, S. Vasudevan

Proliferation arrest and distinct developmental stages alter and decrease general translation yet maintain ongoing translation. The factors that support translation in these conditions remain to be characterized. We investigated an altered translation factor in three cell states considered to have reduced general translation: immature oocytes, mouse ES cells, and the transition state of proliferating mammalian cells to quiescence upon growth-factor deprivation. Our data reveal a transient increase of eIF5B in these conditions. eIF5B promotes 60S ribosome subunit joining and pre-40S subunit proofreading. eIF5B has also been shown to promote the translation of viral and stress-related mRNAs and can contribute indirectly to supporting or stabilizing initiator methionyl tRNA (tRNA-Meti) association with the ribosome. We find that eIF5B is a limiting factor for translation in these three conditions. The increased eIF5B levels lead to increased eIF5B complexes with tRNA-Meti upon serum starvation of THP1 mammalian cells. In addition, increased phosphorylation of eukaryotic initiation factor 2α, the translation factor that recruits initiator tRNA-Meti for general translation, is observed in these conditions. Importantly, we find that eIF5B is an antagonist of G0 and G0-like states, as eIF5B depletion reduces maturation of G0-like, immature oocytes and hastens early G0 arrest in serumstarved THP1 cells. Consistently, eIF5B overexpression promotes maturation of G0-like immature oocytes and causes cell death, an alternative to G0, in serum-starved THP1 cells. These data reveal a critical role for a translation factor that regulates specific cell-cycle transition and developmental stages.

Poster Number 70

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A REDD1/TXNIP pro-oxidant complex regulates ATG4B activity to control stress-induced autophagy and sustain exercise capacity

Investigators: S. Qiao, M. Dennis, X. Song, D. D. Vadysirisack, D. Salunke, Z. Nash, Z. Yang, M. Liesa, J. Yoshioka, S. Matsuzawa, O. S. Shirihai, R. T. Lee, J. C. Reed, L. W. Ellisen

Macroautophagy (autophagy) is a critical cellular stress response, yet the intricate signal transduction pathways controlling autophagy induction in response to stress are poorly understood. Here we reveal a new and essential mechanism of autophagy control whose deregulation disrupts mitochondrial integrity and energy homeostasis in vivo. Stress conditions including hypoxia and exercise induce reactive oxygen species (ROS) through up-regulation of a protein complex involving REDD1, an inhibitor of mTORC1, and the pro-oxidant protein TXNIP. Decreased ROS in cells and tissues lacking either REDD1 or TXNIP increases catalytic activity of the redox-sensitive ATG4B cysteine endopeptidase, leading to enhanced LC3B delipidation and failed autophagy. Conversely, REDD1/TXNIP complex expression is sufficient to induce ROS, suppress ATG4B activity and activate autophagy. In Redd1-/- mice, deregulated ATG4B activity and disabled autophagic flux result in accumulation of defective mitochondria, leading to impaired oxidative phosphorylation, muscle ATP depletion and poor exercise capacity. Thus, ROS regulation through REDD1/TXNIP is physiological rheostat controlling stress-induced autophagy.

DEVELOPMENTAL BIOLOGY

Poster Number 71

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Gut epithelium-derived sonic hedgehog regulates the extracellular matrix during formation of the enteric nervous system

Investigators: N. Nagy, A. M. Goldstein

The enteric nervous system (ENS) is principally derived from vagal-level neural crest cells that migrate rostrocaudally along the entire length of the gastrointestinal tract, giving rise to neurons and glial cells in two ganglionated plexuses. Incomplete migration of vagal-derived enteric neural crest cells (ENCCs) leads to Hirschsprung disease (HD), a congenital disorder characterized by the absence of enteric ganglia along variable lengths of the distal intestine. Inductive interactions between gut epithelium and mesenchyme have been suggested to regulate the migration and differentiation of ENCCs. However, little is known about the function of epithelial derived factors, such as Sonic hedgehog (Shh), how they influence hindgut mesenchyme derived factors and how they regulate extracellular matrix expression during ENS development.

Hindgut from 6 day old chicken embryo was cultured in the presence of Shh protein or Shh overexpressing RCAS-virus. In presence of Shh the hindgut is aganglionic, while in the presence of Shh inhibitor large, ectopic ganglia developed. Shh treatment strongly induced the expression of extracellular matrix versican and collagen type IX, whereas cyclopamine reduced the expression pattern of these inhibitory matrix molecules. These results indicate that versican and collagen IX is a candidate for mediating the effects of Shh on ENCC migration. Shh also inhibited the proliferation and promoted the differentiation of ENCCs. Abnormalities of NCC migration and extracellular pattern formation are characteristic of two human intestinal disorders, HD and intestinal neuronal dysplasia. Our results support an essential role for epithelial-mesenchymal interactions in these aspects of ENS development.

Poster Number 72
ePoster#11

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Live intracellular calcium dynamics during podocyte development and injury in zebrafish

Investigators: R. Tomar, E. Merkel, A. Vasilyev, I. A. Drummond

We aim to understand podocyte calcium signaling during development using live imaging of zebrafish embryos. Transgenic zebrafish expressing the calcium biosensor GCaMP3 in podocyte were generated. Larvae were treated with drugs to determine the source of calcium. Cellular events during glomerulus formation were captured by imaging Tg(wt1b:GFP) and Tg(podocin:Gal4) x Tg(UAS:YFP) embryos. The role of calcium signaling in podocyte differentiation was assayed in zebrafish plce1 morphants.

Immature podocytes are dynamic and interact with the dorsal aorta to form glomerular capillaries. By 4 dpf podocytes stabilize and the filtration barrier is mature. We observed spontaneous intracellular calcium transients in podocytes at 2-3 dpf, which were silenced by 4 dpf suggesting a role for calcium signaling in podocyte maturation. Elimination of filtration pressure by blocking heartbeat resulted in glomerular collapse but did not block podocyte calcium transients. Calcium transients were blocked by cyclopiazonic acid, thapsigargin and 2APB but not by cilnidipine or nifedipine, indicating calcium release from intracellular stores. plce1 knockdown resulted in podocyte defects, disorganized capillaries, and loss of podocin expression, further suggesting a requirement for calcium signaling in podocyte differentiation. Moreover, we showed that wt1a and osr1 are required for podocyte plce1 expression. Significantly, calcium transients were reinitiated in mature podocytes (6dpf) by puromycin injury.

The dynamic behavior of developing podocytes is associated with spontaneous calcium transients that occur independently of vascular stretch. Stable podocytes lack calcium transients, however, transients are reinitiated upon injury.

Poster Number 73

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Vertebral Strength and Estimated Fracture Risk in Women with Anorexia Nervosa and Overweight/Obese Women

Investigators: K. N. Bachmann, A. G. Bruno, M. A. Bredella, E. A. Lawson, C. M. Gill, E. Meenaghan, A. V. Gerweck, M. L. Bouxsein, A. Klibanski, K. K. Miller

Paradoxically, fracture risk is elevated in both obesity and anorexia nervosa (AN). Factor-of-risk (Φ) (applied load/bone strength) is a biomechanically-based method to estimate fracture risk (higher Φ associated with increased fracture risk).

We estimated vertebral strength [by the combination of integral volumetric BMD (Int.vBMD) and cross-sectional area (CSA) from QCT], applied load, and Φ at L4 in 176 women: 65 AN, 45 lean controls, 66 obese (OB). Loads were estimated for: 1) standing; 2) holding 5 kg in each hand (holding); 3) 45° trunk flexion-5 kg in each hand (lifting); 4) 20° trunk lateral bend-10 kg in right hand (bending).

AN had lower, and obese similar, Int.vBMD and vertebral strength than controls. Across all groups, LBM was positively associated with Int.vBMD and vertebral strength (R 0.28-0.45, $p \leq 0.0001$). VAT was negatively associated with Int.vBMD and vertebral strength within OB (R -|0.36-0.38|, $p<0.003$). Applied load was highest in OB and lowest in AN for standing, holding, and lifting, but was highest in AN for bending ($p<0.02$). OB had higher Φ for standing and lifting than controls and AN, whereas AN had higher Φ for bending than controls and OB ($p<0.0001$). AN and OB had higher Φ for holding than controls ($p<0.03$).

Therefore, AN had higher estimated vertebral fracture risk (Φ) for holding and bending, due to inferior strength. Despite normal vertebral strength, OB had higher Φ for standing, holding, and lifting, due to higher applied loads from higher weight. The load-to-strength ratio helps explain fracture risk in both low-weight and obese women.

Poster Number 74
ePoster#12

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A novel mouse model for acquired hypoparathyroidism and its application for studying long-acting parathyroid hormone (LA-PTH)

Investigators: R. Bi, T. Watanabe, Y. Fan, T. J. Gardella, M. Mannstadt

Hypoparathyroidism (HP) is the only endocrine deficiency disorder without replacement therapy. Novel PTH analogs that can mediate sustained normalization of blood and urine calcium are now being explored as potential replacement therapies. Evaluation of such therapies requires an effective animal model. PTX surgery in mice is challenging due to small gland size and variable gland location. To facilitate this procedure, we developed a mouse model ("PTH-GFP") in which the parathyroid gland cells have been engineered to express green fluorescent protein. PTX surgery, as guided by GFP, is greatly facilitated (surgery times < 20 mins./mouse and no mortality in 80 PTX mice so far). Blood analyses revealed that three days after surgery, GFP-PTX mice, as compared to sham surgery mice, exhibited a dramatic decrease in serum PTH (31 vs. 570 pg/mL), low blood Ca++ (1.05 vs. 1.31 mmol/L) and elevated serum phosphorus (9.5 vs. 7.6 mg/dL). We then used the model to evaluate the efficacy of LA-PTH, a new long-acting PTH analog, for normalizing blood Ca in HP. Single s.c. injection of LA-PTH at 1.5, 3, 5, 10, or 20 nmol/kg into GFP-PTX mice led to a dose-dependent increase in blood Ca++ (1.21, 1.23, 1.23, 1.25, 1.26, 1.26 mmol/L). Importantly, a single injection of LA-PTH was able to normalize serum calcium and phosphate levels for more than 72 hours. These results thus show that GFP-PTX mice provide a novel model of acquired HP, and they further highlight the potential utility of novel long-acting PTH analogs as therapies for this disease.

Poster Number 75

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A Shared Genetic Basis for Constitutional Delay of Puberty and Idiopathic Hypogonadotropic Hypogonadism

Investigators: Y-M. Chan, J. Zhu, L. Plummer, M. R. Palmert, J. N. Hirschhorn, S. B. Seminara

Background: Constitutional delay of puberty (CDP) is a common condition in which puberty starts late but progresses normally once started. Although CDP appears to be heritable, no specific genetic cause for CDP has yet been reported. In contrast, many genetic causes have been found for idiopathic hypogonadotropic hypogonadism (IHH), a rare disorder in which defects in GnRH secretion or action result in absent or stalled pubertal development. We hypothesized that variants in IHH genes contribute to the pathogenesis of CDP.

Methods: Two cohorts of subjects with CDP were studied: 1) CDP individuals with no family history of IHH were screened for variants in IHH genes by whole-exome sequencing, with ethnically matched control subjects drawn from the Exome Aggregation Consortium. 2) CDP individuals who are also family members of an IHH proband were screened by targeted sequencing to determine if they shared the proband's variant in an IHH gene; unaffected family members served as controls.

Results: In CDP subjects with no family history of IHH, 14% (8/56) had potentially pathogenic variants in IHH genes vs. 5.6% (1,907/33,855) of controls (P = 0.01), with variants in IL17RD and TAC3 found in multiple CDP subjects. In CDP subjects identified as a family member of an IHH proband, 53% (10/19) shared the proband's variant vs. 12% (4/33) of unaffected family members (P = 0.003).

Conclusions: These findings suggest that IHH genes are also involved in the pathogenesis of CDP. Thus, at least in some cases, CDP shares an underlying pathophysiology with IHH.

Poster Number 76

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Discerning Causal Genetic Variants in Isolated GnRH Deficiency in a Heterozygous Context

Investigators: K. H. Cox, L. M. Oliveira, L. Plummer, R. Balasubramanian, W. F. Crowley

Background: Mutations in PROKR2, which encodes Prokineticin Receptor 2, cause Isolated GnRH Deficiency (IGD). IGD-associated PROKR2 "mutations" are overwhelmingly missense and heterozygous, and they often segregate incompletely within pedigrees, raising the question of whether they are truly causal. The goals of this study were to comprehensively assess the functional effects of all known variants in PROKR2 and to model the heterozygosity seen in IGD.

Methods: Fifty-nine rare missense variants (<1% MAF) were studied: 13 found in IGD patients, 31 reported in controls, and 15 seen in both. Mutant and wild-type (WT) PROKR2 plasmids were either transfected alone or co-transfected into HEK293 cells at fixed ratios, and PROKR2 signaling via the mitogen-activated protein kinase (MAPK) pathway was assessed using an Egr1-luciferase reporter assay. Protein expression of mutant and WT V-5 tagged PROKR2 and trafficking of GFP-tagged PROKR2 were also investigated using western blots and confocal microscopy.

Results: When transfected alone, 41 mutant proteins showed loss of function (LOF); however, the burden of LOF mutations was not different between controls and IGD patients. Interestingly, when mutant/WT PROKR2 were co-transfected, different mechanisms of protein interactions became apparent, including 4 mutations exerting dominant negative effects on WT signaling.

Conclusions: Here we identify complex mutant/WT PROKR2 protein interactions and introduce novel mechanisms for disease-causing PROKR2 variants. Surprisingly, the majority of variants tested benign in co-transfection assays, including some reported in IGD patients, suggesting that they may represent false positive associations. These studies reiterate the importance of performing context-specific functional assays to determine pathogenicity of mutations.

Poster Number 77

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Body Composition in Pituitary, Adrenal and Iatrogenic Cushing's Syndrome and Effects of DHEAS Levels

Investigators: L. E. Dichtel, M. A. Bredella, C. M. Gill, A. D. Riccio, B. M. Russell, K. K. Miller

Hypercortisolemia is associated with abdominal adiposity and muscle wasting, as is hypoandrogenemia. However, it is not known whether visceral adiposity is present in Cushing's syndrome of different etiologies or whether body composition is modulated by DHEAS levels, which vary widely depending on Cushing's etiology. We hypothesized that Cushing's syndrome would be associated with visceral adiposity and muscle wasting whether of pituitary, adrenal or iatrogenic origin, and that these body composition abnormalities would be attenuated in women with relatively higher DHEAS levels. We conducted a retrospective review of women with Cushing's of pituitary (N=25), adrenal (N=13) or iatrogenic (N=12) etiology and controls, matched 1:1 for BMI and age. Abdominal fat depots and psoas muscle mass were assessed from abdominal CT. Mean BMI was comparable among all groups. Urine free cortisol (UFC) or UFC equivalent was comparable in the pituitary, adrenal and iatrogenic groups. Visceral adipose tissue (VAT) and the VAT/subcutaneous adipose tissue (SAT) ratio were higher in the pituitary and adrenal Cushing's groups than their controls in multivariate models adjusted for age and BMI. There was a trend toward higher VAT in pituitary versus iatrogenic Cushing's groups when controlling for age (p=0.06). DHEAS was a negative determinant of TAT, VAT and VAT/SAT ratio, and a positive determinant of muscle mass in multivariate analysis. In conclusion, hypercortisolemia is associated with visceral adiposity in Cushing's of pituitary and adrenal etiology. Higher DHEAS levels may confer relative protection from abdominal fat accumulation and muscle wasting in women with Cushing's syndrome.

Poster Number 78

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The Study to Understand the Genetics of the Acute Response to Metformin and Glipizide in Humans (SUGAR-MGH): Genetic Risk Scores (GRS) to predict the response to type 2 diabetes medications

Investigators: M. Fernandez, N. Colomo, A. Muhammad, L. DeVita, N. Landa, S. Srinivasan, J. Todd, G. A. Walford, M. Hudson, A. B. Goldfine, J. C. Florez

Despite the recent explosion of published genetic associations with glycemic traits, the biological and clinical relevance of different genetic variants is unknown. SUGAR-MGH aims to address this problem by perturbing two different arms of the glucose homeostasis system to uncover the physiology of variants for which no mechanism is known, and to explore if there is a genetically determined response to glipizide or metformin.

Subjects naive to antidiabetic treatments receive 5mg of Glipizide while fasting, and blood is collected at various time intervals. Six days later, they start a 4-dose course of 500 mg metformin and undergo a 75g oral glucose tolerance test. We genotyped variants associated with glycemic traits and constructed a genetic risk score (GRS) for fasting glucose (FG), fasting insulin (FI) and β-cell function (HOMA-B) for 667 individuals. Each locus was assigned a value of 0, 1 or 2 according to the number of trait-raising alleles present, and summed to provide the overall genetic risk for each diabetes-related phenotype.

The 3 GRSs showed the expected associations with their respective baseline traits. During the glipizide challenge, the FG GRS was also significantly associated with glucose trough, even after adjusting for age, sex, BMI, race/ethnicity and baseline fasting glucose. FI and HOMA-B GRSs, however, were not significantly associated with the selected endpoints. None of the GRSs was significantly associated with metformin response.

In this preliminary analysis, SUGAR-MGH shows that response to glipizide beyond fasting glucose levels is genetically determined, which might help predict hypoglycemia to a sulfonylurea.

Poster Number 79

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Impact of common variation at diabetes trait loci MTNR1B and CRY2 on sleep, circadian and melatonin physiology

Investigators: J. M. Lane, A. M. Chang, . CARe consortium, D. Aeschbach, S. W. Cain, C. A. Czeisler, E. B. Klerman, S. W. Lockley, M. St. Hilaire, S. A. Shea, J. F. Duffy, O. M. Buxton, S. Redline, F. A. Scheer, R. Saxena

Abnormalities in sleep quantity, quality, circadian alignment, and melatonin regulation increase the risk of type 2 diabetes (T2D). Common genetic variants at loci harboring a receptor for the circadian-regulated hormone melatonin (MTNR1B) or the core clock gene Cryptochrome2 (CRY2) are associated with increased fasting blood glucose and T2D risk. Whether sleep or circadian disruption mediates this risk is unknown. Our aim was to determine if MTNR1B and CRY2 risk variants associate with measures of sleep or circadian physiology in two populations with complementary strengths: 1) intensive in-laboratory protocols (n=193) with precise measures of endogenous circadian physiology and sleep; cross-sectional questionnaire (n up to 10,332) and polysomnongraph (n=3,026) data from 7 community-based cohorts participating in the Candidate Gene Association Research (CARe) consortium. The MTNR1B variant associated with a greatly delayed circadian phase of dim-light melatonin offset (1.37h) and a substantially longer duration of elevated melatonin levels (41 min) in in-laboratory studies. The effect of MTNR1B rs10830963 on dim-light melatonin offset was partially mediated through delayed offset of melatonin synthesis. We did not identify associations with sleep measures. Our preliminary results show alterations in the melatonin profile by genotype suggesting a mechanism whereby variation in MTNR1B could influence fasting glucose and risk of T2D. Ultimately this research could lead us towards new therapeutic interventions which adjust the timing of melatonin, thereby modifying cardiometabolic risk.

Poster Number 80

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Modulation of LH Pulse Amplitude: Interaction of Continuous Kisspeptin and Estrogen

Investigators: M. F. Lippincott, Y. M. Chan, S. B. Seminara

Introduction: When administered as an IV bolus, kisspeptin-10 can be a potent stimulus for GnRH-induced LH secretion. To explore the impact of sex steroids on kisspeptin responsiveness, continuous kisspeptin-10 was administered to postmenopausal women ± physiologic estradiol replacement.

Methods: Eight post-menopausal women, four on physiologic estradiol replacement, received kisspeptin 12.5 mcg/kg/h IV x 24 h. Blood sampling was performed q10-60min. Mean LH levels; number of LH pulses, and LH pulse amplitude during 3 time windows were compared 1) PRE infusion (hr 0-6) 2) START infusion (hr 6-12), 3) END infusion (hr 22-28).

Results: All post-menopausal women not treated with estradiol experienced a modest drop in LH levels by 13-36% from START compared to PRE infusion, $p<0.05$; attributable to a decrease in LH pulse amplitude from PRE (5.9±3.5 mIU/mL) to START (2.5±1.4 mIU/mL),$p<0.05$. In contrast, all post-menopausal women on estradiol experienced a rise in LH levels from 143% to almost 200% from PRE to END ($p<0.05$%) attributable to an increase in LH pulse amplitude from PRE (4.5±1.8 mIU/mL) to END (9.3±6 mIU/mL),$p<0.05$. There was no change in LH pulse frequency in either group.

Conclusions: The administration of continuous kisspeptin to post-menopausal women results in a moderate suppression of LH. In contrast, estradiol treated post-menopausal women experienced a marked increase in LH levels due to increased LH pulse amplitudes during the kisspeptin infusion. Estradiol positively modulates the influence of kisspeptin on LH pulse amplitude thereby changing LH levels. These data highlight the role of sex steroids in shaping kisspeptin signaling.

Poster Number 81

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Cortisol Measures Across the Weight Spectrum

Investigators: M. Schorr, E. A. Lawson, A. Klibanski, K. K. Miller

Context: There are conflicting reports of increased vs decreased hypothalamic-pituitary-adrenal (HPA) activation in obesity; the most consistent finding is an inverse relationship between BMI and morning cortisol. In anorexia nervosa (AN), cortisol measures are elevated.

Objective: To investigate cortisol measures across the weight spectrum

Design: Cross-sectional

Setting: Clinical Research Center

Participants: 60 women, 18-45 yo: AN (N=18); overweight/obese (OB, N=21); normal-weight controls (HC, N=21)

Measures: Body composition was assessed by DXA and HPA dynamics by UFC, mean overnight serum cortisol, 8 AM serum cortisol and CBG, morning and late-night salivary cortisol, and dexamethasone-CRH testing.

Results: Cortisol measures demonstrated a U-shaped relationship with BMI, nadiring in the overweight-mildly obese range, and body fat, with higher mean cortisol levels in AN than OB. There were weak negative linear relationships between lean mass and some cortisol measures. Despite HPA activation in both AN and OB, there was a negative linear relationship between BMI and 8 AM serum cortisol after dexamethasone suppression (R= -0.36, p=0.009). Most cortisol measures were negatively associated with total hip, femoral neck, and PA spine BMD.

Conclusions: Here we show that cortisol measures are lowest in overweight-mildly obese women —lower than in lean women. With more significant obesity, cortisol levels rise, although not as high as in AN. Therefore, extreme underweight and overweight states may activate the HPA axis, and hypercortisolemia may contribute to increased adiposity in the setting of caloric excess. Hypercortisolemia may also contribute to decreased BMD in the setting of both caloric restriction and excess.

Poster Number 82

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A "Thrifty" MicroRNA Genetically Linked to Obesity, Type 2 Diabetes and Human Positive Selection Regulates Metabolism and Energy Homeostasis

Investigators: L. Wang, A. Naar

Human populations have undergone positive selection for SNPs/mutations in genes/genomic regions, such as "thrifty" genes, which by virtue of elevating fat storage may have conferred an advantage during famines frequent in ancient times. An improved understanding of the genetic and molecular underpinnings of human thrifty phenotypes may facilitate novel therapeutic strategies to counter negative effects of genetic adaptations that are no longer beneficial in the modern world with a profound food abundance, and which indeed may promote human metabolic disease states. MicroRNAs are critical and evolutionarily ancient components of gene regulation. We have identified miR-128-1 as a thrifty microRNA contributing to human positive selection by acting as a switch from energy expenditure to energy/fat storage. We found that the miR-128-1 locus is also linked to obesity and type 2 diabetes. Consistent with these genetic links, antisense antagonism of miR-128-1 in preadipocytes and myoblast promotes brown adipocyte lineage differentiation and elevated expression of energy expenditure genes. Accordingly, once-weekly injection of LNA antisense oligonucleotides targeting miR-128-1 over 16 weeks in Diet-Induced Obese (DIO) mice results in dramatically inproved obesity, pre-diabetes, and hepatic steatosis due to increased energy expenditure.

Taken together, these findings suggest that non-genic actors such as microRNAs emanating from the non-coding portion of the human genome may contribute to human evolutionary adaptations, and act more broadly to control metabolism and energy homeostasis and contribute to human metabolic disorders.

Poster Number 83

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Rejection of an allograft through non-MHC antigens does not translate to the rejection of a different allograft from the same donor in mixed chimeras

Investigators: K. Shinoda, D. Ndishabandi, N. Oh, C. M. Chase, P. S. Russell, J. C. Madsen, R. B. Colvin, A. Alessandrini

We have shown in allotransplant studies involving mixed chimeras that tolerance of non-MHC alloantigens involve Foxp3+ regulatory T cells, while tolerance of MHC antigens do not. The current study investigates whether non-MHC antigens are shared between different types of allografts and whether rejection of one type of allograft affects alloresponses to a different organ from the same donor.

