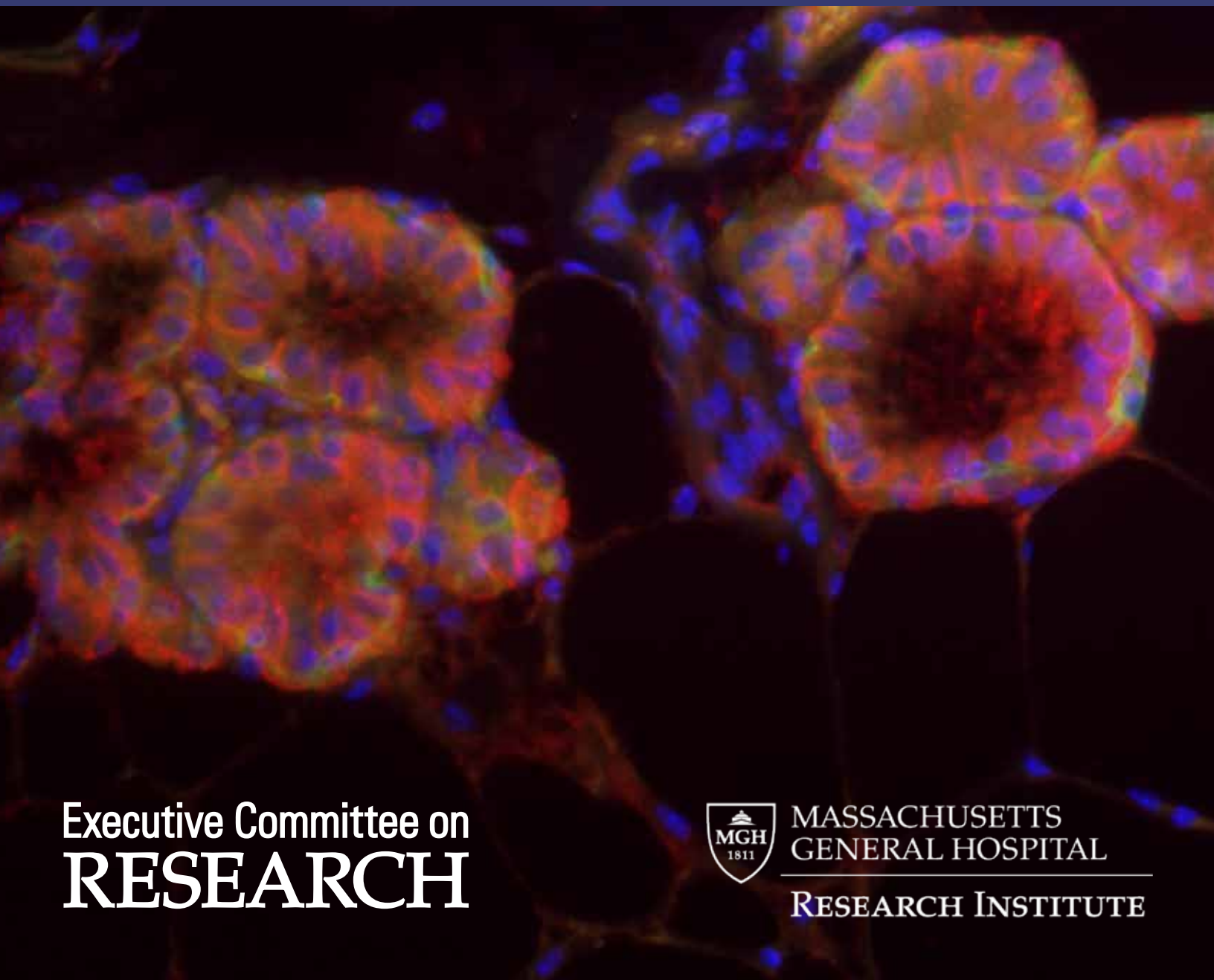
70th Annual Meeting of the MGH Scientific Advisory Committee

SAC 2018

March 28, 2018 | Simches Research Building

Poster Session Abstracts



Executive Committee on RESEARCH

MASSACHUSETTS GENERAL HOSPITAL
RESEARCH INSTITUTE

Contents

Agenda

Wednesday, March 28, 2018
Simches 3.110

Celebration of SCIENCE

10:00 am - 1:30 pm — **POSTER SESSION** — *Simches, Floor 2*
10:00 - 11:30 am Session 1
12:00 - 1:30 pm Session 2

2:00 - 5:00 pm — **CELEBRATION OF SCIENCE** — *Simches, 3.110*

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

Opening Remarks
David N. Louis, MD, Chair, Executive Committee on Research (ECOR)

2:15 - 2:55 pm — **ECOR Report**
David N. Louis, MD, Chair, ECOR

2:55 - 3:30 pm — **2018 Goodman Fellowship**
Role of heterochromatin in cellular dormancy
Mo Motamedi, PhD

3:30 - 3:50 pm — **BREAK**

3:50 - 4:25 pm — **2018 Martin Prize for Clinical Research**
Hematopoietic Stem-Cell Gene Therapy for Cerebral Adrenoleukodystrophy
Florian S. Eichler, MD

4:25 - 5:00 pm — **2018 Martin Prize for Fundamental Research**
Cancer-Specific Retargeting of BAF Complexes by a Prion-like Domain
Miguel N. Rivera, MD

Agenda

Thursday, March 29, 2018
Simches 3.110

9:00 - 9:05 am — **Welcome and Introduction**
David N. Louis, MD, Chair, Executive Committee on Research (ECOR)

9:05 - 9:55 am — **Wyss Institute: A New Model for Crossing the Academic-Industrial Interface**
Donald E. Ingber, MD, PhD, Founding Director, Wyss Institute for Biologically Inspired Engineering at Harvard University

9:55 - 10:25 am — **Overview of the Research Institute**
Harry W. Orf, PhD, Senior Vice President for Research
Susan A. Slaugenhaupt, PhD, Scientific Director, MGH Research Institute (RI)

10:25 - 11:05 am — **Externally Promoting the Research Institute**
Moderator: Susan A. Slaugenhaupt, PhD, Scientific Director, MGH RI
Panelists: Misty Hathaway, Chief Marketing Officer
Peggy Slasman, Senior Vice President, Public Affairs

11:05 - 11:15 am — **BREAK**

11:15 - 11:55 am — **Fundraising for the Research Institute: Advancement Session**
Moderator: Robert E. Kingston, PhD, Chief, Molecular Biology
Panelists: Susan Buchanan, Senior Director of Development
Britain W. Nicholson, MD, Senior Vice President for Development
Susan A. Slaugenhaupt, PhD, Scientific Director, MGH Research Institute (RI)

12:00 - 1:00 pm — **SAC MEMBERS & FACULTY LUNCH**

1:15 - 1:55 pm — **Partnering with Industry: Strategic Alliances Session**
Moderator: Bob Tepper, MD, Third Rock Ventures, Research Institute Advisory Council