We took mixed chimeras (DBA/2(H2d)xB6.Foxp3DTR/DTR) with long-term accepted donor-type DBA/2 skin allografts and depleted donor and recipient-derived Foxp3+ cells with diphtheria toxin. Skin allografts were rejected without loss of hematopoietic chimerism. We re-challenged with a second DBA/2 skin graft and the allograft rejected without depletion of Foxp3+ cells. Splenocytes were harvested 4 weeks after rejection skin grafts and adoptively transferred to B6.Rag1 recipients that were transplanted with DBA/2 skin allografts (SG) (Group A, n = 4) or DBA/2 heart allografts (HG) (Group B, n = 5).

All recipients in Group A rejected both donor (DBA/2) and third-party (C3H) skin grafts. All recipients in Group B continued to accept donor-type heart allografts for more than 8 weeks. Adoptive transfer of splenocytes from non-rejecting mixed chimeras to B6.Rag1 mice transplanted with DBA/2 and C3H skin allografts resulted in the rejection of only the C3H skin (n=5).

We conclude that T effector memory develops to non-MHC antigens to the rejected allograft and not to any other allograft from the same donor. These data have implications with regards to our understanding of how antigen-specific regulatory T cells are induced in mixed chimera tolerance protocols.

Poster Number 84

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Targeting leukocyte integrin CD11b/CD18 ($\alpha M\beta 2$) with a novel mAb salvaged renal function following otherwise irreversible ischemic insult in Cynomolgus monkeys

Investigators: A. B. Cosimi, A. Dehandi, R. N. Smith, X. Li, M. A. Arnaout

Delayed allograft function resulting from ischemia reperfusion injury (IRI) is associated with increased peri-transplant costs and morbidity, as well as inferior long-term survival rates. Leukocyte-mediated tissue injury is an important pathway contributing to pathogenesis of IRI. We have evaluated, in a nonhuman primate renal IRI model, a novel ligand-mimetic monoclonal antibody (mAb 107) that blocks leukocyte CD11b/CD18-mediated proinflammatory functions by acting as a "pure" antagonist, i.e. it lacks the detrimental "partial agonism" of current anti-integrin drugs.

Methods: A 120-minute unilateral renal warm ischemic insult was induced in eight Cynomolgus monkeys by hilar cross clamping. The contralateral ureter was ligated. Four animals received mAb 107 intravenously, and four controls received saline or irrelevant mAb. Post procedure monitoring included serial renal function studies and renal biopsies at 2 days, 1 month and at terminal euthanasia (6-9 months).

Results: All study animals required release of the contralateral ureteral ligature at 2 days because of severe ATN in the IRI kidney. Progressive tubulointerstitial fibrosis was seen in IRI kidney in the four controls. Re-ligation of the contralateral (normal kidney) ureter 2 days prior to planned observation termination resulted in rapid rise in serum creatinine, confirming loss of life-sustaining function by the IRI kidney. In contrast, mAb107-treated animals showed progressive reversal of ATN histopathology, and pre-terminal ligation of the contralateral ureter resulted in no or minimal serum creatinine rise.

Conclusion: Inhibition of CD11b/CD18 at the onset of severe kidney IRI by the pure antagonist mAb 107 alleviated progressive tubulointerstitial renal fibrosis and salvaged kidney function.

Poster Number 85

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Specialized roles for Argonautes 2 and 4 in mammalian antiviral defense

Investigators: M. G. Basavappa, D. Harjanto, E. C. Gantman, D. A. Cronkite, J. T. Prior, H. Reinecker, P. Hertzog, S. Cheloufi, R. Darnell, N. Papavasiliou, K. L. Jeffrey

The four mammalian Argonautes (AGO1-4) are thought to share overlapping functions in micro(mi) RNA regulation, yet only AGO2 possesses the catalytic activity for RNA interference (RNAi). Here, we identify an interferon (IFN)-independent, RNAi-dependent mode of mammalian antiviral defense, in which AGOs 2 and 4 are assigned unique and specialized roles. Sequencing of RNA attached to AGOs in a panel of Influenza A-infected cells selectively deficient in RNAi-effector proteins, revealed influenza-derived RNAs preferentially bound to AGO2 and AGO4. These small RNA species require Dicer as well as AGO4 for processing, but are dependent on AGO2 for virus suppressive activity. AGO2 catalytic inactive cells or AGO4 deficient cells and mice are hyper-susceptible to infection by multiple viruses, independent of type I IFN. Conversely, infected AGO1 or AGO3 deleted cells displayed no difference in viral titers. Together, our data demonstrate that, AGOs 2 and 4 specifically scavenge viral RNA using it for defense against pathogenic viruses.

Poster Number 86

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Autotaxin-independent lysophosphatidic acid (LPA) generation in pulmonary fibrosis

Investigators: K. E. Black, G. Bain, E. Berdyshev, I. Gorshkova, F. V. Castelino, C. K. Probst, B. A. Fontaine, D. Lagares, N. Ahluwalia, R. S. Knipe, V. Natarajan, A. M. Tager

Pulmonary fibrosis is a lethal condition marked by fibroblast accumulation and deposition of extracellular matrix. Lysophosphatidic acid (LPA) mediates this process through effects on fibroblasts, epithelial cells, and endothelial cells. In the circulation LPA is generated by the action of autotaxin, a lysophospholiapse D, on lysophosphatidylcholine (LPC). We therefore examined autotaxin's role in pulmonary fibrosis. We challenged C57BL/6 mice with intratracheal bleomycin, then measured autotaxin concentration and activity in bronchoalveolar lavage (BAL) and plasma as well as autotaxin mRNA expression in whole lung. Multiple species of LPA and LPC (autotaxin's preferred substrate) were analyzed in BAL and plasma, using targeted LC-MS/MS. We then administered PAT-048, a potent autotaxin inhibitor, to bleomycin-injured mice and analyzed the effects.

Autotaxin and LPA levels were elevated in the BAL but not plasma of bleomycin-injured mice. Lung autotaxin expression was however lower in bleomycin-injured mice. In bleomycin-challenged mice, 16:0 LPA and 18:0 LPA were the most abundant species of LPA in the plasma and of LPC in the BAL. However, the most abundant species of LPA in BAL was 22:5 LPA, followed by 22:6 LPA, suggesting that neither LPA in plasma nor LPC in BAL contributed to the increased BAL LPA. Furthermore, PAT-048, a potent autotaxin inhibitor, decreased neither BAL LPA nor pulmonary fibrosis induced by bleomycin.

These data suggest that the increase in LPA that contributes to pulmonary fibrosis is not due to the action of autotaxin. Autotaxin-independent pathways to LPA synthesis may therefore be potential therapeutic targets for fibrotic lung disease.

Poster Number 87

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Pathogen-Secreted Proteases Activate a Novel Plant Immune Pathway

Investigators: Z. Cheng, F. M. Ausubel

Mitogen-Activated Protein Kinase (MAPK) cascades play central roles in innate immune signaling networks in plants and animals. In plants, however, the molecular mechanisms of how signal perception is transduced to MAPK activation remain elusive. We report that pathogen-secreted proteases activate a previously unknown signaling pathway in Arabidopsis thaliana involving the heterotrimeric G-protein complexes, which function upstream of a MAPK cascade. In this pathway, Receptor for Activated C Kinase 1 (RACK1) functions as a novel scaffold that binds to the Gβ subunit as well as to all three tiers of the MAPK cascade, thereby linking upstream G protein signaling to downstream activation of a MAPK cascade. The protease-G protein-RACK1-MAPK cascade modules identified in these studies are distinct from previously described plant immune signaling pathways such as the one elicited by bacterial flagellin, in which G proteins function downstream of or in parallel to a MAPK cascade without the involvement of the RACK1 scaffolding protein. The discovery of the novel protease-mediated immune signaling pathway described here was facilitated by the use of the broad host range, opportunistic bacterial pathogen Pseudomonas aeruginosa. The ability of P. aeruginosa to infect both plants and animals makes it an excellent model to identify novel types of immunoregulatory strategies that account for its niche adaptation to diverse host tissues and immune systems.

Poster Number 88

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Donor specific expansion of regulatory T-cells was associated with allograft tolerance induced by transient mixed chimerism in nonhuman primates

Investigators: K. Hotta, A. Aoyama, T. Oura, M. Tonsho, Y. Yamada, J. S. Allan, J. C. Madsen, B. Cosimi, G. Benichou, T. Kawai

Background: We have reported successful induction of solid organ allograft tolerance in nonhuman primates (NHP) via a mixed chimerism approach. Since these recipients achieved allograft tolerance with transient chimerism, peripheral mechanisms are considered playing a major role in the maintenance of tolerance. In this study, we evaluated the role of regulatory T cells (Treg) in NHP that achieved long-term allograft survival without immunosuppression (IS).

Method: All recipients underwent combined solid organ and donor bone marrow transplantation. Seven allograft recipients [kidney (5) and lung (2)] that achieved long-term (>300 days) allograft survival (LTS) without IS were investigated. Six recipients [heart (4) and kidney (2)] that acutely rejected their allografts were evaluated as controls (AR). T-cell responses were evaluated by mixed lymphocyte responses (MLR) with CFSE/CD4, CD8 and Foxp3 staining.

Results: While CD8+ T cell hypo-responsiveness was observed in LTS (but not in AR), significant anti-donor CD4+ T cell proliferation was observed in both LTS and AR. However, among these proliferated CD4+ cells, significantly higher Foxp3+ cell proliferation was observed in LTS, comparing with those in AR ($P<0.01$). The FoxP3+ cell proliferation in LTS was donor specific, as significantly higher Foxp3+ cell proliferation was observed against the donor than the third party stimulators ($P<0.01$). Interestingly, these proliferated Tregs appeared to be converted from Non-Tregs, as significant proliferation of Foxp3+ cells was observed only after anti-donor MLR with sorted CD4+CD25-, but not with sorted CD4+CD25high cells.

Conclusion: Donor specific expansion of Tregs was associated with long-term allograft tolerance induced after transient mixed chimerism.

Poster Number 89

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Dectin-1 Controls TLR9 Trafficking to Phagosomes containing β-1,3 glucan

Investigators: N. S. Khan, P. V. Kasperkovitz, M. K. Mansour, J. M. Tam, M. Seward, S. Puranam, J. L. Reedy, M. Feliu, J. M. Vyas

Dectin-1 and TLR9 play distinct roles in the recognition and induction of innate immune responses to Aspergillus fumigatus and Candida albicans. Dectin-1 is a receptor for the major fungal cell wall carbohydrate β-1,3 glucan and induces inflammatory cytokines through Card9 and controls phagosomal maturation through Syk activation. In contrast, TLR9 modulates the inflammatory cytokine response. In this study, we demonstrate that β-1,3 glucan beads are sufficient to induce dynamic redistribution of TLR9 to phagosomes and results in accumulation of the cleaved form of TLR9. Trafficking of TLR9 to A. fumigatus and C. albicans phagosomes required Dectin-1 recognition. Similar to CpG beads, inhibition of phagosomal acidification blocks TLR9 accumulation on phagosomes containing β-1,3 glucan. Dectin-1 mediated Syk activation was required to license TLR9 trafficking to β-1,3 glucan-containing phagosomes, A. fumigatus and C. albicans phagosomes. Collectively, our study demonstrates that recognition of β-1,3 glucan by Dectin-1 triggers TLR9 trafficking to β-1,3 glucan-containing phagosomes and may be critical in coordinating innate antifungal defense.

Poster Number 90

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TGF-beta-producing regulatory B cells induce regulatory T cells and promote transplantation tolerance

Investigators: J. I. Kim, W. Liu, L. Liu, K. Lee, R. T. Stott, H. Yeh, J. F. Markmann

Regulatory B (Breg) cells have been shown to play a critical role in immune homeostasis and in autoimmunity models. We have recently demonstrated that combined anti-TIM-1 and anti-CD45RB antibody treatment results in tolerance to full MHC-mismatched islet allografts in mice by generating Breg cells that are necessary for tolerance. Breg cells are antigen-specific and are capable of transferring tolerance to untreated, transplanted animals. Here we demonstrate that adoptively transferred Breg cells require the presence of Treg cells to establish tolerance, and that adoptive transfer of Breg cells increases the number of Treg cells. Interaction with Breg cells in vivo induces significantly more Foxp3 expression in CD4+CD25- T cells than with naive B cells. We also show that Breg cells express the TGF-beta associated latency-associated peptide (LAP) and that Breg-cell-mediated graft prolongation post-adoptive transfer is abrogated by neutralization of TGF-beta activity. Finally, in contrast to wild-type recipients, combined anti-CD45RB / anti-TIM1 antibody treatment does not significantly prolong graft survival in B cell-specific TGF-beta-deficient recipients. Collectively, these findings suggest that in this model of antibody-induced transplantation tolerance, Breg cells promote graft survival by promoting Treg-cell development through the production of TGF-beta.

Poster Number 91
ePoster#13

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Quantification and Characterization of Cell-Mediated Lympholysis

Investigators: G. M. La Muraglia II, M. J. O'Neil, L. M. Madariaga, S. G. Michel, K. S. Mordecai, F. I. Preffer, J. S. Allan, I. M. Hanekamp, J. C. Madsen

Cell-mediated lympholysis (CML) is an established in vitro assay to detect the presence of cytotoxic effector T-lymphocytes precursors. 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 establish a novel isotope-free approach to the detection of CML that utilizes the statistical power of flow cytometry, while also providing qualitative benefits of cell imaging. Primary porcine lymphocyte targets labeled with eFluor670 underwent CML by in vitro generated effector CTLs from peripheral blood mononuclear cells stimulated across a full MHC mismatch. Percent specific lysis was determined by total loss of target cells from the samples in comparison to known positive and negative controls. Normalization of acquisition was provided through the use of flow count beads to account for differential spontaneous effector cell death. We demonstrate detection of target-specific lysis versus non-responsiveness equivalent to the 51Cr-based assay. In addition, the use of quantitative cell imaging can detect and quantify the presence of accessory cells involved in the cytotoxic pathway. Our innovative technique establishes an improved method that permits the quantification and qualification of cell-mediated lysis that can be widely applied in models of transplantation tolerance and rejection. This method improves upon the gold standard 51Cr assay because it is capable of providing further insight and characterization of the interactions between effector and target cells, such as the presence of helper and/or accessory cells, changes in cell morphology and apoptotic indices for specific immune cell subsets.

Poster Number 92

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Neural control of intestinal host defense by a Wnt-acetylcholine neuroimmune axis

Investigators: S. Labed, M. Najibi, J. Irazoqui

A large body of evidence indicates that many aspects of immunity are regulated by the nervous system, but the underlying mechanisms and their physiological significance remain unclear. Using C. elegans as a pre-clinical model organism, we discovered that a Wnt signaling pathway operates in the nervous system to distally induce the expression of host defense genes in the intestinal epithelium during intestinal infection. Neural control of such response is mediated by muscarinic acetylcholine signaling. We show that Wnt induction results in enhanced acetylcholine signaling, which induces a transcriptional host response in the intestinal epithelium mediated by conserved transcription factors that we identified in a genetic screen. Thus, we define a novel pathway that connects pathogen detection in the intestine with nervous system activation, followed by distal communication with the intestinal epithelium and transcription factor activation for host defense. These observations have potential implications for human intestinal innate immunity and inflammation, because Wnt signaling is conserved in human neurons, because the enteric nervous system is poorly understood in relation to innate immunity, and because the human intestinal epithelium expresses receptors for acetylcholine. Therefore, it is highly likely that a similar neural Wnt-Acetylcholine axis may operate in humans for the control of intestinal immunity and inflammation.

Poster Number 93

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Immunomodulatory Strategies Directed Towards Tolerance of Vascularized Composite Allografts

Investigators: M. L. Madariaga, K. Shanmugarajah, S. G. Michel, V. Vincenzo, G. M. La Muraglia, R. Torabi, D. A. Leonard, M. A. Randolph, R. B. Colvin, K. Yamada, J. C. Madsen, C. L. Cetrulo, Jr, D. H. Sachs

Background: Achieving tolerance of vascularized composite allografts (VCAs) would improve the risk-to-benefit ratio in patients who undergo this life-enhancing, though not life-saving, transplant. Kidney co-transplantation along with a short course of high-dose immunosuppression enables tolerance of heart allografts across a full MHC mismatch. In this study, we investigated whether tolerance of VCA across full MHC disparities could be achieved in animals already tolerant of heart and kidney allografts.

Methods: Miniature swine that were tolerant of heart and/or kidney allografts long-term underwent transplantation of myocutaneous VCA across the same MHC barrier. Prior to VCA transplant, Group 1 (n=3) underwent Class I-mismatched kidney transplantation; Group 2 (n=3) underwent two sequential Class I-mismatched kidney transplantations; Group 3 (n=2) underwent haploidentical MHC-mismatched heart/kidney transplantation; and Group 4 (n=2) underwent full MHC-mismatched heart/kidney transplantation.

Results: All three animals in Group 1 and two of three animals in Group 2 showed skin rejection ≤85 days; one animal in Group 2 showed prolonged skin survival >200 days. Animals in Groups 3 and 4 showed skin rejection ≤30 days and regained in vitro evidence of donor responsiveness.

Conclusion: This is the first pre-clinical study in which hearts, kidneys, and VCAs have been transplanted into the same recipient. Despite VCA rejection, tolerance of heart and kidney allografts was maintained. These results suggest that regulatory tolerance of skin is possible but not generally achieved by the same level of immunomodulation that is capable of inducing tolerance of heart and kidney allografts. Achieving tolerance of skin may require additional immunomodulatory therapies.

Poster Number 94
ePoster#14

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Presence of Donor Leukocytes and Donor MHC-Cross-Dressed Recipient Cells and its Relationship to Direct T cell Alloresponses after Allotransplantation

Investigators: J. Marino, P. Crosby, J. Paster, A. Trowell, K. Briggs, L. Maxwell, G. Benichou

We have studied the kinetics of donor cell trafficking from the graft to recipient's lymphoid organs and its correlation to the direct T cell alloresponse in BALB/c mice transplanted with fully allogeneic B6 skin, heart or pancreatic islets. The recipients' spleen, ipsilateral and contralateral lymph nodes were analyzed via imaging flow cytometry (Amnis ImageStream X Mark II) at different time points during the first week after transplantation. No leukocytes of donor origin were found in the recipient's organs after skin transplantation. Alternatively, after islet or cardiac transplantation, some donor leukocytes were detected but only at frequencies not superior to 0.02% of analyzed cells. In contrast, with all transplants, high frequencies (0.5-4%) of recipient cells displaying donor MHC class I and II molecules were observed as early as day 1 post-transplantation and increasing in number towards day 7. Kinetics of T cell alloresponse, measured by ELISPOT, showed an expansion of pro-inflammatory gIFN-producing T cells activated via direct allorecognition as early as 48 hours after transplantation. Taken together, these results suggest that presentation of allogeneic MHC molecules on recipient cross-dressed cells rather than donor passenger leukocytes is the driving force behind direct T cell alloreactivity post-transplantation.

Poster Number 95
ePoster#15

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Dynamic Imaging of the Arthritic Joint Uncovers New Mechanisms and New Targets

Investigators: Y. Miyabe, C. Miyabe, T. T. Murooka, E. Y. Kim, N. D. Kim, T. R. Mempel, A. D. Luster

Rheumatoid arthritis (RA) is characterized by neutrophil recruitment into the joint. Activated neutrophils within the joint release many potent mediators that drive RA pathogenesis directly or indirectly by recruiting and activating other cells. Thus, the control of leukocyte entry into the joint represents a major point at which new therapeutics could be developed to attenuate inflammatory arthritis.

We have found that at least four different chemoattractant receptors (CKRs), BLT1, CCR1, CXCR2 and C5aR, contribute to neutrophil recruitment into the inflamed joint. However, the precise role for each CKR in the process of neutrophil recruitment into the joint remains unclear.

To elucidate the role of each CKRs in the arthritic joint, we imaged the joint by multiphoton intravital microscopy (MP-IVM), and analyzed the role of CKRs on the neutrophil migration cascade.

Neutrophils could easily be seen outside of blood vessels, and were observed to be actively mobile within the inflamed joints of wild-type (WT) mice, while neutrophils could not be observed outside of blood vessels in BLT1-KO mouse. In short term competitive homing experiments in WT arthritic mice, there were fewer BLT1-deficient neutrophils that arrested and transmigrated (TEM) on joint vessels compared to WT neutrophils. In contrast, there is no difference between CXCR2-, CCR1/CXCR2- or C5aR-deficient and WT neutrophils in competitive homing experiments in WT arthritic mice.

Thus, BLT1 plays an important role in both the arrest and TEM of neutrophils across blood vessels of the inflamed joint, and could be a potential target for RA therapy.

Poster Number 96

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PKC and PKD have an evolutionarily conserved role in the regulation of TFEB in response to infection

Investigators: M. Najibi, S. A. Labed, J. E. Irazoqui

Translocation of Transcription Factor EB (TFEB) has recently been found to be a master transcriptional regulator of autophagy and lysosomal biogenesis throughout phylogeny. In addition, we discovered that TFEB activity is important in the regulation of the host response to infection. We previously showed that TFEB is a key transcription factor for host defense in invertebrate and vertebrate hosts. TFEB is activated shortly after gram-positive and gram-negative infection in both systems. Furthermore, we found that in nematodes, TFEB drives the expression of close to 80% of host response genes, including antimicrobial and autophagy genes that are essential for host tolerance to infection. In murine macrophages, we found that TFEB is involved in the induction of pro-inflammatory signals IL1b, IL6, TNFa, and CCL5. Therefore, identifying the mechanisms that control TFEB activation is important to understand transcriptional host responses to infection in mammals. In the present study, our goal was to identify potential upstream regulators of TFEB. In nematodes, an RNAi screen covering more 430 protein kinases revealed that Protein Kinase D (PKD) homolog, dkf-1 is required for TFEB activation during infection. In parallel, a small molecule screen of more than 150 inhibitors covering 30 kinases revealed that Protein Kinase C (PKC) and PKD are also required in murine macrophages. Thus, both C. elegans genetics and drug screens identified this family of kinases as essential for TFEB activation. In conclusion, we propose that activation of PKC and PKD is a necessary upstream step in the activation of TFEB by bacterial pathogens.

Poster Number 97

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Kidney Plasmacytoid Dendritic Cells Display Distinct Cell Surface Markers and May Explain the Induction of Tolerance by Kidney Allografts

Investigators: N. A. Oh, M. Tonsho, D. K. Ndishabandi, R. B. Colvin, J. C. Madsen, A. Alessandrini

Background: Murine studies have demonstrated spontaneous acceptance of full MHC-mismatched kidney allografts and exhibit distinct regulatory T cell-rich organized lymphoid structures (TOLS). Immunohistochemical analysis of accepted kidney allografts confirmed the presence of plasmacytoid dendritic cells (pDCs) in TOLS. Non-human primates (NHPs) studies have demonstrated co-transplantation of kidney and heart allografts from the same donor leads to the acceptance of the heart allograft. This study is focused on understanding this apparent systemic tolerization by the kidney and the role of kidney pDCs.

Methods: Various tissues in a mixed-chimeric NHP tolerant to heart and kidney allografts were analyzed for pDCs using flow cytometry. pDC populations were also analyzed in mice. Tissue specific pDC characteristics were examined for differences in cell markers.

Results: pDCs in the tolerant NHP kidney were found to be BDCA2hi (cell marker unique to pDCs in primates) while bone marrow pDCs in native NHPs were BDCA2low and undetectable in native NHP kidneys. Unlike in NHP, pDCs are present in murine native kidneys pDCs and were found to be B220lowPDCA1low (cell markers characteristic of pDCs in mice), whereas native bone marrow pDCs were B220hiPDCA1hi. Furthermore, isolated kidney pDCs were able to convert naïve T-cells into suppressor, FoxP3+ regulatory T cells.

Conclusions: Here we show that kidney pDCs are unique and their presence may help explain heart allograft acceptance in kidney co-transplantations in NHPs and in spontaneous kidney allograft acceptance in mice. Current studies are underway to further elucidate the role kidney pDCs in allotransplant tolerance in NHP and mice.

Poster Number 98

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Detection of Cell-Mediated Lympholysis in Human Transplantation

Investigators: G. M. La Muraglia II, M. J. O'Neil, M. L. Madariaga, P. J. Spencer, K. S. Mordecai, F. I. Preffer, J. S. Allan, J. C. Madsen, I. M. Hanekamp

A. Human transplantation lacks an assay to assess direct cell-mediated anti-donor response. Current methods employed in the detection of cell-mediated lympholysis (CML) are based upon quantification of traceable elements (51Cr) released from target cells. Here we have adapted the traditional chromium-based CML assay using a flow cytometric approach to assess human CML.

B. Primary human lymphocyte targets labeled with eFluor670 underwent CML by in vitro generated effector cytotoxic lymphocytes from peripheral blood mononuclear cells stimulated across ABO incompatibilities. Percent Specific Lysis was determined by total loss of target cells from the samples correlated with known experimental controls. Positive controls consisted of mismatched effector blood donor types; while negative controls were constructed using donor peripheral blood mononuclear cells activated with self MHC irradiated stimulators.

C. We demonstrate quantifiable and reliable detection of target-specific lysis from ABO incompatible human stimulators and targets. We are also able to characterize specific immunophenotypes within the effector and target cell populations.

D. Our novel approach permits analysis of cell-mediated lysis that can be used in models of human transplantation. This method improves upon the gold standard 51Cr assay because it allows for specific characterization of effector and target cells. Further work will apply this method to investigate mechanisms of transplantation tolerance.

Poster Number 99
ePoster#16

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B-Cell Tolerance to Donor Alloantigens

Investigators: A. R. Patil, M. Climov, A. J. Matar, A. A. Musa, E. P. Harrington, D. H. Sachs, R. Duran-Struuck, C. A. Huang

Pre-existing panel-reactive antibodies and development of donor-specific alloantibodies are known to play important roles in graft dysfunction and deterioration. Preventing and/or eliminating alloantibody responses would dramatically improve organ transplantation outcomes. In our large animal experience of hematopoietic cell transplantation with reduced intensity conditioning, alloantibody responses are not detected even in swine recipients that do not engraft and eventually lose chimerism. Here we report that use of cytokine mobilized peripheral blood stem cells is not necessary and intravenous exposure to unmobilized donor peripheral blood cells under the same novel reduced intensity conditioning protocol consisting of 100cGy total body irradiation, CD3-immunotoxin mediated T-cell depletion and a short course of Cyclosporin A reliably results in stable donor-specific B-cell tolerance across swine leukocyte antigen barriers in miniature swine. Transient T-cell hyporesponsiveness to donor cells is observed followed by regain of T-cell alloresponses after the loss of chimerism. B-cell unresponsiveness persists despite development of sensitized T-cell alloresponses following a donor leukocyte infusion, rejection of donor allografts, and multiple subcutaneous injections of donor cells. The humoral tolerance established is donor specific with preserved antibody responses to third party antigens as tested by immunization with vaccines, complete Freund's adjuvant and keyhole limpet hemocyanin. Even animals with evidence for pre-existing cytotoxic antibodies to donor cells prior to transplantation are successfully tolerized at the B-cell level using this protocol. These results show that robust B-cell tolerance can be established through intravenous exposure to donor hematopoietic cells under certain mildly immunosuppressive conditions even in presensitized recipients.

Poster Number 100

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Local and systemic effects of UVB exposure to skin

Investigators: Y. Sano, J. Park

UVB is a component of solar radiation primarily responsible for causing damage and cancer in skin, and disrupting immune homeostasis. Inflammation is a key mechanism underlying UVB's various detrimental effects. Due to its limited penetration, UVB can only inflict primary damage in the epithelial cells of the body surface. Some physiological effects of UVB are, however, not merely skin deep; an obvious example is the suppression of immune responses in lymphoid tissues following environmental or therapeutic exposure to UVB. Here we show that activation of the protein kinase p38a is restricted to the epidermis in UVB-exposed skin, and that p38a ablation targeted to the epithelial compartment is sufficient to suppress UVB-induced inflammation. Mechanistically, loss of epithelial p38a attenuates the expression of genes required to induce vascular leakage and edema, and also increases the steady-state abundance of epidermal $\gamma\delta$ T cells, which are known to promote the repair of damaged epidermis. These effects of p38a deficiency delineate a molecular network operating at the organism–environment interface, and reveal conditions crucial to preventing the pathology resulting from sun-damaged skin. We also observed that skin exposure to UVB or subcutaneous tumor growth resulted in a massive proliferation of TER-119+CD71+ erythroid progenitors in the spleen. Correspondingly, the UVB-irradiated and tumor-bearing mice produced increased amounts of renal and circulating erythropoietin (EPO). Furthermore, erythroid cells expressing EPOR were detected in the dermis of UVB-irradiated skin and in the tumor. These findings indicate that UVB-exposed skin and tumors can signal to induce EPO production, and mobilize erythroid cells.

Poster Number 101
ePoster#17

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Imaging the role of CXCR3 and its ligands in the interaction of T cells with the brain vasculature in cerebral malaria

Investigators: E. W. Sorensen, J. Lian, I. C. Hirako, F. Marangoni, T. R. Mempel, A. D. Luster

Malaria is still one of the world's most important human pathogens, and cerebral malaria (CM) is its most deadly complication. The pathogenesis of CM remains incompletely understood, which has hindered the development of therapeutics. Our previous work has demonstrated that CXCR3 and its ligands, CXCL9 and CXCL10, are required for the development of CM in mice following infection with Plasmodium berghei ANKA (PbA). Effector T cells are known to be required for murine CM and these infected mice had fewer T cells accumulating in the brains. However, how CXCR3 and its ligands regulate the accumulation of T cells in the brains of PbA-infected mice is not known. We have now employed multi-photon intravital microscopy (MP-IVM) to determine how the CXCR3 chemokine influences the interactions of T cells with the brain vasculature in live mice with CM. Using DPE-GFP transgenic reporter mice in which GFP is expressed in all T cells, we found that there were significantly fewer T cells moving at higher velocities within the vasculature in the brains of CXCR3-/- mice on day 8-9 post-infection compared to WT. Further, the percent of arrested T cells and their duration of arrest were lower within the vasculature of CXCR3-/- infected mice compared to infected WT mice. Using our CXCL9 and CXCL10 dual reporter mouse, we found that CXCL10 was strongly induced in the brain endothelium as early as 4 days post-infection. Experiments are in progress to elucidate the mechanism by which CXCL10 is induced within the brain vasculature.

Poster Number 102
ePoster#18

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IgG4-Related Disease: Baseline clinical and laboratory features in 125 patients with biopsy-proven disease

Investigators: Z. S. Wallace, V. Deshpande, H. Mattoo, V. Mahajan, M. Kulikova, S. Pillai, J. H. Stone

Purpose: IgG4-related disease (IgG4-RD) is an immune-mediated fibroinflammatory condition that can affect nearly any organ. No detailed clinical and laboratory assessments have been reported in large numbers of patients with IgG4-RD diagnoses established by strict clinicopathological correlation.

Methods: We reviewed the baseline features of 125 patients with biopsy-proven disease. The diagnosis was confirmed by pathology review according to consensus diagnostic criteria. Disease activity and damage were assessed by the IgG4-RD Responder Index (RI). Flow cytometry was used to assess levels of circulating plasmablasts.

Results: Of the 125 patients, 103 had active disease and 86 were on no treatment. Only 51% of the patients with active disease had elevated serum IgG4 concentrations. However, patients with active disease and elevated serum IgG4 concentrations were older, had a higher RI, a greater number of organs involved, lower complement levels, a higher absolute eosinophil count, and higher IgE levels compared to those with active disease but normal serum IgG4 ($P<0.01$ for all comparisons). The correlation between IgG4+ plasmablast level and RI ($R=0.45$, $P=0.003$) was stronger than that of total plasmablasts and RI. Seventy-six (61%) of the patients were male, but no significant differences according to gender were observed with regard to disease severity, organ involvement, or serum IgG4 concentrations. Glucocorticoids failed to produce sustained remission in the majority of patients.

Conclusion: Nearly 50% of this patient cohort with biopsy-proven, clinically-active IgG4-RD had normal serum IgG4 concentrations. Serum IgG4 elevation identify a subset with more inflammatory features. IgG4+ plasmablasts correlate well with disease activity.

Poster Number 103

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Extracellular miRNAs induce complement factor B via TLR7-MyD88 in bacterial sepsis

Investigators: L. Zou, G. Xu, Y. Feng, W. Chao

Severe sepsis involves massive activation of the complement system including classical, lectin and alternative pathway (AP). Complement factor B (cfB) is an essential component of AP activation. We have recently found that cfB contributes to cardiac dysfunction and mortality in a mouse model of polymicrobial sepsis. However, the upstream signal leading to cfB production and septic cardiomyopathy remains incompletely understood. microRNAs (miRNAs) are endogenous non-coding RNA of 19-22 nucleotides that play a role in post-transcriptional gene regulation. miRNAs are released into extracellular space under certain stress conditions such as tissue ischemia and bacterial sepsis. Here we show that in a mouse model of polymicrobial sepsis (cecum ligation and puncture, CLP), there was an increase in multiple plasma miRNAs. Among 68 miRNAs tested, 6 of them were found to increase by more than 2-fold, including miR-146a, -145, -34a, -122, -192, -210. When treated with miR-146a, -145, -34a, -122, but not miR-192 and -210, macrophages produced cfB. The increase in cfB production in miR-treated macrophages was mediated by TLR7-MyD88 signaling, as deletion of either TLR7 or MyD88 was able to completely block the miRNA-induced cfB production. In vivo, CLP induced significant and specifically increase of cfB, not C3, C5, gene expression in the heart compared to Sham control. Systemic RNase A administration, which eliminated miRNAs in the blood, led to reduce cardiac cfB level during sepsis. Together, our data indicate that extracellular miRNAs acts as an inducer for cfB via TLR7-MyD88 in sepsis.

Poster Number 104

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Assessment of renal function in kidney transplant recipients through therapeutic drug monitoring of vancomycin

Investigators: N. Elias, C. Varughese, T. Gift, S. Shao

Serum creatinine is often utilized as a marker for transplant success and a tool for assessing renal function. Many additional variables may influence the estimated glomerular filtration rate (GFR) in kidney transplant recipients. The most accurate method of assessing a kidney transplant recipient's renal function remains unclear. The purpose of this study is to assess kidney transplant recipients' renal function by assessing the therapeutic drug monitoring (TDM) of vancomycin to identify the optimal predictor of renal function in kidney transplant recipients. The institutional review board approved the study prior to initiating data collection. A retrospective chart review of kidney transplant patients between January 2011 and June 2014 who received vancomycin was performed. Donor type such as living donor, deceased donor, standard criteria donor (SCD), expanded criteria donor (ECD), donation after cardiac death and donation after brain death were determined for each recipient. Dosing of vancomycin received, dosing of calcineurin inhibitor (CNI) received, CNI serum levels at admission and vancomycin serum levels obtained were evaluated. Providers prescribed vancomycin using either a hospital-approved vancomycin nomogram, or dosing may have been selected by provider's preference. Vancomycin trough levels were collected based on the initial dosing regimen. Dose adjustment requirements to reach therapeutic troughs of 15-20 mcg/mL were evaluated. Here we show, renal function may be better in patients with an average tacrolimus trough level less than 5, as 78% of those patients required vancomycin dose increase, and those who received a transplant from a standard criteria donors, as 80% of those patients required

Poster Number 105

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Experience with an Extended Interval Once-Daily Dosing Tobramycin Program Used in Adult and Pediatric Patients with Cystic Fibrosis (CF) at MGH

Investigators: A. Alruwaili, C. Varughese, L. Yonker, K. Brazauskas, A. Levine, T. Arpino, S. Moskowitz

Background: Tobramycin is a widely used antimicrobial agent in the treatment of pulmonary exacerbations in patients with cystic fibrosis CF. Traditionally, Tobramycin is administered in thrice daily dosing; however, recently extended-interval once-daily dosing has proven to be as safe and efficacious. In 2010, MGH initiated tobramycin extended once daily dosing program for both adults and pediatric patients. However, the pharmacokinetic parameters and safety of this dosing regimen in patients with CF at our center remains unclear

Aims: Determine whether initial doses of 10 mg/kg or 12 mg/kg in adults and pediatrics achieved desired peak levels, estimate the proportion of patients with CF who needed dose adjustments to reach therapeutic target levels, using the standardized tobramycin dosing regimen and to assess renal safety of the standardized tobramycin dose in patients with CF

Methods: Retrospective chart review of all patients admitted to MGH with a diagnosis of CF from May 2010 to December 2013, who received intravenous tobramycin, a pharmacy aminoglycoside consult and aged 5 years. Exclusion criteria include patients with CrCl 20 mL per min, received concomitant inhaled tobramycin and patient who readmitted during the study period. Patient demographic data, serum creatinine at baseline and after tobramycin therapy, concomitant use of nephrotoxic drugs, extrapolated peak, AUC, half-life, and clearance were collected.

Conclusion: The majority of patients required doses higher than the 10 mg/kg, and 12 mg/kg to achieve target concentrations. Recommend using higher initial tobramycin dose for both adult and pediatric patients with CF and continue patient-specific pharmacokinetic monitoring.

Poster Number 106

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Bacterial quorum sensing excreted molecule promotes host tolerance via chromatin modification

Investigators: A. Bandyopadhaya, A. Tsurumi, D. Maura, K. Jeffery , L. G. Rahme

Bacterial quorum sensing (QS) signals are important mediators of immunomodulation. However, how microbes utilize immunomodulatory signals to maintain long-term persistent infection remain unclear. The opportunistic pathogen Pseudomonas aeruginosa (PA) QS regulated small volatile molecule 2-amino acetophenone (2-AA) has been proposed as a biomarker for PA colonization in chronically infected tissues. We have discovered that 2-AA modulates immune responses in a manner that allows bacterial persistence and enabling tolerance to infection. Since 2-AA produces immunomodulation through global control of cytokine expression, we hypothesized that it may employ epigenetic mechanisms.

We detected significant increases in bulk histone acetylation levels in 2-AA stimulated human monocytes compared to 2-AA–pretreated cells. Enrichment of H3K18ac, but not H3K9ac or H3K9me3, was observed specifically at the TNF-alpha promoter following 2-AA stimulation in non-pretreated cells and this enrichment was attenuated by 2-AA-pretreatment in human monocytes. The phosphorylation status of p65 was inhibited by 2-AA, and concomitantly, the protein-protein interaction between p65 and CBP/p300 was disrupted and conversely, the interaction between p50 and histone deacetylase (HDAC)1 was enhanced in 2-AA pretreated cells. We have shown that 2-AA's anti-immune influence is achieved by regulating HDAC1, resulting in H3K18 hypoacetylation at pro-inflammatory cytokine loci. HDAC1 inhibition counters the immunomodulatory effect of 2-AA.

These observations provide the first mechanistic example of a QS molecule that regulates the host epigenome to promote bacterial persistence and a solid understanding of the mechanisms will benefit the development of therapeutic interventions, especially given that epigenetic processes are reversible.

Poster Number 107

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Improving clinical outcomes in patients with methicillin-sensitive Staphylococcus aureus bacteremia and reported penicillin allergy

Investigators: K. G. Blumenthal, R. A. Parker, E. S. Shenoy, R. P. Walensky

Importance: Methicillin-sensitive Staphylococcus aureus (MSSA) bacteremia is a morbid infection. First-line MSSA therapies (nafcillin, oxacillin, cefazolin) are generally avoided in the 10% of patients reporting penicillin allergy, but most of these patients are not truly allergic.

Objective: To determine the optimal evaluation and treatment for patients with MSSA bacteremia and reported penicillin allergy.

Design: Decision tree with sensitivity analyses.

Participants: Simulated inpatients with MSSA bacteremia and reported penicillin allergy.

Interventions: Our model simulates three strategies: 1) no allergy evaluation; give vancomycin (Vanc); 2) allergy history-guided treatment; if history excludes anaphylactic features, give cefazolin (HX-Cefaz); and 3) complete allergy evaluation with history-appropriate penicillin skin testing, if skin test negative, give cefazolin (ST-Cefaz).

Main Outcomes: 12-week MSSA cure, recurrence and death; allergic reactions including major, minor, and potentially iatrogenic; adverse drug reactions (ADRs)

Results: Vanc results in the fewest patients achieving MSSA cure and the highest rate of recurrence: (Vanc 67.3%/14.8% vs. HX-Cefaz 83.4%/9.3% and ST-Cefaz 84.5%/8.9%) as well as the greatest frequency of allergic reactions (Vanc 3.0% vs. HX-Cefaz 2.4% and ST-Cefaz 1.7%) and highest rates of ADRs (Vanc 5.2% vs. HX-Cefaz 4.6% and ST-Cefaz 4.7%). Even in a "best case for Vanc" scenario, Vanc yields the poorest outcomes. ST-Cefaz is preferred to HX-Cefaz though sensitive to input variations.

Limitation: Parameter uncertainty limits definitive projection of the best allergy strategy.

Conclusions: Patients with MSSA bacteremia and a reported penicillin allergy should have the allergy addressed for optimal treatment. Full allergy evaluation with skin testing seems preferred although more data are needed.

Poster Number 108

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The Scaffold Protein IQGAP2 is Essential for the Innate Control of HCV Infection in Hepatoma Cells

Investigators: C. Brisac, S. Salloum, V. Yang, S. Chevaliez, E. A. Schaefer, J. Hong, N. Alatrakchi, A. Lidofsky, D. N. Fusco, L. F. Peng, W. Lin, R. T. Chung

Type I Interferon (IFN) provides fundamental cellular defense mechanisms and is licensed worldwide for the treatment of various viral infections, including Hepatitis C virus (HCV), malignant cancers and immune disorders. At the molecular level, it is now clear that JAK/STAT is not the only activated pathway and our recent unbiased genome-wide siRNA screen identified a hundred previously unreported genes essential for IFN antiviral activity against HCV in cell culture. Among those genes was the IQ-motif containing GTPase activating protein 2 (IQGAP2), a liver abundant scaffold protein recently described as a tumor suppressor protective against the development of several cancers, including hepatocellular carcinoma (HCC). Here we show that IQGAP2 regulates permissiveness to HCV in an IFN-dependent manner in vitro. IQGAP2 doesn't act through the JAK/STAT pathway but rather physically interacts with the RelA/p65 subunit of the NF-kB complex. Our data indicate that both IQGAP2 and RelA are essential for the full induction of a subset of IFN-Stimulated-Genes with anti-HCV properties in hepatoma cells.

Taken together, these data indicate that IQGAP2 is a novel IFN effector gene acting independently of the classical JAK/STAT pathway. Because IQGAP2 is predominantly expressed in the liver, this novel pathway may open new avenues for the treatment of hepatotropic pathogen infections.

Poster Number 109

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Anti-VEGF treatment normalizes tuberculosis granuloma vasculature and improves small molecule delivery

Investigators: M. Datta, L. E. Via, W. S. Kamoun, C. Liu, W. Chen, G. Seano, D. M. Weiner, D. Schimel, K. England, J. D. Martin, X. Gao, L. Xu, C. E. Barry, R. K. Jain

Tuberculosis (TB) causes almost 2 million deaths annually, and an increasing number of patients are resistant to existing therapies. TB patients require lengthy chemotherapy, possibly because of poor penetration of antibiotics into granulomas where the bacilli reside. Granulomas are morphologically similar to solid cancerous tumors in that they contain hypoxic microenvironments and can be highly fibrotic. Here we show that TB-infected rabbits have impaired small molecule distribution into these disease sites due to a functionally abnormal vasculature, with a low molecular weight tracer accumulating only in peripheral regions of granulomatous lesions. Granuloma-associated vessels are morphologically and spatially heterogeneous, with poor vessel pericyte coverage in both human and experimental rabbit TB granulomas. Moreover, we found enhanced vascular endothelial growth factor (VEGF) expression in both species. In tumors, anti-angiogenic, specifically anti-VEGF, treatments can "normalize" their vasculature, reducing hypoxia and creating a window-of-opportunity for conjunctive chemotherapy; thus, we investigated vessel normalization in rabbit TB granulomas. Treatment of TB-infected rabbits with the anti-VEGF antibody bevacizumab significantly decreased the total number of vessels while normalizing those that remained. As a result, hypoxic fractions of these granulomas were reduced and small molecule tracer delivery increased. These findings demonstrate that bevacizumab treatment promotes vascular normalization, improves small molecule delivery, and decreases hypoxia in TB granulomas, thereby providing a potential new avenue to improve delivery and efficacy of current treatment regimens.

Poster Number 110

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Plasma leptin levels in children hospitalized with cholera in Dhaka, Bangaldesh

Investigators: B. W. Falkard, T. Uddin, M. A. Rahman, M. F. Franke, A. Aktar, T. R. Bhuiyan, D. T. Leung, R. C. Charles, R. C. LaRocque, J. B. Harris, S. B. Calderwood, F. Qadri, E. T. Ryan

Cholera is caused by the noninvasive gram-negative bacterium, Vibrio cholerae, that causes diarrheal symptoms by inducing rapid dehydration in the small intestine. Leptin, a critical hormone in human metabolism, has more recently been shown to be important for developing adequate immune responses in healthy individuals and in response to infections. In particular, malnourished children with low leptin levels have reduced T-cell responses. We have studied the impact of naturally occurring variations in leptin on immune responses to V. cholerae. We determined serum leptin levels on day 2, 7 and 30 in Vibrio cholerae-O1 infected children living in Dhaka, Bangladesh and compared these levels with respect to matched healthy controls, as well as day 180 levels within the cholera patients themselves. We found that patients at the acute stage of cholera had significantly lower serum leptin levels than matched controls, and that these acute-phase leptin levels rose through convalescence. We also evaluated the association between leptin on day 2 and immunological responses that developed at later time points to cholera toxin B subunit (CtxB), a T-cell dependent antigen, and V. cholerae lipopolysaccharide (LPS), a T cell independent antigen. If considering their baseline nutritional status, we found a significant association of acute phase leptin levels to immune responses to CtxB. Our results suggest that leptin may play a role during cholera, especially in maturation of immune responses to T-cell dependent antigens and malnourished children, that have low leptin levels, may be impaired in their ability to develop such responses.

Poster Number 111

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Functional Impact of a long non-coding RNA on HIV control

Investigators: S. V. Kulkarni, A. Lied, V. Kulkarni, P. McLaren, M. N. Carrington

In the three decades of the HIV epidemic, it has become clear that HIV-1 can be well controlled by drugs, but not eliminated. Alternative strategies, which target host factors important in HIV infection and offer protection from or cure of HIV-1 infection, need to be developed. Genome-wide association studies (GWAS) in HIV infected patients have identified a multitude of host genetic determinants of HIV viral control and/or disease progression. However, the majority of the disease-associated SNPs identified are in non-coding regions and the functional mechanisms mediating these associations are still unknown. A single nucleotide polymorphism (SNP; rs1015164) in close genomic proximity of a long intergenic non-coding RNA (lincRNA) gene associated with HIV viral control and progression to AIDS in two distinct GWAS studies. The rs1015164 A allele, which associated with higher viral loads and more rapid disease progression, correlated with higher expression levels of the novel lincRNA, CCRL2-5′AS. Here we show that the lincRNA (CCRL2-5′AS) augments expression of an HIV co-receptor, CCR5. Thus, variation in the expression levels of CCRL2-5′AS may contribute to HIV viral control through enhanced CCR5 expression and greater susceptibility to HIV infection. These data are likely to result in the finding of yet undiscovered host factors important in HIV viral control. The lincRNAs based diagnostic and therapeutic modalities against cancer are being developed and some have showed promising results in human clinical trials. Our study is the first to dissect the role of a lincRNA in HIV pathogenesis and will likely identify new therapeutic targets.

Poster Number 112

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Inhibiting Fungal Multidrug Resistance by Disrupting an Activator-Mediator Interaction

Investigators: J. L. Nishikawa, A. Boeszoermenyi, L. A. Vale-Silva, D. Sanglard, M. Sanguinetti, G. Wagner, H. Arthanari, A. M. Naar

Eukaryotic transcription activators stimulate the expression of specific sets of target genes through recruitment of co-activators such as the RNA polymerase II-interacting Mediator complex. While aberrant function of transcription activators has been implicated in a number of diseases, therapeutic targeting efforts have been hampered by a lack of detailed molecular knowledge of the mechanisms of gene activation by disease-associated transcription activators. We previously identified an activator-targeted three-helix bundle KIX domain in the human MED15 Mediator subunit that is structurally conserved in Gal11/MED15 Mediator subunits in fungi. The Gal11 KIX domain is engaged by pleiotropic drug resistance transcription factor (Pdr1) orthologues, key regulators of the multidrug resistance (MDR) pathway in S. cerevisiae and the clinically important human pathogen Candida glabrata. Drug-resistant clinical isolates of C. glabrata most commonly harbour point mutations in Pdr1 that render it constitutively active, suggesting that this transcriptional activation pathway may represent a lynchpin in C. glabrata MDR. Facilitated by the NMR solution structure of the Cg Gal11A KIX domain we have carried out sequential biochemical and in vivo high-throughput screens to identify small molecule inhibitors of the interaction between the CgPdr1 transactivation domain and the Cg Gal11A KIX domain. The lead compound (iKIX1) represses Pdr1-dependent gene activation and re-sensitizes drug-resistant C. glabrata to azole therapy in vitro and in animal models for disseminated C. glabrata infection. We have demonstrated the feasibility of small molecule targeting of a transcription factor-binding site in Mediator as a novel therapeutic strategy in human diseases.

Poster Number 113

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Outcomes of concomitant fractures of the radial head and capitellum: The 'kissing lesion'

Investigators: F. M. Claessen, A. R. Kachooei, K. K. Verheij, G. P. Kolovich, C. S. Mudgal

Background/ Purpose: Radial head and capitellum fractures both result from an axial load onto an extended elbow with the forearm in pronation. Radial head compression against the capitellum in this position may cause concomitant fracture of the capitellum. A concomitant radial head and capitellum fracture is a rare event and only a few reports have been described. We have called this concomitant occurrence a 'kissing lesion'. It remains uncertain as to whether radial head fracture type is correlated with the presence of a kissing lesion. We aim to assess outcomes of patients with contiguous fractures of the radial head and capitellum.

Methods: Using data from 5 hospitals, we retrieved records of patients over 18 years of age who underwent treatment for capitellum fracture and radial head fracture between January 1993 and 2014. Patients with olecranon fractures or trochlea fractures were excluded.

Results: A total of 11 patients with a radial head fracture and a concomitant capitellum fracture were included. Based on the operative reports, 10 radial head fractures were classified as Hotchkiss Modification of the Mason Classification type II, and 1 was classified as type I.

Conclusion: Surgeons have to be alert to capitellar damage in case of a Hotchkiss type II radial head fracture. An additional computer tomography should be performed, and during operative treatment of the radial head, a careful inspection of the capitellum is recommended to avoid a missed diagnosis and subsequent complications.

Poster Number 114

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Association of Perioperative Allogeneic Blood Transfusion with Survival in Patients with Metastatic Long-Bone Fractures

Investigators: S. J. Janssen, Y. Braun, J. E. Ready, K. A. Raskin, S. A. Lozano-Calderon, M. L. Ferrone, F. J. Hornicek, J. H. Schwab

Background: Previous studies demonstrated a detrimental effect of perioperative blood transfusion on cancer recurrence and survival after resection of primary malignancies. The question arises whether this association also exists in patients with already disseminated disease undergoing surgery for metastatic long-bone fractures.

Purposes: Our primary null-hypothesis is that perioperative allogeneic blood transfusion is not associated with survival after operative treatment of long-bone metastatic fractures accounting for clinical, laboratory, and treatment factors.

Patients and Methods: We included 845 consecutive patients with 911 operatively treated metastatic long-bone fractures from two institutions in this retrospective study. Overall survival was compared between blood transfusion groups: "no transfusion", "1 to 2 units", "3 to 4 units", and "5 or more units" using log-rank analysis. We used Multivariable Cox regression analysis to account for clinical, laboratory, and treatment factors.

Results: Bivariate analysis showed a difference in survival among transfusion groups (P = 0.001). However, none of the perioperative allogeneic blood transfusion groups: "1 to 2 units" (hazard ratio [HR] 1.02, standard error [SE] 0.10, 95% confidence interval [CI]: 0.84 – 1.24, P = 0.84), "3 to 4 units" (HR 1.16, SE 0.15, 95% CI: 0.90 – 1.50, P = 0.26), and "5 or more units" (HR 1.29, SE 0.22, 95% CI: 0.93 – 1.81, P = 0.13), as compared to the "no transfusion" group were independently associated with survival.

Conclusion: With the numbers available we found no association of perioperative allogeneic blood transfusion with survival in patients operatively treated for metastatic long-bone fractures.

Poster Number 115

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Anesthesia Related Complications in Muscular Dystrophy and Autophagy Dysfunctions

Investigators: A. Kashiwagi, S. Hosokawa, R. Ueki, Y. Norimatsu, J. A. Martyn, S. Yasuhara

Duchenne Muscular Dystrophy (DMD) patients are affected by progressive muscle damage and receive orthopedic surgeries to correct the deformities caused by muscle mass loss (scoliosis and limb contracture). DMD patients are, however, at high risk for anesthesia-related complications including rhabdomyolysis, hyperkalemia, and sudden cardiac arrest. Doctors perform surgery to improve the patients' life but the patients die from anesthesia complications. This is a true tragedy and has to be solved, but the detailed mechanisms were totally unclear. Part of the technical difficulty derives from the unpredictable nature of anesthesia-induced complications in these patients.

Previous evidence has established that general anesthesia causes mitochondrial damage. Autophagy/mitophagy functions as a cytoprotection mechanism defending cells from many forms of stresses. No previous studies, however, have shown the clear link between anesthesia and autophagy/mitophagy in DMD. When autophagosomes are formed, they travel along the microtubule network to meet and fuse with lysosomes for maturation. The roles of microtubules network in the autophagy maturation, however, have remained unclear in DMD.

Here we show that using mdx mice, the murine equivalent of DMD, a highly reproducible model of anesthesia-induced hyperkalemia and rhabdomyolysis is established. In wild type mice, anesthesia per se induces autophagy/mitophagy with dynamic reorganization of microtubule network on which autophagosomes and lysosomes are mutually associated. In mdx mice, however, despite the normal induction of autophagy, microtubule network formation is defective and association of autophagosomes and lysosomes are compromised. This defect leads to perturbed maturation of autophagosomes and results in hyperkalemia and rhabdomyolysis.

Poster Number 116

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Numerical surgery and finite element analysis of sagittal split ramus osteotomy

Investigators: Z. Liu, Y. Qian, Y. Zhang, Y. Fan

Many surgeons preferentially used sagittal split ramus osteotomy (SSRO) to correct the mandibular deformities, but sometimes postoperative temporomandibular joint disorder (TMD) occurred. The problems might be associated with the changes of stress distributions in the temporomandibular joint (TMJ).

A three-dimensional finite element model of the mandible and articular fossa-eminence complexes was established based on the preoperative CT of a patient with mandibular prognathism. The articular discs and the cartilages were constructed based on the anatomical structures. The numerical surgery was simulated according to the actual surgery plans of SSRO. Then the model of postoperative 6 months was established. Contact elements were used to simulate the interaction among the discs and articular cartilages. Nonlinear cable elements were used to simulate the disc attachments and the ligaments. Muscle forces and boundary conditions corresponding to the central occlusion were applied on the two models.

The results showed that the intermediate zone of the disc in the preoperative model mainly sustained tensile stress, but compressive stresses were dominant in the anterior and posterior band of the discThe maximum stress of the disc was located at the intermediate zone in the postoperative model. The postoperative model had the lower maximum stress than the preoperative model. The maximum tensile stresses in the intermediate zone of the discs in the postoperative and preoperative models were 0.475 MPa and 1.067 MPa, respectively. Some TMJ disorders were improved after the SSRO, and appropriate functional training can help to reduce or even avoid postoperative TMDs.

Poster Number 117
ePoster#19

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Factors Associated with Reoperation after Fixation of Displaced Olecranon Fractures

Investigators: F. M. Claessen, Y. Braun, R. Peters, J. N. Doornberg, G. Dyer, D. C. Ring

Background: Olecranon fractures are prone to reoperation, mostly for implant removal.

Hypothesis: We hypothesized that there are no factors associated with reoperation after open reduction and internal fixation of a fracture of the olecranon.

Methods: Three hundred and ninety two adult patients that had operative treatment of a displaced olecranon fracture not associated with other fractures, dislocation, or subluxation at 2 area hospitals between January 2002 and May 2014 were analyzed to determine factors associated with reoperation.

Results: Ninety-nine of 392 fractures (25%) had a second operation, 92 (93%) at least in part for implant removal (12 for wire migration [3% of all fractures, 12% of reoperations]). In multivariable analysis younger age and women were significantly associated with reoperation and implant removal, but technical factors were not.

Conclusions: Reoperation after olecranon fracture fixation seems to relate more to preferences than to adverse events. Implants might be more prominent in younger women or perhaps they have a stronger preference for implant removal no matter the prominence.

Level of Evidence Level IV, retrospective study

Poster Number 118

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The prognostic significance of a pathologic fracture in patients with osteosarcoma

Investigators: O. D. Palthe, T. A. Meijs, S. J. Janssen, J. H. Schwab, S. A. Lozano-Calderon

Over the last years limb salvage has earned a more prominent role in the local treatment of osteosarcoma. Limb-sparing surgery results in a better function of the limb compared to amputation, while the oncological outcomes are similar. Therefore the current standard treatment of osteosarcoma is wide resection in combination with neoadjuvant and adjuvant chemotherapy. Approximately 5% to 10% of patients with an osteosarcoma sustain a pathologic fracture, either at presentation or during neoadjuvant chemotherapy. The presence of a pathologic fracture in patients with osteosarcoma is thought to be one of the factors associated with a poor prognosis in patients with osteosarcoma and considered a possible contraindication for limb salvage.

The purpose of this study was to determine the prognostic value of a pathologic fracture on survival, local recurrence, and metastasis in patients with osteosarcoma and to assess whether limb salvage can be safely performed in patients with pathologic fracture.

The study cohort consisted of 42 patients with pathologic fracture and 179 patients without pathologic fracture. Overall survival in the pathologic fracture group was significantly lower than in the non-fracture group

Limb salvage was not associated with a difference in survival or local recurrence rate compared to amputation in patients with pathologic fracture.

Pathologic fracture was associated with worse overall survival and a higher rate of metastasis in patients with osteosarcoma, but had no influence on local recurrence. It seems that limb salvage can be safely performed in patients with pathologic fracture if other prognostic characteristics are favorable.

Poster Number 119

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A new therapy for itch, and possibly inflammation and drug reactions

Investigators: E. Azimi, V. B. Reddy, E. A. Lerner

Background: Substance P (SP) is a neuropeptide and key mediator of histamine-independent itch. NK1 is the classical receptor for SP but NK1 antagonists are ineffective for treating itch and inflammation. Mrgprs, a family of receptors expressed on mast cells and dorsal root ganglia, are pivotal for histamine-independent itch and drug reactions. We asked if SP activates Mrgprs and if blocking Mrgprs would prevent SP-induced scratching.

Methods: Itch induced by SP was compared between NK1 knock out (KO) and Mrgpr KO mice. The interaction between SP and mouse Mrgprs was studied in cultured dorsal root ganglion neurons (DRGs) from NK1 KO mice. The interaction between SP and human Mrgprs was studied by heterologous expression in HeLa cells.

Results: Itch was evoked by SP in NK1 KO mice but not in Mrgpr KO mice. SP activated mouse MrgprA1 and human-MRGPRX2. We identified a tripeptide inhibitor of these Mrgprs. The tripeptide blocked activation of NK1 KO DRGs and itch in wild type mice. In contrast, NK1 specific antagonists had no significant effect on itch.

Conclusion: SP and Mrgprs are critical to itch, drug reactions and neurogenic inflammation. We show that SP is an endogenous ligand for human-MRGPRX2. Human-MRGPRX2 is expressed on DRGs, keratinocytes, and mast cells and transmits itch and orchestrates the crosstalk between the skin, nervous and immune systems. The tripeptide that we have identified, or a derivative, has the potential to be a specific treatment for itch, drug reactions and conditions associated with neurogenic inflammation such as eczema and asthma.

Poster Number 120
ePoster#20

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The anosmia - cilia connection: development of a new sensory cilia assay using genetically encoded calcium biosensors in zebrafish

Investigators: J. G. Bergboer, C. Wyatt, C. Austin-Tse, E. Yaksi, I. A. Drummond

Ciliopathies represent a range of complex human syndromes, all having mutations leading to abnormally formed and/or dysfunctional cilia. We aimed to devise an in vivo, zebrafish-based method to measure sensory cilia function. We studied responses of olfactory sensory neurons (OSNs) present in the olfactory epithelium after stimulation with different odorants. In the zebrafish olfactory epithelium four types of OSNs are present, one of these is ciliated. The ciliated OSNs respond to bile acids. To measure OSN activity we used the genetically encoded fluorescent calcium biosensor GCaMP5 under the general neuronal HuC promoter. We used the zebrafish ift88 cilia mutant to study the effect of cilia malformation on olfactory functioning. Ift88 is crucial during cilia formation and maintenance. We tested the responses of wild-type Tg(HuC:GCaMP5), ift88 mutants and siblings (N=10 and 9 fish) to different odorant stimuli. Our results show that in 2.5 day old embryos, OSNs show robust and reproducible calcium signals upon odorant stimulation.

In ift88 cilia mutants the OSN response towards bile acids, sensed via ciliated OSNs, was significantly reduced to 62% compared to their siblings, while the response to amino acids, sensed via non-ciliated OSNs, did not change. Similar results were obtained using morpholino induced knock-down of another cilia gene: ift172.

In conclusion, we developed a novel method to study sensory cilia function in zebrafish. This method will be used to further explore mechanisms of sensory cilia function in vertebrate neurons and will provide a platform for in vivo screens for therapeutic compounds to treat ciliopathies.

Poster Number 121

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Dysregulation of cytokines mediated painful diabetic neuropathy

Investigators: B. M. Yanik, J. R. Dauch, H. T. Cheng

Patients with diabetic neuropathy from type 2 diabetes report a significantly decreased quality of life caused by painful diabetic neuropathy (PDN). Our goal is to study the roles of cytokine-mediated inflammation in PDN to establish a novel treatment. Previously, we established that db/db mouse is a mouse model of PDN with the development of mechanical allodynia at 8-12 wk of age. To study the regulation of pro-inflammatory and anti-inflammatory cytokines in PDN, we first studied the cytokine expression profile in lumbar dorsal root ganglion (LDRG) neurons during the period of mechanical allodynia. We detected reduced expression of interleukin (IL)-10, an anti-inflammatory cytokine and elevated tumor necrosis factor (TNF)-alpha, a pro-inflammatory cytokine during the period of PDN in db/db mice. To determine if the reduced IL-10 expression mediates the mechanical allodynia in db/db mice, we administered IL-10 (1 g/kg), an anti-inflammatory cytokine or saline (control) intraperitoneally to the control db/+ and db/db mice starting at 8 wk of age. IL-10 treatment was repeated every other day for 2 wk. IL-10 treatment significantly reduced mechanical allodynia and thermal hyperalgesia in db/db mice at 10 wk of age. In addition, IL-10 treatment inhibits the upregulation of TNF-alpha and nerve growth factor in LDRG. These results suggest that dysregulation of cytokine-mediated inflammation contributes to the development PDN in db/db mice. Treatments that target this mechanism could be effective for treating PDN.

Poster Number 122

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Associations between white matter lesion microstructure and imaging markers of Alzheimer's disease

Investigators: J. P. Coutu, A. E. Golblatt, D. S. Salat

White matter lesions, or leukoaraiosis, are typically identified in vivo as white matter signal abnormalities (WMSA) on magnetic resonance imaging. WMSA are apparent in up to 95% of adults older than 60 years old and are associated with small vessel disease and several vascular risk factors. While WMSA volume has been shown to be increased in Alzheimer's disease (AD), limited evidence exists to demonstrate that the WMSA present in AD are similar in nature to those observed in non-demented older individuals. Additionally, little is known about how this typically vascular-associated tissue damage relates to more classical imaging markers of AD pathology such as cortical and hippocampal atrophy which could provide important information about the role of this tissue damage in the neurodegenerative pathology of AD. We used a large publicly-available dataset from the Alzheimer's Disease Neuroimaging Initiative which included 74 controls, 97 participants with MCI and 48 participants with AD with T1-weighted and diffusion-weighted imaging datasets. Associations were investigated between diffusion tensor imaging measures and the following imaging markers of AD: total WMSA volume, total white matter volume, ventricular volume, hippocampal volume and the average cortical thickness of regions most affected in early AD. We find that while regions like the parahippocampal white matter are indeed related to markers of cortical degeneration such as hippocampal volume and cortical thickness, WMSA tissue properties are unrelated to this phenomenon and are instead associated with mechanisms related to ventricular enlargement and total WMSA volume.

Poster Number 123

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Massively Expedited Genome-wide Heritability Analysis (MEGHA)

Investigators: T. Ge, T. E. Nichols, P. H. Lee, A. J. Holmes, J. L. Roffman, R. L. Buckner, M. R. Sabuncu, J. W. Smoller

The discovery and prioritization of heritable phenotypes is a computational challenge in a variety of settings, including neuroimaging genetics and analyses of the vast phenotypic repositories in electronic health record systems and population-based biobanks. Classical estimates of heritability require twin or pedigree data, which can be costly and difficult to acquire. Genome-wide Complex Trait Analysis (GCTA) is an alternative tool to compute heritability estimates from unrelated individuals, using genome-wide data that is increasingly ubiquitous, but is computationally demanding and becomes difficult to apply in evaluating very large numbers of phenotypes. Here we present a novel, fast and accurate statistical method for high-dimensional heritability analysis using genome-wide single nucleotide polymorphism (SNP) data from unrelated individuals, termed Massively Expedited Genome-wide Heritability Analysis (MEGHA), and accompanying nonparametric sampling techniques that enable flexible inferences for arbitrary statistics of interest. MEGHA produces estimates and significance measures of heritability with several orders of magnitude less computational time than existing methods, making heritability-based prioritization of millions of phenotypes based on data from unrelated individuals tractable for the first time. As a demonstration of application, we conducted heritability analyses on global and local morphometric measurements derived from brain structural magnetic resonance imaging (MRI) scans, using genome-wide SNP data from 1,320 unrelated young healthy adults of non-Hispanic European ancestry. We also computed surface maps of heritability for cortical thickness measures and empirically localized cortical regions where thickness measures were significantly heritable. Our analyses demonstrate the unique capability of MEGHA for large-scale heritability-based screening and high-dimensional heritability profile construction.

Poster Number 124

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MicroNeurotrophins as potential therapeutics for the treatment of Amyotrophic Lateral Sclerosis

Investigators: K. E. Glajch, K. A. Mueller, N. A. Rauf, L. G. Rycyna, C. R. Vanderburg, M. M. Maxwell, A. Gravanis, G. Sadri-Vakili

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder caused by loss of motor neurons. ALS patients experience rapid deterioration in muscle function with an average lifespan of 3-5 years after diagnosis. Currently, the most effective therapeutic for the treatment of ALS only extends lifespan by a few months. Neurotrophic factors (NTFs) are a group of molecules involved in neuronal development, maintenance, and survival. NTF treatment has previously shown efficacy in pre-clinical ALS models. However, clinical trials using NTFs produced no major improvements in ALS patients, due in part to the limited penetration of NTFs across the blood brain barrier (BBB). Microneurotrophins are small neurotrophic-like compounds that cross the BBB and bind to tyrosine kinase receptors mimicking the pro-survival effects of endogenous neurotrophic factors. In this study, we sought to determine the potential therapeutic efficacy of two microneurotrophins, BNN23 and BNN27, in a mouse model of ALS expressing the G93A mutation in the superoxide dismutase (SOD1) gene. Our results demonstrate that administration of BNN23 and BNN27 in mutant SOD1 mice resulted in trends towards the improvement of disease phenotypes and delayed disease progression relative to vehicle treatment. In particular, BNN27 demonstrated consistent trends in both male and female mutant SOD1 mice. Currently we are planning a follow-up pre-clinical study to further determine the therapeutic effects of BNN27 in SOD1 mice using a larger cohort.

Poster Number 125
ePoster#21

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A novel patient-adjustable reconfigurable MRI head-coil technology for enhanced imaging of patients with brain implants

Investigators: L. Golestani Rad, B. Keil, G. Bonmassar, L. L. Wald

Deep brain stimulation (DBS) is an FDA approved neurostimulation procedure that has emerged as the gold-standard treatment for a variety of highly disabling drug-resistant neurological and psychiatric disorders. Despite the general effectiveness of DBS, its underlying mechanisms of action are still unclear. Uncertainties remain about which circuits are affected, which exact fiber bundles need to be targeted and the most efficacious stimulation protocol. In this regard, magnetic resonance imaging (MRI) appears excellently poised as a high-resolution, non-invasive imaging tool which could help address these open questions. However, the interaction of the radiofrequency (RF) fields of MRI scanners and the implanted electrodes imposes serious safety hazards that restrict the post-operative applicability of MRI for DBS patients. We are developing novel MRI methodologies tailored and validated for each patient-specific geometry, which will bring MRI to bear on the clinical questions regarding the mechanism and targeting of DBS treatment. Specifically we (1) develop and validate reconfigurable MRI transmit technologies that reduce the unwanted interaction of RF fields and implanted electrodes up to 100 fold below levels produced by available systems, while increasing the signal-to-noise ratio (SNR) up to 5 times at the level of cortical structures, and (2) validate these methodologies with comprehensive electromagnetic simulations and phantom experiments to determine the safe range of imaging parameters and optimize clinical imaging protocols. Our work will enable clinicians for the first time to safely acquire high-resolution functional and structural images of patients with DBS implants and obtain useful information about function of affected brain networks.

Poster Number 126

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Superior time perception for lower musical pitch explains why bass-ranged instruments lay down musical rhythms

Investigators: M. J. Hove, C. Marie, I. C. Bruce, L. J. Trainor

The auditory environment typically contains several sound sources that overlap in time, and the auditory system parses the complex sound wave into streams or voices that represent the various sound sources. Music is also often polyphonic. Interestingly, the main melody (spectral/pitch information) is most often carried by the highest-pitched voice, and the rhythm (temporal foundation) is most often laid down by the lowest-pitched voice. Previous work using electroencephalography (EEG) demonstrated that the auditory cortex encodes pitch more robustly in the higher of two simultaneous tones or melodies, and modeling work indicated that this high-voice superiority for pitch originates in the sensory periphery. Here, we investigated the neural basis of carrying rhythmic timing information in lower-pitched voices. We pre- sented simultaneous high-pitched and low-pitched tones in an isochronous stream and occasionally presented either the higher or the lower tone 50 ms earlier than expected, while leaving the other tone at the expected time. EEG recordings revealed that mismatch negativity responses were larger for timing deviants of the lower tones, indicating better timing encoding for lower- pitched compared with higher-pitch tones at the level of auditory cortex. A behavioral motor task revealed that tapping synchroni- zation was more influenced by the lower-pitched stream. Results from a biologically plausible model of the auditory periphery suggest that nonlinear cochlear dynamics contribute to the observed effect. The low-voice superiority effect for encoding timing explains the widespread musical practice of carrying rhythm in bass-ranged instruments and complements previously established high-voice superiority effects for pitch and melody.

Poster Number 127

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HDAC6 Inhibitors Modulate Lys49 Acetylation and Membrane Localization of β-Catenin in Human iPSC-Derived Neuronal Cells

Investigators: J. Iaconelli , J. H. Huang , S. S. Berkovitch, S. Chattopadhyay , R. Mazitschek, S. L. Schreiber , S. J. Haggarty , R. Karmacharya

We examined the effects of isoform-specific histone deacetylase (HDAC) inhibitors on β-catenin posttranslational modifications in neural progenitor cells (NPCs) derived from human induced pluripotent stem cells (iPSCs). β-catenin is a multifunctional protein with important roles in the developing and adult central nervous system. Activation of the Wnt pathway results in stabilization and nuclear translocation of β-catenin, resulting in activation of multiple target genes. In addition, β-catenin forms a complex with cadherins at the plasma membrane as part of the adherens junctions. The N-terminus of β-catenin has phosphorylation, ubiquitination, and acetylation sites that regulate its stability and signaling. In the absence of a Wnt signal, Ser33, Ser37, and Thr41 are constitutively phosphorylated by glycogen synthase kinase 3β (GSK3β). β-Catenin phosphorylated at these sites is recognized by β-transducin repeat-containing protein (βTrCP), which results in ubiquitination and degradation by the ubiquitin-proteasome pathway. The N-terminal regulatory domain of β-catenin also includes Ser45, a phosphorylation site for Casein Kinase 1α (CK1α) and Lys49, which is acetylated by the acetyltransferase p300/CBP-associated factor (PCAF). The relevance of Lys49 acetylation and Ser45 phosphorylation to the function of β-catenin is an active area of investigation. We find that HDAC6 inhibitors increase Lys49 acetylation and Ser45 phosphorylation but do not affect Ser33, Ser37, and Thr41 phosphorylation. Lys49 acetylation results in decreased ubiquitination of β-catenin in the presence of proteasome inhibition. While increased Lys49 acetylation does not affect total levels of β-catenin, it results in increased membrane localization of β-catenin.

Poster Number 128

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Temporal properties of retinal ganglion cell responses to repetitive stimulation

Investigators: M. Im, S. I. Fried

Recent clinical reports indicate that stimulation rates in the range of 5-7 Hz are most preferable to users of retinal prosthetics. This is surprising given that previous in-vitro studies suggest that such rates have a diminishing effect on the response to electric stimulation in retinal ganglion cells (RGCs) and suggests that the responses to repetitive stimulation may be heterogeneous across different types of RGCs. If so, the possibility exists that some types of RGCs may contribute more to psychophysical percepts than others and a more systematic investigation into the responses to repetitive stimulation might help to explain specific clinical observations. We measured the electrically-evoked responses of RGCs in the isolated rabbit retina as a function of stimulation rate and explored the differences across cell types.

We found the electric responses of RGCs to repetitive stimuli have clear distinctions between ON and OFF types. The responses of all OFF cells (n=9/9) showed the reduced sensitivity to subsequent stimuli. In contrast, the responses of all ON cells (n=8/8) exhibited a novel 'reset' behavior that has not been described previously; the new stimulus suppressed all pending responses to any previous stimuli and initiated its own response that is highly similar to the response from a single stimulus in isolation. Also, theses contrasts between ON and OFF cells created different temporal properties of electrically-elicited responses to repetitive stimuli in various stimulation rates.

Our results reveal the fundamental differences between responses in ON and OFF types of RGCs, offering insights to psychophysical results.

Poster Number 129

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Deciphering the role of site specific phosphorylation of huntingtin protein in Huntington's Disease

Investigators: A. S. Iyengar, R. Vijayvargia, R. Jung, B. Shin, M. E. MacDonald, I. Seong

HD is a dominantly inherited brain disorder characterized by progressive deterioration of motor and cognitive functions caused due to the abnormal expansion of the CAG trinucleotide repeat region in Exon 1 of the HTT gene. Although numerous studies focus on the N-terminal fragment associated toxicity of the protein, much is not known of the full length protein function and structure. Post-translational modifications, including phosphorylation, plays an important role in the biological regulation of proteins and recent studies have suggested that phosphorylation plays a critical part in regulating protein-protein interaction as well as sub-cellular localization of huntingtin protein. Preliminary evidence utilizing purified phospho-mutant htt proteins and anti-phospho htt antibodies generated in the lab suggests that phosphorylation at ser434, 1181, 1201 and 2116 have interdependency or 'cross talk' which suggests a functional role to modifications at these sites. Moreover, immunofluorescence and immunoblot results strongly indicate differences in the pattern of localization and expression between these sites in human neural progenitor cells (hNPCs). Interestingly, when studied in hNPCs, phosphorylation of htt is significantly enhanced in actively dividing cells compared to cells in the resting state suggesting that phosphorylation of htt might be involved in cell division. Previous studies augmented by our observations also point towards a possible relationship between htt phosphorylation and its interaction with microtubules in dividing cells. Our systematic biochemical approach at deciphering the structural and functional role of phosphorylation of htt will enhance our understanding of the contribution of protein modifications to HD pathogenesis.

Poster Number 130

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Aberrant growth of sensory neurons expressing mutant SPT is mediated by phosphorylation of ERM proteins by p38 MAPK: Implications for Hereditary Sensory Neuropathy Type 1

Investigators: B. Jun, A. Chandra, B. P. Schmidt, F. S. Eichler

The enzyme serine palmitoyltransferase (SPT), which is responsible for the de novo synthesis of sphingolipids, has been shown to be mutated in patients with hereditary sensory neuropathy type 1 (HSN-1). Mutant SPT exhibit enzymatic promiscuity, leading to the incorporation of L-alanine over its canonical substrate, L-serine. While this atypical metabolic activity of mutant SPT is thought to cause HSN-1, little is understood about its specific downstream effects on neuronal development and growth. Here, we characterized sensory neurons of the dorsal root ganglia isolated from transgenic SPTLC1C133W mice expressing mutant SPT. Mutant neurons exhibited an increase in growth and branching compared to wild-type, which was rescued after treatment with L-serine. Interestingly, the aberrant morphology observed in the mutant neurons occurred in conjunction with increased expression of cytoskeletal scaffolding proteins at the neural growth cone, namely, the phosphorylated-ERM proteins (ezrin/radixin/moesin). Subsequent analyses of mutant neurons detected an increase in the expression and activation of p38 mitogen-activated protein kinases (p38 MAPK). Of note, the p38 MAPK pathway is responsible for mediating responses to cellular stress such as inflammation or injury. Our present data suggest that mutant SPT may induce increases in expression of p38 MAPK and pERM, leading to altered growth of sensory neurons during initial development. Collectively, these findings help elucidate possible early pathological mechanisms of HSN-1 and identify the p38 MAPK signaling cascade as a potential therapeutic target.

Poster Number 131

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Optogenetic rescue of disrupted slow oscillations in an animal model of Alzheimers disease

Investigators: K. V. Kastanenka, N. Shakerdge, S. Hou, S. Wegmann, B. J. Bacskai

Slow oscillations are important for consolidation of memory during sleep, and Alzheimers Disease (AD) patients experience both sleep and memory disturbances. Thus, we examined slow oscillation activity in an animal model of AD, APP/PS1 mice, using voltage sensitive dye Rh 1691 through cranial windows. APP/PS1 mice exhibit aberrant slow oscillation activity prior to plaque deposition, with a cohort of mice lacking slow oscillations altogether. Abberant excitatory activity within the cortical circuit was responsible for slow oscillation dysfunction, since topical application of GABA restored slow oscillations in APP/PS1 mice. In addition, month-long treatment with light activation of Channelrhodopsin-2 (ChR2) expressed in excitatory cortical neurons restored slow oscillations by synchronizing neuronal activity and facilitating state transitions. Driving slow oscillation activity at the normal frequency with ChR2 halted amyloid plaque deposition and prevented calcium overload associated with this pathology. Thus, targeting slow oscillatory activity in AD patients might prevent neurodegenerative phenotypes and slow disease progression.

Poster Number 132

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The development of novel Huntington's disease knock-in mice to study the effect of CAG repeat interruption on disease phenotype

Investigators: M. V. Kovalenko, J. StClaire, R. Mouro Pinto, J. L. Neto, B. Lager, G. Flynn, J. Beltran, M. Kwan, A. Ghavami, M. Mazzela, L. Menalled, D. Brunner, D. G. Monckton, D. Fischer, S. Kwak, D. Howland, V. C. Wheeler

The expanded CAG repeat in the Huntington's disease (HD) gene HTT is the major contributor to disease onset and severity. The HTT CAG repeat expands somatically in a time-dependent and cell-type/tissue-dependent manner, with medium-spiny neurons of the striatum exhibiting particularly dramatic expansions. Genetic knockout studies in HTT CAG knock-in mice have demonstrated that genes in the mismatch repair pathway are absolutely required for somatic HTT CAG expansion and that the same genes enhance the HD pathogenic process. These findings strongly suggest that somatic expansion itself is a disease modifier, with important implications for the development of novel disease-modifying therapies.

Here, we have developed a novel series of HD knock-in mice with the purpose of gaining insight into the role of somatic HTT CAG expansion on phenotypic expression independent of the effects of mismatch repair gene knockouts. The mice harbor either pure CAG tracts (HttCAG45, HttCAG78, HttCAG104) or CAG tracts interrupted with CAA residues (Htt[CAGCAACAGCAACAA]9, Htt[CAGCAACAGCAACAA]16, Htt[CAGCAACAGCAACAA]21. The pure CAG knock-in mice exhibit somatic expansion of the CAG repeat that increases with increasing mouse age and constitutive CAG repeat length and occurs in a tissue-specific manner. In contrast, the [CAGCAACAGCAACAA] repeat configuration results in complete repeat stabilization in all tissues tested. RNA and protein expression analyses indicate that full-length mutant huntingtin is expressed from all of the alleles. Histological and behavioral phenotyping of the mice is ongoing. Together, we expect that integrated molecular and phenotypic data from these mice will provide new insight into the role of somatic expansion in HD.

Poster Number 133

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Magnetic Stimulation of PFC pyramidal neurons using micro-coils

Investigators: S. Lee, S. I. Fried

Transcranial magnetic stimulation (TMS) uses a time-varying magnetic field to non-invasively modulate neural activity of the brain. Both TMS and repetitive TMS (rTMS) are used to investigate neuronal pathways as well as to treat neurological disorders. Ongoing advancements with these techniques have been hindered by a lack of understanding of the mechanisms underlying neuronal activation and the limitations associated with small animal models. Here we show that micro-coils can be used to probe the responses of cortical pyramidal neurons in the mouse brain in vitro. In contrast to much previous work indicating that the proximal axon is the target of TMS, we found that the apical dendrite was also highly sensitive to magnetic stimulation. We also found that excitability was strongly influenced by coil orientation. Altering the size and location of the coil allowed the sensitivity of targeted neurons to be probed, e.g. thresholds of the axon, soma and apical dendrite could all be determined (both field strength and field-gradient strength). Because small coils target only small volumes of neuronal tissue, it is likely that a coil-based implant would provide more precise control of cortical responses than conventional electrodes.

Poster Number 134
ePoster#22

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Functional re-wiring of neural circuits in post-traumatic epileptogenesis

Investigators: K. P. Lillis, Z. Wang, K. J. Staley

In secondary epilepsy, a seizure-prone neural circuit forms following an insult to the brain (e.g. traumatic brain injury). The nature of both the epileptogenic changes that take place during this "latent period" and the continuing evolution that occurs following the onset of seizures remain relatively unknown. To elucidate the nature of re-wiring following injury, we use hippocampal slice cultures which, within a week after the "injury" of slicing, sprout to form new synapses and spontaneously develop seizures. We have developed a custom imaging apparatus that allows us to continuously monitor activity in the slice as it develops first interictal bursts, then seizures. Using genetically encoded calcium indicators, which fluoresce more brightly when a neuron fires, we track changes in spiking patterns in thousands of individual neurons for up to four weeks. This combination of tools provides us with long-term time lapse movies of anatomical changes (e.g. cell death) and periodic high speed movies of network activity. We are using this voluminous data to identify epileptogenic changes in functional network architecture. We first determine significantly correlated neurons by computing the cross-correlation between all pairs of calcium traces. We then compute betweenness centrality, mean path length, and degree distribution to classify networks as random, uniform, or scale-free. We are extending these findings, using genetic targeting of fluorescent proteins, to identify cell-type specific changes in functional connectivity and homeostatic regulation. Preliminary data indicate that, following injury, functional connectivity transitions from sparse, to scale-free, and finally to uniform following the onset of seizures.

Poster Number 135

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Neuroimaging and Psychophysiological Responses in Fear Extinction Recall: Do Anxiety Disorders Differ From Post-Traumatic Stress Disorder?

Investigators: M. F. Marin, H. Song, A. J. Landau, B. L. Rosenbaum, R. G. Zsido, P. L. Rosencrans, S. C. Divatia, E. F. Pace-Schott, W. D. Kilgore, S. P. Orr, R. K. Pitman, N. M. Simon, M. R. Milad

Individuals diagnosed with post-traumatic stress disorder (PTSD) exhibit impaired fear extinction recall . This behavioral deficit has been linked to hypoactivations of the ventromedial prefrontal cortex (vmPFC) and the hippocampus, along with hyperactivations of the dorsal anterior cingulate cortex (dACC) and the amygdala. At this point, it is unknown whether these behavioral and neural activations patterns are specific to PTSD or also characterize other anxiety disorders. Fifty-two participants went through a clinical diagnosis interview where they were categorized based on their main diagnosis (17 social anxiety disorder (SAD), 20 generalized anxiety disorder (GAD) and 15 specific phobia (SP)). They were all exposed to a 2-day fear conditioning and extinction paradigm in an fMRI setting. Data were compared to previous cohorts of healthy controls (HC, n=18) and PTSD (n=18). At a psychophysiological level, no group differences were observed. At a neuroimaging level, both GAD and SAD groups exhibited less dACC, vmPFC and hippocampal activations compared to HC. SP had significantly less dACC activation than HC. Relative to PTSD, SAD showed a similar pattern of brain activations whereas GAD showed lower amygdala and dACC activations. SP showed higher vmPFC, but lower amygdala activations, relative to PTSD. These preliminary results show that although not apparent at the psychophysiological level, the neuroimaging results during extinction recall reveal similarities and differences in various anxiety disorders relative to PTSD and HC. SA, and GAD to a lesser extent, share similar signatures than PTSD whereas the SP exhibits neural patterns that are closer to HC.

Poster Number 136
ePoster#23

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Intergenerational instability in Huntington's disease: insights from mouse models

Investigators: J. L. Neto, V. C. Wheeler, R. Mouro-Pinto

Huntington's disease (HD) is a neurodegenerative disorder caused by the expansion of a CAG trinucleotide repeat in the HTT gene. The size of this trinucleotide expansion is extremely important as it affects disease penetrance and age-of-onset. In humans, repeat sizes up to 35 CAGs are present in non-affected individuals, while alleles in the 36-39 range are associated with reduced disease penetrance, and repeats over 39 CAGs are fully penetrant. Repeat instability is present in patients and mouse models of the disease, and is observed both intergenerationally and somatically.

Our laboratory has collected over a decade's worth of intergenerational transmissions of the Htt CAG repeat in genetically precise HD knock-in models, over a broad range of CAG sizes and on six different mouse background strains. These data are currently being analyzed in order to confirm and further disentangle several aspects of trinucleotide instability in intergenerational transmissions, namely: the effect of parental CAG allele size, the perceptible parent-of-origin effect, and the effect of different genetic backgrounds/strains.

Preliminary analyses have shown an obvious allele size effect on intergenerational instability, with small alleles (18 CAGs) exhibiting virtually no instability, while very long alleles (>99 CAGs) result in over 80% unstable transmissions. A parent-of-origin effect is also observed with unstable maternal transmissions trending toward contraction events while paternal ones are expansion-biased. Additionally, the ratio of unstable:stable transmissions seems to differ when comparing different congenic strains, suggesting that genetic variants may influence intergenerational repeat instability.

Poster Number 137

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A functional role for BDNF in Familial Dysautonomia

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Familial Dysautonomia (FD) is an autosomal recessive disorder with extensive sensory and autonomic nervous system involvement present at birth. Neuropathological studies show a marked reduction of neurons in the sympathetic and sensory ganglia. All FD patients have an intronic splice site mutation in the IKBKAP gene, the scaffolding member of the Elongator protein complex involved in transcriptional elongation. This mutation results in tissue-specific skipping of exon 20 in the mRNA with aberrant splicing most pronounced in neuronal tissues. The alternative splicing defect leads to reduced production of normal IKAP protein in FD patients. Neurotrophic factors are implicated in the survival and differentiation of several neuronal populations. Previous in vitro studies have indicated reduced neurotrophic activity in human fibroblasts from FD patients and defective expression of genes encoding neurotrophic factors in mice leads to similar neuronal phenotypes to those observed in FD patients. Here we show that reduced IKAP expression impacts brain-derived neurotrophic factor (BDNF) transcription in FD patient fibroblast cells, and we demonstrate a functional consequence of BDNF misregulation using our induced pluripotent stem (iPS) cell model system of human neural development. We found insufficient neurotrophic support from FD cells on the development of human iPS cell-derived neurons. Interestingly, the reduced biological activity from FD patient cells was rescued pharmacologically using the kinetin, which corrected aberrant mRNA splicing of IKBKAP. Our data suggests that aberrant regulation of BDNF due to IKAP reduction may contribute to the developmental defects in neural development observed

Poster Number 138

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Investigating G-rich DNA aptamer binding to huntingtin

Investigators: G. E. Owens, R. Vijayvargia, R. Jung, M. E. MacDonald, I. S. Seong

Huntington's disease is an autosomal dominant neurodegenerative disorder caused by a polyglutamine expansion (>35 glutamines) in the N-terminus of the huntingtin protein. Huntingtin is a large soluble protein (350 kDa) and predicted to have a predominant HEAT/HEAT-like repeat secondary structure. Huntingtin's large size and extensive post-translational modifications have made expression and purification of the full-length recombinant protein difficult. Our lab has recently successfully created a method for purification of full-length huntingtin, as well as large fragments of the huntingtin protein, in an insect cell expression system. We have recently also completed cloning an allelic series of N-terminal 65 kDa fragments of huntingtin. This has created an opportunity to study the biophysical properties of the huntingtin protein with the essential post-translational modifications present in eukaryotic cells. Using an aptamer microarray, we have discovered several synthetic guanine(G)-rich DNA molecules that bind to purified full-length huntingtin with high affinity and specificity in a polyglutamine length-dependent manner. Preliminary western blot data suggest that these G-rich aptamers bind to an epitope in the C-terminal portion of the full-length huntingtin protein, quite far in primary sequence from the N-terminal polyglutamine repeat, even though these aptamers bind in a polyglutamine length-dependent manner. This suggests that the polyglutamine repeat expansion leads to structural modifications throughout the huntingtin protein, and these G-rich aptamers may represent a novel way to study the structure of full-length huntingtin.

Poster Number 139
ePoster#24

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High-throughput mental health drug discovery using zebrafish fear responses

Investigators: A. J. Rennekamp, R. T. Peterson

Freezing in response to threatening stimuli is a behavioral trait observed in most animals. The ability to choose an appropriate defense response during a threatening situation has consequences not only for survival, but also psychiatric health. Because zebrafish larvae can be easily used for high-throughput chemical screening, we designed a high-throughput behavioral fear assay to rapidly and cost-effectively identify new chemicals that affect brain function. Here we show the discovery of several new classes of chemical compounds that mimic or counteract the effects of several hallucinogens. Target-based drug discovery approaches have the potential to miss systems-modulating compounds, which may be useful for the treatment of complex polygenic diseases such as schizophrenia. In contrast, our whole-organism drug discovery approach is both holistic and target-agnostic and therefore has the potential to lead us down new therapeutic avenues.

Poster Number 140

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Where do neuroblasts go after a focal cortical injury in a brain like kids' brains?

Investigators: C. M. Smith, S. R. Taylor, C. P. Dodge, A. C. Duhaime, B. A. Costine

Traumatic brain injury (TBI) is the leading cause of death and disablement in children and no effective therapies currently exists. In early postnatal life, neuroblasts are generated in the subventricular zone (SVZ) migrate through the brain and populate specific brain regions. In rodent models of TBI, neuroblasts divert from their normal course, target the lesion site, and contribute to repair; however, it is not known if neuroblasts target the lesion in the gyrencephalic brain. Previously we have shown that a cortical impact on postnatal day 7 increased the area of the SVZ, but the number of neuroblasts in the white matter leading to the lesion was not increased at 7d post-injury. Here, we aim to determine if neuroblasts born around the time of injury (5-bromodeoxyuridine injected at injury; BrdU) target the injured rostral gyrus. In this interim analysis, the number of neuroblasts in the rostral gyrus was not different in injured vs. sham piglets. A greater number of neuroblasts born at the time of injury (BrdU+) were present in the white matter of the rostral gyrus of injured piglets compared to uninjured piglets, though few in number ($p = 0.04$). We observed chains of neuroblasts from the SVZ to the lateral gray matter ("lateral gyri"). Injured piglets tended to have a greater number of neuroblasts in this actively populating site than sham animals ($p = 0.09$). After TBI, the gyrencephalic brain may allocate neuroblasts to actively populating regions (lateral gyri) rather than "throwing good after bad" at the injury.

Poster Number 141
ePoster#25

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A new perspective on dystonia from a mouse with a mutation discovered in the Lamb1 gene, a laminin subunit implicated in synaptic plasticity

Investigators: Y. B. Liu, A. Tewari, J. Salameh, E. Arystarkhova, T. G. Hampton, A. Brashear, L. Ozelius, K. Khodakhah, K. J. Sweadner

Dystonia (which causes abnormal postures and twisting movements) is normally thought to be a disorder of basal ganglia and cerebellum. A new mouse line arose here by spontaneous mutation. It exhibits intermittent hyperkinetic hindlimb movements and curvy tail, and electromyography (EMG) showed co-contraction of opposing muscle groups, a hallmark of dystonia. Electrophysiology detected abnormal firing rates in Purkinje neurons and deep cerebellar nuclei in awake mice. More surprisingly, hindlimb hyperextension occurred in sleep and anesthesia, conditions where descending activity from the brain is suppressed. Spinal transection actually enhanced contractions and hyperreflexia. This indicates fundamental spinal abnormalities that manifest when inhibitory activity descending from the brain is impaired. The new perspective is that dystonia may result not from abnormal excitatory patterns generated by the brain, but instead from failure of the brain to properly coordinate the inhibition of intrinsic spinal circuits that synchronize opposing muscle groups.

Inheritance was dominant. The gene was mapped by SNP analysis, and a nonsense mutation producing a C-terminal truncation of Lamb1, laminin beta1 subunit, was identified by whole exome sequencing and validated. The truncation is predicted to perturb binding of the assembled laminin trimer. Lamb1 is expressed in only selected neurons in CNS and spinal cord, conspicuously Purkinje cells, some nuclei, certain striatal and dorsal horn interneurons, and sensory neurons. Laminins, along with other extracellular matrix components, are implicated in synaptic structure and plasticity. Lamb1 is the third extracellular matrix component gene to be implicated in an inherited movement disorder after SGCE, epsilon-sarcoglycan, and Reln, reelin.

Poster Number 142

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Novel synaptotagmin 1- presenilin 1 interactions and their implications in Alzheimer's disease pathogenesis

Investigators: K. M. Zoltowska, A. Kuzuya, M. Arimon, X. Li, S. Svirsky, O. Berezovska

Synaptic loss is the strongest correlate of memory deterioration in Alzheimer's disease (AD). Synaptic dysfunction is caused by local accumulation of amyloid β (Aβ), and in particular neurotoxic Aβ42. Aβ is a proteolytic product of a subsequent processing of the amyloid precursor protein by two enzymes - β-secretase and presenilin 1 (PS1)/γ-secretase. Continuous, default or experimentally induced neuronal activity causes an increase in Aβ production, which is strongly related to intracellular calcium flux and synaptic vesicle exocytosis.

To gain further insight into Ca2+-dependent regulation of the Aβ production, we performed a mass spectrometry screen and found synaptotagmin 1 (Syt1) as a novel PS1 interactor that binds directly to PS1 in high Ca2+. Syt1 is a calcium sensor in neurotransmitter release and is involved in trafficking of synaptic vesicles at the active zone of the synapse. The interaction was confirmed in vitro and in vivo by co-immunoprecipitation and Förster resonance energy transfer (FRET) experiments. The role of Syt1 in Aβ40 and Aβ42 production, and in the stability and trafficking of β- and γ-secretases was investigated using Syt1 knock-down and overexpression approaches.

Our experiments demonstrate that PS1 interacts with Syt1 in a Ca2+-dependent manner, and that Syt1 overexpression and knock-down result in increased and decreased levels of secreted Aβ, respectively. Hence, they bring together important players in the AD pathogenesis: synapse, Ca2+, PS1 and Aβ production. The discovery that Syt1 (via its Ca2+-induced interaction with PS1) affects Aβ at the synapse opens new avenues for therapeutic interventions focusing at the synapse.

Poster Number 143

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Psychometric Evaluation of the Staff Perception of the Disruptive Patient Behavior Scale

Investigators: R. Lipkin-Orlando, D. A. Jones, M. Duffy, A. Weiss, D. L. Carroll

Disruptive patient behavior (DPB), including physical or verbal aggression toward healthcare providers, has a major impact on work environment safety within hospitals. The rate of DPB on staff, particularly nurses, has increased in recent years. Despite the importance of this issue, there is no standardized approach to capturing staff perceptions of DPB. Therefore the purpose of this study was to psychometrically evaluate the Staff Perception of Disruptive Patient Behavior (SPDPB) scale.

A mixed-methods approach was used to develop/psychometrically evaluate the SPDPB scale. Individual items were generated from a free text survey completed by 770 health care providers. A prototype 66-item instrument was developed and content validity was obtained. An evaluation of the psychometric properties of the SPDPB was completed with 558 nurses. The evaluation included internal consistency reliability of the 66-item SPDPB scale, principal component analysis (PCA) of this scale, and the internal consistency reliability of PCA-derived SPDPB subscales to refine/confirm the final SPDPB scale.

The SPDPB scale is a multi dimensional measure that focuses on nurse perception of DPB. PCA with varimax rotation identified 6 components that explained 54.1% of the variance. There was 1 item with a component loading of <.30 making the final scale 65 items with an alpha of .95 with a range of .78 to .93.

The SPDPB scale focuses on perceptions, including areas of concern/needs associated with DPB. The SPDPB scale demonstrated psychometric adequacy and can be recommended to measure perceptions of DPB.

Poster Number 144

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Genome-wide off-target cleavage profiles of CRISPR-Cas RNA-guided nucleases defined by GUIDE-Seq

Investigators: S. Q. Tsai, Z. Zheng, N. T. Nguyen, M. Liebers, V. V. Topkar, V. Thapar, N. Wyvekens, C. Khayter, A. J. Iafrate, L. P. Le, M. J. Aryee, J. K. Joung

Clustered regularly interspaced short palindromic repeat (CRISPR)-CRISPR-associated (Cas) RNA-guided nucleases (RGNs) are robust and programmable tools that can be customized to introduce targeted DSBs. Athough it has been shown that some monomeric RGNs can cleave off-target sites with up to 5 mismatches, the global specificity profiles of these nucleases has remained unknown. To identify CRISPR-Cas induced DSBs in the genomes of living human cells, we developed a novel method called Genome-wide Unbiased Identification of DSBs Enabled by Sequencing (GUIDE-seq), which is based on efficient integration of an end-protected double-stranded oligodeoxynucleotide tag into RGN-induced breaks. When performed with 10 RGNs in 2 human cell lines, we identified all known off-target cleavage sites of these RGNs as well as many novel sites. Additionally, in non-nuclease treated control cells, GUIDE-seq identified background 'hotspots' of DSBs. Finally, in validation experiments in which we sequenced across GUIDE-seq detected off-target cleavage sites, we found evidence of indels as well as translocations between on-target, off-target, and background 'hotspot' DSBs. The ability of the GUIDE-seq method to sensitively detect DSBs on a genome-wide scale will make it broadly useful not only for identifying sites of nuclease-induced DNA cleavage but also for studying naturally occurring DNA damage and repair.

Poster Number 145

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Identification and characterization of long noncoding RNAs regulated by TGF-β signaling in hepatic stellate cells

Investigators: C. Zhou, S. R. York, J. Y. Chen, K. Daneshvar, J. Pondick, A. C. Mullen

Hepatic fibrosis is the underlying cause of cirrhosis and liver failure in nearly every form of chronic liver disease, and hepatic stellate cells (HSCs) are the primary cell type responsible for this fibrosis through a process driven by TGF-β signaling. Long noncoding (lnc) RNAs are increasingly recognized as regulators of development and disease, but they have not been defined in human HSCs, and little is known about their role in hepatic fibrosis. We established a computational pipeline to assemble lncRNA transcripts expressed in HSCs under conditions including induction by TGF-β signaling. This analysis identified ~4000 of lncRNA loci in HSCs and showed that over 60% of these lncRNAs are divergently transcribed from protein-coding genes. Expression of individual lncRNAs was confirmed by PCR. Their full length transcripts were then defined by rapid amplification of cDNA ends (RACE) and localized by RNA fluorescent in situ hybridization (FISH). More than 150 lncRNAs were found to be directly induced by TGF-β signaling, and over 70% of lncRNAs located at super enhancers were also directly induced by TGF-β signaling. Approximately 800 of the identified lncRNAs were uniquely enriched in HSCs compared to 38 other human tissues. Co-expression network analysis also revealed that lncRNAs were enriched in networks involved in extracellular matrix biosynthesis and/or transcription regulation. This analysis defines the lncRNAs expressed in human hepatic stellate cells and allows us to focus on identifying the function of lncRNAs with the greatest potential to regulate hepatic fibrosis.

Poster Number 146

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"Crisscrossed" Clinicopathologic Profiles in Two Cases of FTLD-tauopathies: A Focus on Primary Progressive Aphasia and Corticobasal Syndrome

Investigators: T. D. Gefen, J. C. Sherman, E. T. Hedley-Whyte, M. Frosch, B. D. Dickerson

Background: Correlating clinical phenotype to underlying neuropathology is a necessary but challenging practice in the diagnosis of non-Alzheimer's disease (AD) dementias. This is particularly challenging in Primary Progressive Aphasia (PPA)/Frontotemporal Dementia (FTD)-spectrum disorders given evolving symptoms, the heterogeneity of possible underlying non-Alzheimer's pathologies, and the absence of biomarkers that detect these pathologies during life.

Case History: Patients with PPA can ultimately show underlying non-Alzheimer's tauopathy, including Pick's disease (frontotemporal lobar degeneration; FTLD-tau), Progressive Supranuclear Palsy (PSP), or Corticobasal Degeneration (CBD). In CBD and PSP, motor symptoms consistent with the underlying pathology often emerge. Patients with corticobasal syndrome (CBS) can show a variety of pathologies including CBD, PSP, FTLD, or AD. We present two non-Alzheimer's cases followed extensively until death. In both cases, CSF AD biomarkers (Aβ42, T-tau, P-tau181) were inconsistent with Alzheimer's pathology. Case 1 (right-handed, onset=age 66) had a ~5-year history of an atypical form of Wernicke-like PPA with right>left atrophy in lateral temporal cortex, later developing behavioral symptoms, with symmetric parkinsonism emerging one year before death. Autopsy demonstrated CBD pathology. Case 2 (right-handed, onset=age 48) had a ~10-year history of classic CBS, with perirolandic hypometabolism and atrophy. Behavioral symptoms and semantic impairment developed late in course. Autopsy demonstrated Pick's disease (FTLD-tau).

Conclusions: These two well-characterized cases that show post-mortem FTLD-tau pathology illustrate currently unclassifiable cases that contradict typical rules-of-thumb when clinicians attempt to predict pathology in patients during life. Sensitive biomarkers that employ tau-PET imaging or CSF may be critical for early detection and treatment of FTLD-tau-spectrum disorders.

Poster Number 147

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Tailored ventilation in morbidly obese patients

Investigators: M. Pirrone, C. Mietto, D. Fisher, D. Chipman, D. Imber, R. Kacmarek, L. Berra

Obesity is a major health threat in the United States, causing more deaths than cancer. In Intensive Care Units (ICU), obesity is associated with failure to liberate from mechanical ventilation, leading to tracheostomy, ventilator dependence and death. In supine obese people, the weight of the abdomen pushes against the diaphragm, causing a cephalad displacement of the muscle. This increased pressure inside the pleural cavity causes atelectasis and hypoxemia, worsening the elastic properties of the respiratory system. The reduction of aerated lung tissue at end-expiration reduces the Functional Residual Capacity (FRC). The application of adequate levels of positive end-expiratory pressure (PEEP) can prevent lung collapse. Current ventilation guidelines do not include specific PEEP titration techniques for mechanical ventilation in obese patients, leading to failed liberation from mechanical ventilation. With this study, we aimed to describe the characteristics of the respiratory system in mechanically ventilated morbidly obese patients at different levels of PEEP.

Adult intubated patients with Body Mass Index > 35 kg/m2 were studied. Patients' respiratory mechanics were studied in supine position at different PEEP levels: 1) baseline, 2) lowest PEEP level with positive end-expiratory transpulmonary pressure before and after a recruitment maneuver and 3) best PEEP identified through a decremental PEEP trial.

Our study showed that routinely set PEEP levels are not adequate in morbidly obese patients, resulting in reduced FRC and worse respiratory mechanics. Esophageal-titrated PEEP and decremental PEEP trial yield the same conclusion. Recruitment maneuvers are able to increase FRC significantly while reducing end-inspiratory transpulmonary pressure.

Poster Number 148

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ST2 Predicts Mortality and Length of Stay in a Critically Ill Non-cardiac Intensive Care Unit Population

Investigators: J. W. Rudolf, E. Lee-Lewandrowski, K. Lewandrowski, J. Januzzi, E. K. Bajwa, J. Baron

Background: Suppression of tumorigenicity 2 (ST2) is a clinical biomarker of cardiac strain secreted by cardiomyocytes under mechanical stress. ST2 has been shown to be an excellent tool for the prediction of mortality in patients with cardiac conditions including heart failure and myocardial infarction. Prior work has demonstrated that high sensitivity troponin T (hsTnT), a marker of cardiac cell death, is frequently elevated and predictive of poor outcomes in critically ill patients without primary cardiac pathology suggesting that critically ill patients may be at risk for unrecognized cardiac injury. We sought to evaluate whether ST2 would also be elevated and predictive of prognosis in non-cardiac, critically ill patients.

Methods: We measured hsTnT and ST2 on excess plasma specimens collected within 12 hours of medical intensive care unit (MICU) admission on 441 patients, and evaluated the prognostic power of ST2 alone and in combination with hsTnT.

Results: 96% of patients had ST levels above the normal reference range. Increased ST2 was highly predictive of hospital and ICU length of stay and in-hospital morality, both by itself and in combination with hsTnT. Patients in the highest quartile of ST2 values had a 4.1 fold higher relative risk of death compared to those in the bottom quartile.

Conclusions: ST2 is increased and predictive of prognosis in most non-cardiac MICU patients. These findings suggest that ST2 and hsTnT may be useful in patient risk stratification and that critically ill patients may often have unrecognized cardiac injury.

Poster Number 149

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Linkage to HIV/TB care in South Africa: a randomized trial of health navigators

Investigators: I. V. Bassett, S. M. Coleman, J. Giddy, L. M. Bogart, C. E. Chaisson, D. Ross, T. Govender, K. A. Freedberg, R. P. Walensky, E. Losina

Background: Many people newly-diagnosed with HIV do not enter care. We investigated the efficacy of health-system navigators for improving linkage to HIV care and TB treatment completion in Durban.

Methods: In a randomized controlled trial, adults (≥18y) enrolled at 4 outpatient sites underwent HIV and TB screening. HIV-infected or HIV/TB-coinfected participants were randomized to a navigator intervention or usual care. Navigator arm participants had a baseline interview addressing barriers to care, and received phone calls and SMS reminders over 4 months. The primary outcome was 3 months on ART and alive for those ART-eligible or completion of 6 months of TB treatment, assessed via study site medical records and the national death registry 9 months after enrollment.

Results: 1,899/4,903 (39%) subjects were HIV-infected (49% female; mean age 34); 1,146 (60%) were ART eligible, and 369 (19%) were co-infected with TB. In the usual care arm, 197 (21% of HIV-infected and 37% of ART eligible) reached the primary outcome, compared to 212 (22% and 34%, respectively) in the navigator arm (p=0.60). 250/1,899 (13%) HIV-infected subjects died; there was no difference in death rates between arms.

Conclusions: <40% of newly-diagnosed HIV-infected, ART-eligible individuals in Durban had evidence of taking ART for ≥3 months or completing TB treatment nine months after diagnosis. This RCT did not demonstrate that a health navigator improves ART initiation or TB treatment completion. Low rates of engagement in care and lack of efficacy of the navigator highlight the urgency of identifying effective strategies for improving HIV/TB care outcomes.

Poster Number 150

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A Tool for Understanding Food Cost Perception in Chelsea, MA

Investigators: F. A. Choudhury, F. Gonzalez, F. A. Khan, H. C. Cheung

Introduction: A common explanation for the perceived links between obesity and poverty involves the low cost of energy-dense foods. However, there is an evident gap between increased purchasing power and buying healthier food.

Objective: This research explores the behavioral economics aspect of food cost perception. The general perception that 'eating healthfully' is more expensive has increasing importance for food consumption forecasting.

Method: We prepared a free listing questionnaire asking Chelsea community members to list twenty (20) basic food preference and rank their perceived expensiveness of each item on a scale from 'Cheap' to 'Expensive. Then we calculated the local aggregate cost of most common food items on a per-pound (or per-pint for juices), per gram of protein basis from random retail stores in Chelsea. Additionally, Likert Scale was used to calculated the mean perceived fair price for various food groups, including fruit, vegetables, starch, seafood, and meat.

Results: According to the data, the participant's perceived food price is significantly higher than the market price for fruits [p<0.0001] (price $1.27/lbs), vegetables [p<0.0001](price $1.68/lbs), and starch [p= 0.006] (price $2.07/lbs). Additionally, the perceived food price for seafood is significantly lower (p<0.02) (price $6.68) than the market price. No significant difference was found for meat (p=0.4) (price $3.79)

Conclusions: Healthier food options costs $0.47/200 kcal more than less healthy option – averaging $550 more in annual food costs per person. Given the prevalence of obesity in Chelsea, efforts to develop programs that offset higher cost of fruits and vegetables in high-risk population are imperative.

Poster Number 151

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Health Services Outcomes Research: A targeted intervention for development of faculty who are under-represented in medicine

Investigators: K. M. Winkfield, A. E. Reid, G. M. Porter, E. B. Olson

In an effort to increase retention and advancement of faculty who are underrepresented in medicine (URM) at Massachusetts General Hospital (MGH), the Multicultural Affairs Office implemented a Minority Faculty Development Award Program (MFDAP) that provides financial support and mentoring to physician/scientists and clinician-teachers. The purpose of this study was to assess the effectiveness of the MFDAP. An on-line survey was administered to faculty receiving MFDAP grants between 2004 and 2012. Two cohorts were surveyed independently, recipients from 2004-2009 and 2009-2012. Semi-structured interviews were conducted with recipients in the 2004-2009 cohort. We assessed qualitative and quantitative measures of academic productivity, commitment to an academic career, importance of and satisfaction with the MFDAP award and mentor relationship. We also compared the retention rates at MGH and in academic medicine of recipients and non-recipients of MFDAP. Of the 83 unique applications, 26 grants totaling $3,120,000 were awarded between 2004-2012. MGH has retained 88% of MFDAP recipients, as opposed to 60% of applicants who did not receive the awards. Fifteen recipients (57.7%) have received academic promotions and 17 (70.8%) have garnered subsequent grants, with amounts totaling $23Mil. The majority of recipients believe they will remain in academic medicine for the next 5 to 10 years. All recipients found the MFDAP very helpful to their careers. The MFDAP is effective and associated with high retention and advancement of URM faculty at MGH and in academic careers, building a more diverse workforce. Recipients also show evidence of academic promotion and career advancement since receiving the award.

Poster Number 152

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GWAS meta-analysis of ten studies identifies five novel loci associated with gallstone disease in European ancestry individuals

Investigators: A. D. Joshi, C. Andersson, M. Gala, S. Stender, A. T. Hansen, B. Nordestgaard, A. R. Folsom, P. L. Lutsey, L. Weng, R. Noordam, A. Teumer, W. Tang, U. Völker, H. Völzke, L. Rose, P. E. Weeke, J. Hempe, D. I. Chasman, S. Buch, A. D. Johnson, A. T. Chan

In 2007, a genome wide association study (GWAS) consisting of 280 cases identified the hepatic cholesterol transporter ABCG8 locus as a susceptibility factor for human gallstone disease. No other population-based GWAS has been reported for this phenotype. Therefore, we performed a large-scale GWAS meta-analysis of 9,298 gallstone cases and 62,007 controls of European ancestry, pooled from 8 cohort and 2 case-control studies to discover additional genetic variants associated with gallstone disease. Inverse variance weighted meta-analysis of age and sex adjusted study-specific estimates was performed to identify SNPs that were independently associated with gallstones disease. Two SNPs in ABCG8, rs11887534 (p=7.2x10-53) and rs4245791 (p=4.0x10-31), were independently associated with gallstone disease. Additionally, we identified novel independent associations for rs1025447 (p=4.7x10-11) in DYNC2LI1, which plays a structural role in cilia formation; rs9843304 (p=3.3x10-9) in TM4SF4; rs2547231 in SULT2A1, which is a sulfo-conjugation enzyme that acts cholesterol-derived sterol bile acids (p=1.9x10-9); rs1260326 (p=1.4x10-8) in GCKR, a glucokinase regulator; and rs6471717 (p=6.03x10-8) near the CYP7A1 gene that codes for an enzyme to catalyze formation of bile salts from cholesterol. Replication was performed for six SNPs in five loci (except DYNC2LI1), and all of them validated in independent replication samples ($p < 5x10-6$; 6,489 cases and 66,366 controls) from three studies. In this large-scale GWAS meta-analysis of gallstone disease, we identified five biologically plausible novel loci, with putative functions in cholesterol transport and metabolism, cilia structure/bile flow, and sulfonylation of bile acids/ hyrdoxysteroids.

Poster Number 153

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Paroxysmal Atrial Fibrillation Poses A Large But Transient Increase In Ischemic Stroke Risk: A Case-crossover Study Using Continuous Heart Rhythm Recording

Investigators: D. E. Singer, P. D. Ziegler, Y. Chang, M. P. Turakhia

Background: Atrial fibrillation (AF) raises stroke risk 5-fold. But the relationship between occasional ("paroxysmal") episodes of AF and stroke risk is not well quantified because AF is frequently asymptomatic and unrecorded. We studied ischemic stroke patients who had implanted cardiac devices (e.g., defibrillators) providing continuous heart rhythm recording. For each patient we compared AF in the 30 days pre-stroke versus the day 91-120 pre-stroke control period (case-crossover design). Intra-individual comparison controls for background stroke risk. The ratio of discordant pairs provides the odds ratio (OR). From prior work, we defined "significant" AF (sigAF) as ≥5.5 hours AF on ≥1 day.

Results: Veterans Administration databases, 2004-2009, provided 187 stroke patients with continuous heart rhythm records: median age 68 years, 99% male. 156 patients had negative (AF irrelevant) and 15 had positive (AF indeterminate) sigAF in both periods. 16 patients had informative discordant sigAF: 13 were positive in the case and 3 were positive in the control periods: OR=4.33 (1.19-23.7). The 13 discordant patients had a median of 100 (IQR: 61-439) minutes/day of AF during the case period versus 0 (IQR: 0-0) minutes/day during the control period. Analysis of sequential 5-day intervals (1-5, 6-10, etc) preceding the stroke revealed OR=17 for days 1-5, declining to background risk by 31 days.

Conclusions: This largest sample of stroke cases with antecedent rhythm monitoring demonstrated that multihour episodes of AF transiently raise ischemic stroke risk. Our results support a trial of transient use of rapidly acting anticoagulants linked to onset and offset of AF episodes.

Poster Number 154

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Liberian Traditional healers' interpretations of psychosis and treatment modality

Investigators: M. Ulloa , M. Ameresekere, B. C. Lange , M. P. Kempeh, D. G. Karnga, M. Fallah, A. Payne , E. Brown , J. B. Menyongai , B. Harris , D. C. Henderson , C. P. Borba , N. V. Christian

Background: Mental and substance use disorders were the leading cause of years lived with disability in sub-Saharan Africa in 2010 accounting for 19% of the disability burden despite an average of only .06/100,000 psychiatrists in the population.1 With a substantial shortage of available mental health providers and treatment options for individuals with psychiatric illnesses, many prefer and depend on traditional healing practices.2 One goal of this study was to understand mental illness in Liberia, to explore the available treatment options for psychotic disorders and to identify traditional healers' methods of delivering care to this population.

Methods: In-depth qualitative research methodology was used during face to face semi-structured interviews with 23 Liberian traditional healers. Interviews were approximately 30 minutes and were recorded and transcribed verbatim. Data was analyzed using the principles of thematic analysis until theoretical saturation was reached.

Results: Several themes emerged about the identification and treatment of psychotic disorders. Psychosis was referred to as "madness" or "craziness." The symptoms of craziness were described as problems starting in the head, the heart, and the eyes. Frustration, war-related events and indigenous beliefs like possession by spirits were considered as possible causes of psychosis. Treatments included counseling and the application of herbs on the skin, intranasally or through the eyes.

Conclusions: A number of traditional healers expressed interest in receiving education on the diagnosis and management of mental illness, specifically psychosis. Further collaboration could provide an opportunity to explore how western interventions can be integrated into traditional medicine practices.

Poster Number 155

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Traditional treatments for substance abuse in Liberia

Investigators: M. Ulloa, M. Ameresekere, B. C. Lange, M. P. Kempeh, D. G. Karnga, M. Fallah, A. Payne, E. Brown, J. B. Menyongai, B. Harris, D. C. Henderson, C. P. Borba, N. V. Christian

Background: From 1989 to 2003, over 250,000 Liberians were killed in two civil wars. Survivors were left with a multitude of physical and psychological issues at the societal and individual level, including substance abuse. Due to Liberian cultural beliefs and the scarcity of mental health care professionals, substance abusers and their families access traditional healers. The objective of this study was to assess the beliefs and practices of traditional healers towards mental illness, including substance abuse.

Method: Face-to-face, semi-structured qualitative interviews with 23 traditional healers, lasting approximately 30 minutes, were recorded and transcribed verbatim. Traditional healers were recruited through Liberian communities and medical centers. Principles of thematic analysis were used to analyze the data until saturation was reached.

Result: Traditional healers reported substance abuse to be a common problem in Liberia often attributed to adverse events experienced during the civil wars. They described treating alcohol, marijuana and narcotic use by forcing abstinence by utilizing physical restraints, and administering herbs and leaves to reduce withdrawal symptoms. Additionally, healers will order patients to over consume the substance to produce a negative reaction to cease future cravings.

Conclusion: Treating substance abuse can be a dangerous process and requires careful monitoring. Future research can benefit from examining not only the efficacy of traditional medicines for substance abuse treatment but also the safety of using herbal remedies. Collaboration with medical professionals to ensure safe treatment practices for patients suffering from life threatening withdrawal could reduce mortality and complications often associated with substance abuse treatment.

Poster Number 156

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Association between tobacco smoking and death by suicide: A competing risks hazard analysis in a large twin cohort with 35-year follow up

Investigators: A. E. Evins, K. Korhonen, T. H. Kinnunen, J. Kaprio

The aim of this study was to estimate the association between smoking and suicide, and the degree to which it is influenced by dose, cessation, psychiatric and somatic morbidity, genetic, and environmental factors. 16,282 twin-pairs born before 1958 in Finland and alive in 1974 were queried with detailed health and smoking questionnaires in 1975 and 1981. Participants were followed for 35 years for psychiatric and medical treatment and vital status, including suicide determination by forensic autopsy. We found that current smokers had a higher cumulative suicide incidence than former or never smokers. Heavy current smokers had higher suicide risk (hazard ratio (HR)=3.47; 95% CI, 2.31-5.22) than current smokers who smoked less (HR=2.30; 95% CI, 1.61-3.23) (p=0.017). Compared to never smokers, current smokers had increased suicide risk (HR=2.84; 95% CI, 1.58-5.10) adjusting for depressive symptoms, alcohol and sedative-hypnotic use, and excluding those with serious somatic or psychiatric illness, while former smokers did not. In the 28 twin-pairs discordant for smoking and suicide, 24 of the suicides were in smokers and 4 in non-smokers (OR=6.0; 95% CI, 2.06-23.8). The results of this study demonstrate that tobacco smoking is associated with completed suicide with a large, dose dependent effect, independent of demographic factors, depressive symptoms, heavy alcohol use, and major psychiatric and somatic illness.These results are consistent with an interpretation that genetic factors do not significantly modify the relationship between smoking and suicide and that tobacco smoking may have a causal role in relation to suicide.

Poster Number 157

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Inattention and disrupted resting state functional connectivity of the cerebellar default node in ADHD

Investigators: A. Kucyi, M. J. Hove, J. Biederman, E. M. Valer

Background: Abnormal spontaneous interactions between the brain's default mode network (DMN) and networks associated with attention to the external environment (sensory, salience and dorsal/ventral attention networks) have been hypothesized to underlie attentional deficits in ADHD. These networks include cerebellar components that are accessible stimulation targets for therapeutic neuromodulation. We thus tested whether spontaneous functional connectivity (FC) of the cerebellar node of the DMN (CerDMN) is disrupted in ADHD and whether this FC relates to individual differences in inattention.

Methods: 23 adults with ADHD and 23 age-, IQ-, and sex-matched healthy controls underwent resting state fMRI (10 minutes) and completed the Adult ADHD Self-Report Scale (ASRS). The mean time series of seed regions in the CerDMN were extracted, and FC with the whole brain was calculated. Within- and between-group differences in FC were assessed. Additionally, relationships between the ASRS inattention subscale and individual differences in FC were assessed for between-group interactions. All analyses were conducted at the whole brain, voxel-wise corrected level (FLAME; $Z > 2.3$, cluster-based $p < 0.05$).

Results: Within both groups, CerDMN was functionally connected with cortical DMN regions. FC of the CerDMN in ADHD compared to healthy controls showed less anti-correlation with widespread regions of salience and dorsal/ventral attention networks. Inattention positively correlated with FC between CerDMN and regions within the dorsal attention network in healthy controls but not in ADHD.

Conclusion: This work provides novel evidence of impaired CerDMN decoupling with cortical networks in ADHD and highlights a role of the cerebro-cerebellar interactions in cognitive function.

Poster Number 158

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A Needs Assessment for Health and Mental Health Education and Awareness for Africans in Lowell

Investigators: L. Parnarouskis, C. E. Oppenheim, A. Stevenson, M. Ulloa, S. Schor, M. Amaresekere, J. Menyongai, B. Chukwuezi, D. Johnson, S. Kouagheu, C. Onyeulo, S. Slopadoe, P. Wennah, D. C. Henderson, C. Borba

Background: Lowell, MA is home to a large international population. A rising number are of African descent, many from war-affected countries. Refugees and immigrants who have been exposed to war and violence face unique challenges including acclimating to a foreign country, culture, and language. Research has shown that they are at higher risk for health and mental health problems.

Methods: N=52 interviews were conducted with adults of African descent living in or near Lowell to assess attitudes about physical and mental health, access to health services, substance abuse, and the recent Ebola outbreak in West Africa. Data were collected through a semi-structured qualitative interview between October-December 2014.

Data were analyzed using rapid analysis. Two raters listened to each qualitative interview and took notes independently, then met with the larger team to compare overall themes.

Results: Participants reported that the most common mental health issues were depression, anxiety or "stress," and PTSD. However, they reported that "crazy" is a widely-used, stigmatized term to describe any serious mental health issue in which a person is believed to have lost control over their physical and/or mental state. Respondents reported many structural and cultural barriers to accessing mental health care including lack of insurance, difficulty navigating health care systems, language barriers, stigma, lack of awareness of treatment options, and aversions to medication.

Conclusions: Mental health awareness and stigma reduction campaigns, insurance and health system education, and dissemination of information about available care options within the community would be useful interventions with this population.

Poster Number 159

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D-cycloserine augmentation of cue exposure therapy for smoking cessation

Investigators: M. Otto, G. Pachas, C. Cather, S. Hoeppner, S. Moshier, B. Hearon, A. E. Evins

Aims: Recent study of D-cycloserine (DCS) augmentation has underscored the importance of adequate exposure and minimization of reconditioning experiences post dosing. We aimed to investigate the effect of a single dose of DCS immediately prior to two smoking cessation cue exposure therapy (CET) sessions under these conditions for maintenance of tobacco abstinence.

Methods: Sixty-two adult smokers attained at least 18 hours abstinence with their choice open-label varenicline or nicotine replacement (NRT) and were randomized to receive 50 mg of DCS (n=30) or identical placebo (n=32) prior to each of 2 sessions of CET, one week apart. During CET, participants were exposed to emotional and external cues for tobacco use in an extinction paradigm. CET I was conducted during the last week of varenicline or NRT; CET II was conducted one week after discontinuation of cessation pharmacotherapy. The primary outcome was 6-week, verified continuous tobacco abstinence. Secondary outcomes were craving and physiologic reactivity to smoking cues.

Results: Recently abstinent smokers who received DCS augmentation of CET for relapse-prevention had triple the rates of continuous abstinence versus those assigned to placebo + CET (30% DCS + CET, 9% placebo + CET, Fishers Exact p = 0.049). Moreover, DCS had an enhanced extinction effect vs. placebo for craving and physiological reactivity to smoking cues in the subsample that were cue reactive and had no post dose smoking-related reconditioning experiences.

Conclusions: This study provides evidence that under optimized conditions for extinction learning, DCS enhanced CET efficacy for prevention of relapse to tobacco smoking.

Poster Number 160

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Nonverbal Signals of Physician Empathy Increase Ratings of Both Warmth and Competence

Investigators: D. A. Reinero, G. T. Kraft-Todd, L. Baer, A. S. Heberlein, J. M. Kelley, H. Riess

Nonverbal behavior plays a major role in communicating and perceiving emotion and empathy. Empathy is beneficial to both the patient-physician relationship and patient health outcomes. Research also suggests that impressions of others are formed along dimensions of "warmth" and "competence," and that these dimensions account for most of the variance in perceptions of everyday social behaviors. In many domains, however, an inverse relationship between judgments of warmth and judgments of competence has been documented, though this has never been tested in medicine. In this report the perception of nonverbal behavior in the context of the patient-physician relationship was explored utilizing the online crowdsourcing tool, Amazon Mechanical Turk. It was hypothesized that physicians displaying nonverbal behaviors associated with empathy would be viewed as more empathic, warm, and competent than physicians who displayed unempathic nonverbal behaviors. Participants viewed a scripted patient-physician encounter (with either a female or male physician) with accompanying photographs while imagining themselves as the patient and then made judgments about the physician. Results confirmed the hypothesis and, importantly, there was no warmth/competence tradeoff, as has been shown in other domains. Consistent with prior research, participants showed a same-sex preferential bias, rating the physician that was the same sex as themselves, higher on warmth and competence. In addition, an empathic female physician was rated higher on warmth and competence than an empathic male physician. The findings suggest that to build positive patient-physician relationships, physicians should consider the importance of communicating empathy through their nonverbal behaviors.

Poster Number 161

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Personality Traits Predicting Hair Pulling Severity

Investigators: E. S. Tung, C. A. Flessner, E. M. Altenburger, M. A. Blais, D. L. Pauls, M. R. Frank, N. J. Keuthen

To date, trichotillomania researchers have not examined the relationships between personality traits and severity and control over hair pulling. Thus, it is unclear if the strength of certain personality traits are associated with the severity of the pulling urges/behavior and control over them. We examined whether specific personality traits (NEO Five-Factor Traits: neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness) can predict hair pulling severity and control.

Our results indicate that neuroticism is a significant predictor of both hair pulling severity and control even after controlling for depression and anxiety severity. This makes sense given that affective variables are known triggers for hair pulling. In addition, openness and agreeableness were also significant predictors of hair pulling severity. A positive correlation was reported between hair pulling severity and openness. As openness increases, the individual is more aware of, and immersed in, their feelings and thus more likely to feel distress which triggers greater pulling severity. Conversely, a negative correlation was reported between agreeableness and hair pulling severity. Thus, individuals with greater pulling severity are less likely to be interpersonally receptive and trusting, either because they are absorbed in their hair pulling experiences or worried about the responses of others due to their apparent hair loss.

Examination of personality dimensions in TTM parallels the inclusion of dimensional rating scales in DSM-5. This effort promotes the study of broad dimensional characteristics across different diagnostic entities in an effort to understand the overlapping and unique variables that contribute to disorder development.

Poster Number 162

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Latino Americans' access to psychiatric and neurological services: a pilot study

Investigators: M. Ulloa, C. M. Avila-Urizar, C. B. Evans, J. C. Urizar, Z. Chemali

Background/Aims: Disparities exist in accessing mental health services for the Latino population in the United States. Limited access to care can increase the burden of mental illness. In this pilot study, we examine individual and societal factors influencing Latinos access to psychiatric and neurological services in designated centers of the Partners healthcare system in Massachusetts.

Methods: Data was obtained through a 35 item mixed method questionnaire. Twenty (N=3 male; N=17 female) Latino Americans, mean age of 58.3, were recruited. Descriptive statistics, logistic regression models and thematic analysis were used in the analysis.

Results: Majority of the sample reported living in the United States for 5 years or more. Same language providers were preferred over providers of identical Latin American descent and gender. Transportation was a limiting factor in accessing care. Despite low English proficiency, participants self reported good comprehension of illness, treatment, and potential side effects. Primary care physicians were the source for depression and anxiety referrals. Faith and prayer were considered essential in the healing process, however religious and cultural beliefs did not impact seeking mental health services. Citizenship status was associated with a 3.73 fold-increased odds of seeing a psychiatrist for trauma.

Conclusion: This pilot study provided preliminary information on the individual preferences and non-medical aspects that influence Latinos use of psychiatric and neurological services. Additional research is needed on a larger scale to ascertain our results so that health care services can be improved. New data samples should focus on different Latino ethnic subgroups.

Poster Number 163

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Clozapine, Diabetes Mellitus, Cardiovascular Risk and Mortality: Results of a 20-year Naturalistic Study

Investigators: B. Vincenzi, K. L. Nemani, M. C. Greene, M. Ulloa, P. M. Copeland, S. Al-Khadari, D. C. Henderson

Objective: The goal of this 20-year naturalistic study of clozapine treated patients was to examine the cardiovascular risk factors following clozapine initiation and resultant mortality estimates from cardiovascular disease.

Method: Data were collected from medical records from January 1992 to February 2012 and included age, gender, race, diagnosis, family history of diabetes, and age at clozapine initiation for clozapine-treated patients with schizophrenia or schizoaffective disorder. Clozapine dosage and laboratory results were extracted at 12-month intervals.

Results: At the time of clozapine initiation, the mean age of the 96 patients was 36.4 years ±7.6 years; N=27(28%) were women. The Kaplan-Meier estimate for 20-year cardiovascular events was 29%, while the Kaplan-Meier estimate for 20-year mortality from cardiovascular disease was 10%. While there was an increase in mean cardiovascular risk during the first ten years ($p<.01$), there was a slight decrease beyond ten years ($p<.01$). Patients who were involved in cardiometabolic research studies showed a greater decrease in cardiovascular risk factors over 20 years ($p = .05$). The Kaplan-Meier estimate for 20-year all-cause mortality was 22%. Forty-one patients were diagnosed with diabetes (42.7%).

Conclusions: These results support the hypothesis that clozapine-treated patients appear to be at risk for cardiovascular events and death secondary to clozapine directly increasing the risk of medical disorders, such as obesity, diabetes, hypertension, and hyperlipidemia. In this cohort, cardiovascular risk was highest in the first decade; the number of cardiovascular events decreased in the second decade. Clozapine did not continue to account for weight gain after the first decade.

Poster Number 164

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Autonomic and conscious indices of fear generalization: a focus on gender effects

Investigators: R. P. Wolthusen, E. A. Boeke, S. Nasr, M. R. Milad, R. B. Tootell, D. J. Holt

Background: Fear generalization is a process in which a fear response occurs to a stimulus that is similar to a threatening stimulus. Although some degree of fear generalization is adaptive, excessive fear generalization may produce psychopathological states, such as anxiety. Anxiety disorders are more common in females than in males. The reason for this is unknown, but one possibility is that the mechanisms that inhibit fear generalization are weaker in females compared to males. Thus, here we tested for gender differences in autonomic and explicit fear generalization.

Methods: Twenty-three male and eighteen female subjects underwent a Pavlovian fear conditioning procedure, in which one of a pair of objects was either followed (CS+) or not followed (CS-) by a shock. Subjects were then presented with the CS+, CS- and five levels of a CS+-to-CS- morph continuum between the paired stimuli, chosen based on individual discrimination thresholds. Skin conductance was measured throughout. Finally, subjects were asked to rate the percent likelihood that each stimulus had ever been followed by a shock.

Results: The males and females did not differ in demographic variables or symptom levels, levels of differential fear conditioning or skin conductance responses during generalization. However, females showed significantly greater levels of explicit fear generalization than males, with higher shock likelihood ratings of the two morphs that were most similar to the CS+.

Conclusions: Our finding suggests that gender influences conscious, but not unconscious, fear generalization processes. This might contribute to a higher prevalence of anxiety disorders in females.

Poster Number 165
ePoster#26

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Role of VCAM-1+ macrophages in splenic hematopoiesis in acute and chronic inflammation

Investigators: P. Dutta, M. Nahrendorf

Background: Splenic myelopoiesis provides a steady flow of leukocytes to inflamed tissues, and leukocytosis correlates with mortality, for instance in cardiovascular disease. Yet regulation of hematopoietic stem cell (HSC) activity in the spleen is incompletely understood.

Hypothesis: Macrophages retain hematopoietic stem cells in the spleen via VCAM-1.

Results: Nanoparticle-enabled in vivo RNAi silencing of the receptor for macrophage colony stimulation factor (M-CSFR) blocked splenic macrophage maturation ($p<0.01$), reduced splenic VCAM-1 expression ($p<0.001$) and compromised splenic HSC retention. This was consistent with significantly reduced splenocyte count and lighter spleen weight ($p<0.001$) in siMCSF-R-treated mice challenged with LPS. Depleting macrophages in CD169 iDTR mice released HSC from the spleen. Since M-CSFR knockdown significantly reduced VCAM-1 expression in the spleen and splenic macrophages expressed VCAM-1 at high levels, we hypothesized that macrophages retain splenic HSC via VCAM-1. To this end, we selectively silenced VCAM-1 on macrophages, which resulted in mobilization of splenic HSC. This was consistent with the finding that more than 90% of HSPC locate within 10 µm distance from VCAM-1+ macrophages in the spleen. When we silenced either VCAM-1 or M-CSFR in mice with myocardial infarction or in ApoE-/- mice with atherosclerosis, nanoparticle-enabled in vivo RNAi mitigated blood leukocytosis, limited inflammation in the ischemic heart and reduced myeloid cell numbers in atherosclerotic plaques ($p<0.001$).

Conclusions: Red pulp VCAM-1+ macrophages are essential to splenic HSC retention and extramedullary hematopoiesis. These macrophages use the adhesion molecule VCAM-1 to retain HSCs in the spleen.

Poster Number 166

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The histone deacetylase SIRT6 controls embryonic stem cell fate via TET-dependent production of 5-hydroxymethylcytosine

Investigators: J. P. Etchegaray, L. Chavez, Y. Huang, J. Choi, B. Martinez-Pastor, R. M. Walsh, C. Sommer, K. N. Ross, A. Goren, S. Ramaswamy, G. Mostoslavsky, K. Hochedlinger, A. Rao, R. Mostoslavsky

How embryonic stem cells (ESCs) commit to specific cell lineages and ultimately yield all cell types of a fully formed organism remains a major question. ESC differentiation is accompanied by large-scale histone and DNA modifications, but the relations between these two categories of epigenetic changes are not understood. Here, we demonstrate the interplay between the histone deacetylase Sirtuin 6 (SIRT6), which targets acetylated histone H3 at lysine 56 (H3K56ac), and TET (Ten-eleven translocation) enzymes, which convert 5-methylcytosine (5mC) into 5-hyfroxymethylcytosine (5hmC). We found that ESCs derived from SIRT6 knockout (S6KO) mice are skewed towards neuroectoderm development. This phenotype is associated with an increased expression of the core pluripotent genes Oct4, Sox2 and Nanog, which are directly regulated by SIRT6-dependent deacetylation of H3K56ac. We show increased recruitment of OCT4 to regulatory regions of Tet genes in S6KO versus WT ESCs. Genome-wide analysis revealed an upregulated expression of neuroectoderm genes, which are marked with higher levels of 5hmC in S6KO versus WT ESCs, thereby implicating TET enzymes as responsible for the neuroectoderm-skewed differentiation of S6KO ESCs. Strikingly, knockdown of TET1 and TET2 fully rescued the differentiation defect in S6KO ESCs. Additionally, we found the role of SIRT6 in ESC differentiation to be conserved in human ESCs (hESCs). Overall, we demonstrate a new role for SIRT6 as a chromatin regulator safeguarding the balance between ESC pluripotency and differentiation through TET-mediated oxidation of 5mC into 5hmC.

Poster Number 167
ePoster#27

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Wnt signaling is required for zebrafish kidney regeneration

Investigators: C. N. Kamei, Y. Liu, N. A. Hukriede, I. A. Drummond

Kidney regeneration in zebrafish occurs by repair of existing nephrons as well as by de novo generation of new nephrons from adult organ progenitor cells by neonephrogenesis. We report here that Wnt signaling plays an essential role in adult zebrafish kidney regeneration. Newly forming nephron condensates in the developing mesonephros as well as those formed in the adult in response to gentamicin injury express the Wnt receptor frizzled9b (fzd9b) and the canonical Wnt target gene lef1. In the Tg(lhx1a:gfp) nephron progenitor reporter line, both individual lhx1a-positive adult kidney progenitor cells and nephron condensates are specifically marked by expression of fzd9b. Pharmacological blockade of Wnt signaling using IWR1 and IWP2 led to a decrease in lhx1a+ nephron condensates after injury. EdU labeling in Tg(lhx1a:gfp) fish after gentamicin injury reveals that newly forming condensates are actively proliferating and this proliferation is blocked by Wnt inhibition. A new Wnt reporter line Tg(TCF/Lef-miniP:dGFP) combined with EdU labeling shows distinct zones of hi vs low Wnt activity and proliferation in new nephron formation. Our results demonstrate an essential role for Wnt signaling in adult zebrafish kidney regeneration. Identification of fzd9b as a new marker of adult kidney progenitor cells opens new avenues to investigate their developmental origins and regenerative potential.

Poster Number 168

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Hematopoietic Stem Cell Functional Heterogeneity is Cell Autonomous

Investigators: V. W. Yu, R. Yusuf, T. Oki, J. Wu, B. Saez, X. Wang, C. Cook, M. Ziller, H. Gu, A. Meissner, C. Lin, P. Kharchenko, D. Scadden

Genetic tagging has indicated that normal and malignant tissues are comprised of functionally heterogeneous cell populations, yet the spectrum of heterogeneity and how tissue stem cells commit to fate choices is poorly understood. We used an endogenous fluorescence clonal labeling strategy that does not involve integrating viruses or transposon mutagenesis to functionally track, isolate and molecularly profile hematopoietic stem cell subpopulations in vivo and studied their molecular profiles associated with fate choices. Immunophenotypically uniform, endogenous hematopoietic stem cells had clonal variability in differentiation outcome and cell production under homeostatic conditions. Notably, these characteristics were highly stereotypic, conserved under the stress of transplantation and associated with distinctive RNA expression and DNA methylation patterns. Further, fixed patterns of behavior included sensitivity to inflammatory and genotoxic stress. Therefore, a range of distinct functional attributes are present in populations of hematopoietic stem cells under homeostasis that are epigenetically enforced and persist through times of stress. Functional chimerism of subclones of stem cells with fixed characteristics may provide the range of responses observed in tissues with limited niche influence.

Poster Number 169

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Erlotinib, an EGF receptor antagonist, induces aquaporin 2 (AQP2) phosphorylation and increases water reabsorption in lithium treated mice

Investigators: P. W. Cheung, N. Nomura, A. V. Nair, H. Lu, R. Bouley, D. Brown

Impairment of vasopressin receptor type 2 (V2R) signaling in the kidney is detrimental to water homeostasis. However, strategies targeting cAMP signaling to treat diseases associated with water balance have thus far been unsuccessful in humans, prompting a search for alternative pathways that modulate AQP2 trafficking. Our previous study using high-throughput small molecule screening showed that AG-490, an EGFR and JAK-2 kinase inhibitor, enhanced AQP2 membrane expression. Therefore, we tested erlotinib (ERL), an FDA-approved EGFR inhibitor and found that ERL enhanced AQP2 membrane expression in LLC-PK1 cells by increasing AQP2 exocytosis and decreasing endocytosis. This effect was cAMP and cGMP independent, and was not inhibited by PKI, a selective PKA inhibitor. Despite apparently bypassing cAMP pathways, ERL resulted in phosphorylation of AQP2 in a dose dependent manner at serine 256, an essential step in VP-induced AQP2 membrane accumulation. This suggests that an alternative kinase plays a role in S256 phosphorylation upon ERL treatment. The physiological relevance of ERL was studied in a lithium-induced NDI mouse model. ERL (100 mg/kg/day oral gavage) increased apical AQP2 accumulation in collecting duct cells and significantly reduced urine volume (0.11± 0.03 vs 0.06 ±0.02 g of urine/g of body weight) after 4 days of treatment. In conclusion, EGFR inhibition ameliorates lithium induced NDI in mice, and increases AQP2 membrane expression in a cAMP independent fashion but with downstream AQP2 S256 phosphorylation. We propose that EGFR activation tonically inhibits AQP2 trafficking and this effect is overcome by ERL. Ongoing work is aimed at identifying the kinases involved.

Poster Number 170

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V-ATPase B1 subunit knockout mice have a gender-dependent defect in urine concentrating ability

Investigators: A. V. Nair, T. G. Paunescu, R. Bouley, D. Brown

Men dehydrate more than women during prolonged exercise, but the etiology is unclear. We assessed gender specific water homeostasis in C57BL/6 mice, and found that the basal urine osmolality is higher in female than in male mice (2756±278 vs 2420±185 mOsm/kg: mean±SEM). 12 h water deprivation decreased urinary output by 57 % in females and by 52 % in males (osmolality: 3664±244 vs 3468±318 mOsm/kg). Since β-intercalated cells (ICs) were shown to play a role in body fluid balance (e. g., Gueutin et al, JCI 2013), we studied the water concentrating ability of mice deficient in the B1 subunit of the V-ATPase (B1-/- mice). Urinary volume was 10 % higher in B1-/- males and 29 % higher in B1-/- females compared to wild type mice (WT). Unexpectedly, urine output in water-deprived B1-/- mice decreased by 46 % in females compared to only 17 % in males. Western blot and immunofluorescence staining revealed lower AQP2 expression in B1-/- mice of both sexes compared to WTs. While in dehydrated females AQP2 relocated to the apical membrane region of principal cells (PCs), this effect was much less significant in males, consistent with their weaker concentrating ability. Females have a higher IC/PC ratio than males, and B1-/- females have a higher IC percentage than WTs, whereas B1-/- and WT males do not differ. Our data support the idea that ICs are, via the V-ATPase, involved in body fluid homeostasis in addition to acid/base balance, and that these effects are gender dependent.

Poster Number 171
ePoster#28

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Simulation Model for Transcervical Laryngeal Injection Providing Real-time Feedback

Investigators: T. A. Hron, J. B. Kobler, G. J. Loan, J. A. Burns

Objective: This study aimed to develop and evaluate a model for teaching transcervical laryngeal vocal fold injections to head and neck surgeons. Transcervical injections are used to administer therapeutic medications and tissue fillers to patients with vocal fold disorders, e.g. botulinum toxin for spasmodic dysphonia.

Methods: A 3-dimensional printer was used to create a laryngotracheal framework based on de-identified computed tomography images of a human larynx. The arytenoid cartilages and intrinsic laryngeal musculature were created in silicone from clay casts and thermoplastic molds. The thyroarytenoid (TA) muscle was created with electrically conductive silicone using metallic filaments embedded in silicone. Wires connected TA muscles to an electrical circuit incorporating a cell phone and speaker. A needle electrode completed the circuit when inserted in the TA during simulated injection, providing real-time feedback of successful needle placement by producing an audible sound. Face validation by the senior author confirmed appropriate tactile feedback and anatomical realism. Otolaryngologists pilot-tested the model and completed pre-simulation and post-simulation questionnaires.

Results: The high-fidelity simulation model provided tactile and audio feedback during needle placement, simulating transcervical vocal fold injections. Otolaryngology residents demonstrated higher comfort levels with transcervical thyroarytenoid injection on post-simulation questionnaires.

Conclusion: This is the first study to describe a simulator for developing transcervical vocal fold injection skills. The model provides real-time tactile and auditory feedback that aids in surgical skill acquisition. Otolaryngologists reported increased confidence with transcervical injection after using the simulator.

Poster Number 172

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Cartilage Status at Time of Hip Arthroscopy Predicts Failure in Patients with Hip Dysplasia

Investigators: M. K. Dwyer, J. Lee, J. C. McCarthy

Long-term survivorship following hip arthroscopy has been shown to be dependent upon the severity of cartilage damage at the time of surgery. However, it is unknown whether cartilage damage at the time of arthroscopy can predict failure in patients with dysplasia. We examined whether cartilage damage at the time of arthroscopy predicted conversion to total hip arthroplasty in patients with dysplasia. We identified 228 hips in 185 patients with dysplasia who underwent hip arthroscopy. The articular cartilage of the posterior, superior, and anterior regions of the acetabulum and femoral head were assessed for signs of chondral damage (absent, mild (grades I or II), or severe (grades III or IV)). Sixty-five patients went on to receive total hip arthroplasty at an average of 3.1±3.1 years after arthroscopy. A stepwise multivariable logistic regression analysis was conducted to determine predictors of the eventual need for joint replacement following hip arthroscopy for patients with dysplasia. Logistic analysis revealed presence of mild changes on the posterior femoral head ($p=0.001$) and presence of severe changes on the anterior acetabulum ($p=0.007$) made a significant contribution to the predictor. Patients with mild arthritic changes of the posterior femoral head were 9.97 times (95%CI:2.62,37.99), while patients with severe arthritic changes of the superior acetabulum were 6.12 times (95%CI:1.66,22.58) more likely to convert to joint replacement. Here we show show that the presence of chondral damage on the posterior femoral head and anterior acetabulum are strong predictors of ultimate conversion to total hip replacement in patients with hip dysplasia.

Poster Number 173

ePoster#29

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Novel fluorescent imaging technology for intraoperative detection of occult peritoneal metastasis of colorectal cancer

Investigators: C. Chan, L. F. Liesenfeld, I. Ferreiro-Neira, J. C. Cusack

Background: Colorectal cancer is the third most common cancer; approximately 10% of patients develop peritoneal metastasis, which is considered incurable. A novel fluorescent imaging technology, which relies on the differential expression of Cathepsin proteases between tumor and normal tissues, has been developed for intraoperative cancer detection. In this study, we aim to evaluate the suitability of this technology for intraoperative detection of colorectal peritoneal metastasis in preclinical models.

Methods: Using quantitative reverse transcription PCR, the expression levels of different Cathepsins (CTS-B, -L1, -S, -K, -D, -G, -O, and -F) were determined in human colon cancer cell lines (HT29, LoVo, SW620) in comparison to internal reference genes (HSPCB, YWHAZ, RPS13) in vitro and in xenografts. The Cathepsin expression profiles of these cell lines were also compared to the one of normal human peritoneal tissues.

Results: While HT29 and SW620 expressed two dominant Cathepsins (CTS-B and -D) in vitro, LoVo had three (CTS-B, -D and -L1). Interestingly, the expression levels of all Cathepsins were markedly increased (average of 6.7 to 19-fold) in the LoVo xenografts, in which CTS-B became the dominant Cathepsin expressed. The overall Cathepsin expression level in the LoVo xenografts was also much higher than normal human peritoneal tissue with a 1.2 to 4.2-fold difference in CTS-B, -L1, -S, and –D expression.

Conclusion: The overall Cathepsin expression was much higher in human colon cancer xenografts than normal peritoneal tissues. This finding supports the use of Cathepsin-based fluorescent imaging technology for intraoperative detection of peritoneal metastasis in colorectal cancer patients.

Poster Number 174

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The Financial Impact of Intraoperative Adverse Events

Investigators:

Importance: Little evidence currently exists regarding the clinical or financial impact of intraoperative adverse events (iAEs)

Objective: To study the additional healthcare charges attributable to the occurrence of an iAE.

Design, Setting and Participants: The administrative and ACS-NSQIP databases at our tertiary academic medical center were linked for all patients undergoing abdominal surgery between January 2007 and October 2012.

Exposure: Intraoperative adverse events (iAEs)

Main Outcome and Measures: The primary outcome was total healthcare charges. Secondary outcomes specifically included direct, indirect, operating room, laboratory, radiology, nutrition, and medical therapy charges. The ICD-9-CM based Patient Safety Indicator "Accidental Puncture or Laceration" was used to screen the linked database for iAEs. All iAEs were subsequently confirmed using a standardized review of all flagged medical records. Multivariate analyses were performed to assess the healthcare charges independently attributable to the occurrence of an iAE.

Results: Of 9111 patients, 183 were confirmed to have iAEs. Multivariate analyses controlling for demographics, preoperative co-morbidities/laboratory values, type/approach of surgery, operative complexity (measured in relative value units), and relevant intraoperative factors demonstrated that iAEs independently increased the total hospitalization charges by 41% [95%CI: 30%-52%] (P<0.001). Specifically, the direct, indirect, operating room, laboratory/ radiology, and alimentation/medical therapy charges increased by 42%, 39%, 27%, 54%, and 48%, respectively (all p<0.001).

Conclusions and Relevance: In addition to the morbidity incurred by patients, the occurrence of an iAE is associated with major additional healthcare charges. In an era of cost-containment, understanding and preventing iAEs can lead to major cost savings.

Poster Number 175

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What factors are associated with second opioid prescription after treatment of distal radius fractures with a volar locking plate?

Investigators: I. M. Schol, T. Teunis, N. Stoop, C. J. Park, D. Ring

Background: Patients who take more opioids after fracture treatment report greater pain intensity and less satisfaction with pain relief. Knowledge of the factors associated with a second opioid prescription might inform better pain management protocols and encourage decreased and safer use of opioids after orthopaedic surgery.

Are demographics, prior opioid prescriptions, injury characteristics, or catastrophic thinking associated with a second opioid prescription following treatment of distal radius fractures with a volar locking plate? And are any of those factors associated with disability or pain at suture removal?

Methods: We used data on 206 patients enrolled in two previous prospective studies. Mean age was 53 years (±SD 15, range 19-89) and 60 (30%) were male. Forty-seven (23%) patients received second opioid prescription. We recorded additional demographics, AO fracture type, ASA classification, radiographic parameters at time of injury prior to reduction and after surgery, and catastrophic thinking.

Results: Using multivariable analysis, male sex (OR 2.4, 95%CI 1.1-5.1, partial pseudo R2=0.024, P=0.022), opioid prescription within 90 days prior to injury (OR 7.0, 95%CI 1.6-32, partial pseudo R2=0.031, P=0.010), and greater dorsal angulation of the articular surface on lateral post-injury radiographs (OR 0.98, 95%CI 0.96-1.0, partial pseudo R2=0.032, P=0.035) were associated with a second opioid prescription after surgery.

Conclusion: One measure of fracture severity, dorsal displacement, was independently associated with a second opioid prescription, but alone acounted for 3.2% of variation. Other factors, as patients' expectation prior to surgery, in particular the realization that injury and surgery hurt, might be adressed in future research.

Poster Number 176

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CD49b and LAG-3 are specific markers of T regulatory Type 1 (Tr1) cells in non-human primates

Investigators: R. Yu, M. Tonsho, P. Spencer, S. Bernard-Stoecklin, G. Benichou, J. C. Madsen

Background: T regulatory Type 1 (Tr1) cells are CD4+CD25-FoxP3- adaptive regulatory T cells that inhibit antigen-presenting cell (APC) activation via secretion of IL-10 and TGF-β and via cell-contact dependent mechanisms mediated through their expression of programmed cell death (PD)-1. In bone marrow transplant recipients, Tr1 cells have been shown to prevent allograft rejections well as GVHD. Recently, CD49b and LAG-3 have been identified as Tr1 specific markers in mice and humans, but it is unknown whether these markers can be used to detect and isolate Tr1 cells in non-human primates.

Method: Mononuclear cells were isolated from the peripheral blood of cynomolgus monkeys(PBMCs). First, co-expression of CD49b and LAG-3 was compared among bona fide CD4+CD25highFoxP3+ Tregs and CD4+CD25-Foxp3-using flow cytometry[figure1]. Second, PD-1 and PD-L1 expressions were analyzed in CD49b/LAG-3 double positive NHP PBMCs.

Results: In cynomolgus monkeys, CD49b and LAG-3 were co-expressed on CD4+CD25-Foxp3- lymphocytes but not on CD4+FoxP3+Tregs. After activation, a significant proportion of CD49b+/LAG-3+CD4+ Tr1 cells also expressed PD-L1 and PD-1.

Conclusion: Our data show that the surface markers CD49b and LAG-3 can be used to distinguish Tr1 from "classical" FoxP3+ Tregs in cynomolgus monkeys.

Poster Number 177

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A Small Molecule Screen to Identify Novel Targets of Hepatic Fibrosis

Investigators: J. Y. Chen, S. R. York, K. Daneshvar, R. T. Chung, A. C. Mullen

Background: Hepatic stellate cells (HSCs) are the primary cell type responsible for hepatic fibrosis, the final common pathway leading to cirrhosis and liver failure for nearly every cause of chronic liver disease. Activation of HSCs in response to injury represents the key step in hepatic fibrogenesis, and is characterized by a phenotypic change from a non-fibrogenic, quiescent HSC to a fibrogenic HSC myofibroblast that secretes extracellular matrix proteins responsible for the fibrotic scar. There are currently no treatments available that are directed at the common endpoint of fibrosis.

Methods: We have developed a small molecule screen to identify compounds that revert fibrotic HSC myofibroblasts to the quiescent phenotype through the quantification of perinuclear lipid droplets with fluorescent microscopy.

Results: Conditions have been optimized in a 384-well plate format using culture in Matrigel as a positive control for reversion of activated HSC myofibroblasts into quiescent HSCs. We have demonstrated the feasibility of this approach by performing an unbiased, high throughput screen using a library of 1600 bioactive compounds. We are currently confirming positive hits by quantifying expression of key genes that mark the fibrotic state, including ACTA2. Cells that exhibit formation of lipid droplets and a loss of ACTA2 expression will be considered quiescent HSCs.

Conclusion: Detection of lipid droplets provides a robust readout to screen for small molecules that can induce activated HSC myofibroblasts that revert to the quiescent HSC phenotype.

Poster Number 178

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Chronic tadalafil treatment attenuates increases in intraocular pressure and retinopathy in sGCα1-deficient mice

Investigators: A. C. Dordea, S. Vandenwijngaert, R. E. Tainsh, K. Allen, W. S. Lieb

Primary open angle glaucoma (POAG) is a progressive eye disease characterized by increased intraocular pressure (IOP) and loss of retinal ganglion cells (RGC). The nitric oxide (NO)-soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) pathway has been associated with POAG: genetic and epidemiological studies implicated impaired NO-cGMP as a possible pathogenic mechanism in POAG, and preclinical studies demonstrated the ability of NO-donor compounds (e.g. latanoprostene bunod) to lower IOP. Also, mice deficient in the subunit of sGC, sGCα1, develop increased IOP and retinal neurodegeneration. We hypothesized that enhancing cGMP signaling with the phosphodiesterase 5 inhibitor tadalafil (Cialis) would attenuate the development of glaucoma in sGCα1-deficient mice.

In a pilot study, female sGCα1-deficient mice were fed a tadalafil-containing diet or control diet (n=7 and n=8, respectively) for eight months. IOP was measured bimonthly under isofluorane anesthesia. At the end of the study, eyes were harvested and RGCs were stained with βIII-tubulin for density assessment.

The IOP increase, typically observed in sGCα1-deficient mice, was attenuated in tadalafil-fed mice: over an eight month period, IOP increased 2±1 mmHg in tadalafil-fed compared to 5±1 mmHg in control mice ($P<0.01$). Additionally, RGC counts were higher in 12-month old tadalafil-fed sGCα1-deficient mice compared to age-matched control-fed mice (51±1 vs. 48±1 counts/mm2, respectively, $P<0.05$).

Here we show, that the use of tadalafil attenuated the increase in IOP and neurodegeneration in sGCα1-deficient mice. Tadalafil may therefore serve as a potential therapeutic in glaucoma, particularly in patients presenting with dysfunctional NO-sGC signaling.

Poster Number 179

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PI3K inhibition induces homologous repair defects in endometrioid endometrial cancer patient derived xenografts leading to synergistic anti-tumor activity with PARP inhibitors

Investigators: T. Hall, C. DiGloria, M. Kim, L. Zhang, R. Tambouret, D. R. Borger, R. Foster, W. B. Growdon, B. R. Rueda

Background: Inhibition of the phosphatidylinositol 3-kinase (PI3K) pathway may induce defective homologous repair in breast carcinoma leading to heightened sensitivity of poly-ADP ribose polymerase (PARP) inhibitors. Our objective was to test single agent and combined PI3K and PARP inhibition in endometrial cancer (EnCa).

Methods: Under an institutional protocol, tumors from consenting patients with EnCa were collected at the time of primary surgery and genotyped for common oncogenic mutations utilizing the SNaPshot® platform. Four arm cohorts of NOD/SCID mice bearing xenografts derived from two primary human endometrioid endometrial tumors with and without PIK3CA gene mutations (ENCA1 & ENCA2 respectively) were treated with vehicle or NVP BKM-120 (30 mg/kg) alone and in combination with veliparib (25 mg/kg) and tumor volumes were assessed. Immunoblotting was used to assess molecular alterations in post treatment samples at specific time points.

Results: Two PDX lines were identified, one with a known PIK3CA mutation (R88Q), one with no mutation detected. While NVP BKM120 as a single agent failed to induce significant anti-tumor activity in either model, decreased pAKT, RAD51 and increased γ-H2AX levels were observed. Dual NVP BKM-120 and veliparib therapy significantly impeded tumor growth ($p < 0.01$) in the ENCA1 model, while in ENCA2, significant tumor regression was observed ($p < 0.001$). Combination therapy was associated with reconstitution of baseline RAD51 and increased γ-H2AX levels.

Conclusion: These results suggest PI3K inhibition sensitizes endometrial tumors with and without PIK3CA mutations to a PARP inhibitor possibly due to induced defects in homologous repair mediated by RAD51.

Poster Number 180

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Phosphorylated HER3 associated with trastuzumab resistance in HER2 gene amplified uterine serous carcinoma xenograft tumors

Investigators: F. Hernandez, C. Digloria, J. Groeneweg, D. Borger, R. Foster, B. Rueda, W. Growdon

Background: Uterine serous carcinoma (USC) is an aggressive subtype of endometrial cancer that commonly harbors HER2 gene amplification though clinical trial has demonstrated that USC is impervious to trastuzumab therapy.

Methods: Cohorts of mice harboring xenografts derived from HER2 gene amplified (HER2:Chr 17 > 2.0) non-immortalized USC cell line ARK2 were treated with either vehicle, trastuzumab (10 mg/kg IP BIW), lapatinib (150 mg/kg QD oral gavage) or the combination of trastuzumab and lapatinib for 21 days. Acute and chronic post treatment tumors were assessed for downstream signaling alterations.

Results: Single agent trastuzumab failed to impact xenograft growth and induced rapid elevation in pHER3 levels with unchanged pAKT and pERK expression following acute and chronic treatment. Lapatinib alone resulted in tumorstatic effects ($p < 0.01$) and dual therapy with trastuzumab and lapatinib induced synergistic activity that decreased tumor volume compared to all other arms ($p < 0.01$). Anti-tumor activity observed in the lapatinib and dual lapatinib/trastuzumab arm was not associated with any alterations in pHER3 levels following 21 day treatment, though acute elevations in pHER3 were noted in the dual HER2 blockade arm at 24 hours after treatment.

Conclusion: In HER2 amplified USC, innate trastuzumab resistance was associated with an elevation in pHER3 levels following acute and chronic treatment. In contrast, treatment arms that utilized lapatinib demonstrated significant anti-tumor activity without increase in pHER3 over the course of treatment. These data highlight HER3 activation as a possible trastuzumab resistance mechanism in USC that can be abrogated through the addition of lapatinib.

Poster Number 181

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New-Onset Diabetes Mellitus is Encountered during the Development of a Non-Human-Primate Heart Transplantation Tolerance Protocol

Investigators: K. A. Matthews, M. Tonsho, J. S. Allan, J. C. Madsen

Background: The most robust non-human primate transplant tolerance models have been based on a protocol that involves 4 months of post-transplant triple-drug immunosuppression, followed by bone marrow transplantation with subsequent immunosuppressive withdrawal. Despite considerable experience with this paradigm in transplantation using the Cynomolgus macaque (Macaca fasicularis), side effects are rarely observed. We report here a case of new-onset diabetes arising in a macaque while on triple-drug therapy following an en bloc heterotopic heart and thymus transplant.

Methods: A 5.5 year old male cynomolgus macaque underwent an en bloc heterotopic heart and thymus transplant, while on a post-transplant immunosuppressant regimen consisting of tacrolimus, mycophenolate mofetil, and methylprednisolone. The animal was monitored daily and weighed weekly, with clinical testing performed when indicated.

Results: Fifty-seven days post transplant, the macaque exhibited anorexia and weight loss. The blood glucose (BG) was 727 mg/dl and the HbA1c was 10.1%. Also noted were glucosuria and ketonuria. A diagnosis of diabetes mellitus was made and insulin therapy was initiated. The animal's clinical condition improved, and immunosuppression was weaned off per protocol. Seventy-four days after discontinuation of immunosuppression, BG normalized and the insulin therapy was stopped. The animal's BG and HbA1c have since remained within normal limits.

Conclusions: Reported here is a unique case of new-onset diabetes mellitus during triple-drug therapy—a condition commonly observed in human transplant patients, but not previously described in the non-human primate models. We assume that the development of diabetes mellitus is related to our use of methylprednisolone and tacrolimus in relatively high-doses.

Poster Number 182

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IL-6 signaling blockade enhances regulatory T cell activation and proliferation in giant cell arteritis

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Objectives: We aimed to characterize the effector and regulatory CD4+ T-cell compartments in the peripheral blood of GCA patients treated with tocilizumab (TCZ).

Methods: We evaluated 37 GCA patients classified into one of three categories: 1) active disease (aGCA, n=9); 2) disease remission on corticosteroid (CS) monotherapy (rGCA-CS, n=17); and 3) disease remission on TCZ therapy (rGCA-TCZ, n=11). Nine healthy controls were also included. Using flow cytometry, we determined the percentages (%) of IFN+IL-17- (Th1), IL-17+IFN- (Th17), CD25high (Treg), and CD45RA-Foxp3high (activated Treg, aTreg) cells within the CD4+ lymphocyte population. In addition, we determined the % of Foxp3+ cells expressing Ki67 (proliferating Treg). We assessed Treg function in suppression assays. Serum concentrations of IL-12, IFNγ, IL-6, IL-1β, IL-23, IL-21, TNF-α, CCL20, IL-17A, and IL-10 were measured by Luminex.

Results: The frequencies (mean %) of Th1, Th17, and Treg cells were equivalent across groups. However, the frequency of aTregs was higher in rGCA-TCZ patients (1.3%) compared with rGCA-CS patients (0.7%; p=0.03). Moreover, the number of proliferating Tregs was significantly greater in rGCA-TCZ patients (31.7%) compared to rGCA-CS (15.6%; p=0.003) and aGCA (15.5%; p=0.007) patients. Tregs were functional in all groups. IL-10 levels were significantly increased in the serum of patients with aGCA. There were no significant differences in the serum levels of other cytokines or chemokines measured.

Conclusions: The therapeutic effects of blocking IL-6 receptor signaling in GCA could be mediated by increasing the activation and proliferative potential of Tregs. Further studies are needed to expand these preliminary findings.

Poster Number 183

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Increasing IKAP expression by mRNA splicing modification improves phenotype in a mouse model of Familial Dysautonomia

Investigators: E. Morini, P. Dietrich, M. Salani, M. Nilbratt, I. Dragatsis, S. A. Slaugenhaupt

Familial dysautonomia (FD) is a recessive neurodegenerative disease caused by a splice-mutation in IKBKAP gene which leads to variable skipping of exon 20. We found that kinetin can correct the IKBKAP miss-splicing 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, including brain. Despite these remarkable advances we lacked an animal model in which to test the effect of increasing IKAP on the FD phenotype. In order to create a phenotypic model of FD in which we could manipulate mRNA splicing, we introduced the FD transgene, carrying the human IKBKAP gene with the major FD splice mutation, into Ikbkapdelta20/flox mouse model. FD9/Ikbkapdelta20/flox mouse recapitulates several phenotypic features observed in FD patients, including reduced growth rate, reduction in the number of fungiform papillae, spinal abnormalities, and reduction in the volume of the sympathetic stellate and dorsal root ganglia. The creation of this new model has allowed us to initiate a preclinical trial of kinetin and will permit testing of other strategies aimed at either targeting mRNA splicing or increasing expression of IKBKAP.

Our preliminary results show that kinetin leads to improved IKBKAP splicing and has beneficial effects on the overall growth of FD embryos and adult mice. The completion of this study will allow us to evaluate the spectrum of benefits that modification of IKAP levels by kinetin and other drugs may offer FD patients.

Poster Number 184

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Modulation of soluble mutant huntingtin in blood by PBT2 in the REACH2HD Trial

Investigators: M. Moscovitch-Lopatin, D. Oakes, S. Gao, R. Dorsey, D. Angus, C. Herd, S. M. Hersch, M. Moscovitch-Lopatin, D. Oakes, S. Gao, R. Dorsey, D. Angus, C. Herd, S. M. Hersch, Investigators of REACH2HD

PBT2, a metal attenuating compound with affinity to copper and zinc, has been shown to reduce aggregation of mutant (mt) huntingtin (Htt), extend survival of the R6/2 mouse model of Huntington disease (HD) and improve executive function in Alzheimer disease. In the REACH2HD study, PBT2 was administered double- blinded for 26 weeks to 109 adults with mild to moderate HD, randomized (1:1:1) to PBT2 250 mg once daily, PBT2 100 mg once daily, or placebo at 19 HSG sites in Australia and the USA. We examined the effects of PBT2 treatment on leukocyte huntingtin from blood samples of the subjects participating in REACH2HD. Using a multiplex of antibodies against three epitopes on the mtHtt, we determined by Homogeneous Time Resolved Fluorescence the levels of soluble mtHtt and total (t) endogenous Htt in leukocytes lysates. The soluble mtHtt and tHtt protein normalized values were significantly increased at 12 weeks in subjects taking 100 mg ($p=0.01$ and $p=0.026$) and 250 mg ($p=0.009$ and $p=0.021$) of PBT2 compared to placebo. At 26 weeks, there were no differences in the treatment groups compared to placebo. These findings suggest that PBT2, at 100 mg or 250 mg daily, may directly impact Htt as a therapeutic target, at least in the periphery.

Poster Number 185

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Inhaled Nitric Oxide: an sGC-dependent IOP lowering agent

Investigators: S. Muenster, W. S. Lieb, A. C. Dordea, S. Vandenwijngaert, R. Tainsh, W. M. Zapol, E. S. Buys

The nitric oxide (NO)-soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) pathway regulates intraocular pressure (IOP). Preclinical and clinical studies demonstrated the ability of NO-donor compounds to lower IOP. The use of inhaled NO gas (iNO) is an approved therapy for pulmonary hypertension and is under development as a treatment for other cardiovascular diseases. We hypothesized that breathing NO lowers IOP in an sGC-dependent manner.

IOP was measured in wild type (WT) mice and mice deficient in the α1 subunit of sGC (sGCα1-/- mice) anesthetized with isoflurane (n=9, each), breathing either control gas (N2/O2) or 40 ppm NO in O2. IOP was also measured in awake WT breathing either control gas or 40 ppm iNO for 40 minutes (n=8, each).

Breathing control gas did not affect IOP in WT or sGCα1-/- mice. Breathing iNO decreased IOP in both anesthetized WT mice (9.86±0.31 vs 8.42±0.51 mmHg at baseline and after iNO, respectively) and awake WT mice (14.13±1.95 vs 10.93±1.01 mmHg, at baseline and after 40 min iNO, respectively). In contrast, iNO did not lower IOP in sGCα1-/- mice (9.75±0.31 vs 9.46±0.30 mmHg at baseline and after iNO, respectively)

These findings confirm that NO is an IOP-lowering agent and identify NO-gas as a possible therapeutic approach to acutely lower IOP. In addition, our results identify sGC as the downstream target of NO's ability to lower IOP. sGC stimulators, under development for treatment of cardiovascular diseases, such as the recently approved ADEMPAS®, may be considered as a novel treatment option for elevated IOP.

Poster Number 186

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High HLA class I antigen expression in combination with low PD-L1 expression as a favourable prognostic biomarker in intrahepatic cholangiocarcinoma

Investigators: F. Sabbatino, V. Villani, J. H. Yearley, L. Cai, V. Deshpande, I. T. Konstantinidis, S. Nota, Y. Wang, A. X. Zhu, L. Goyal, D. T. Ting, N. M. El-Bardeesy, T. S. Hong, K. K. Tanabe, C. Moon, S. Ferrone, K. D. Lillemoe, C. R. Ferrone

Novel effective therapies are needed for intrahepatic cholangiocarcinoma (ICC). Immunotherapy with checkpoint molecule-specific monoclonal antibodies (mAbs), such as programmed cell death-1 (PD-1) or programmed cell death-ligand 1 (PD-L1), is effective in ~30% of patients with various solid tumors. For this type of immunotherapy to be effective, patients must be able to mount an immune response to their own tumors. Therefore, the HLA class I antigen processing machinery must be functional in the targeted tumor cells. This background has prompted us to investigate whether ICC patients may benefit from this type of immunotherapy. Tumors from ICC patients were analyzed for i) lymphocyte infiltrate as a measure of a patient's immune response against their own tumor, ii) HLA class I antigen expression, and iii) PD-1 and PD-L1 expression by T cells and ICC cells, respectively. Our data demonstrates that ICC patients can mount a T cell immune response against their own tumor. The efficacy of this anti-tumor immune response is likely to be weakened by the increased PD-L1 expression and presence of HLA class I antigen defects we have observed. These mechanisms provide ICC cells with escape mechanism(s) from immune recognition. This mechanism i) accounts for the potential role of HLA class I antigen and PD-L1 expression levels as prognostic biomarkers in ICC and ii) provides a strong rationale for implementing immunotherapy with checkpoint molecule-specific mAbs in patients bearing ICC tumors without defects in HLA class I antigen expression.

TRANSLATIONAL MEDICINE & EXPERIMENTAL THERAPEUTICS

Poster Number 187

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MYC Gene Amplification Renders Cancers Hypersensitive to NAD+ Depletion

Investigators: K. Tateishi, A. J. Iafrate, Q. Ho, W. T. Curry, T. T. Batchelor, K. T. Flaherty, S. Sundaram, D. P. Cahill, H. Wakimoto, A. S. Chi

MYC is a potent oncogene that drives an aberrant cancer metabolic state adapted for constitutive production of biomolecular precursors to feed rapid tumor cell growth. Here, we demonstrate that cancers genetically driven to activate Myc are exquisitely sensitive to depletion of the metabolite nicotinamide adenine dinucleotide (NAD+), and we identify inhibition of the NAD+ salvage enzyme nicotinamide phosphoribosyl-transferase (NAMPT) as a strikingly selective therapeutic strategy for MYC-amplified cancers. In a panel of patient-derived glioblastoma (GBM) tumorsphere lines, low nanomolar cytotoxicity with NAMPT inhibition was restricted to lines harboring MYC or MYCN gene amplification. NAMPT inhibition significantly inhibited Myc-driven xenograft growth, including invasive intracerebral GBM tumorsphere models. As a basis for this therapeutic selectivity, we found that Myc reprograms NAD+ metabolism. Basal NAD+ levels were significantly lower in MYC/MYCN-amplified GBM tumorsphere cells, while forced expression of c-Myc significantly reduced NAD+ levels in non-MYC-amplified cells and generated a marked dependency on NAD+.

Poster Number 188

ePoster#30

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Heart En Bloc Thymus Transplantation Permits Long-Term, Acute Rejection-Free Cardiac Allograft Survival in Nonhuman Primates (NHPs)

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The role of vascularized thymus on tolerance induction has been well studied in swine recipients of kidney allografts, but not in NHP recipients of heart allografts. Here we investigate whether co-transplantation of vascularized donor thymus would prolong the survival of allogeneic cardiac allografts without chronic immunosuppression.

MHC-mismatched heart en bloc thymus allografts were transplanted into thymectomized cynomolgus macaques treated with equine ATG, anti-IL-6R mAb and conventional immunosuppression, consisting of tacrolimus, mycophenolate mofetil, and methylprednisolone. Starting on POD 166, the immunosuppressive drugs were slowly weaned; and by POD 207, all drugs were stopped. Thymopoiesis was monitored with T cell receptor excision circles (TREC), as well as phenotypic markers of peripheral recent thymic emigrants. The alloreactive T cell and B cell responses were serially assessed, and the grafts were examined by serial biopsies.

The first animal treated this way has achieved acute rejection-free cardiac survival with preserved cardiac function for over 537 days. Thymopoiesis by the donor thymus was detected early after transplantation and donor-specific T cell hyporesponsiveness was observed in IFN-γ ELISPOT. The recipient developed alloantibody and allograft vasculopathy by day 300, but never demonstrated evidence of acute cellular rejection. Two other recipients are currently >97 and >57 days posttransplant without signs of acute rejection.

This is the first demonstration of long-term cardiac allograft survival induced by the co-transplantation of vascularized donor thymus in nonhuman primates. The apparent split tolerance achieved by this strategy may be overcome by adding a B cell depleting agent to the protocol.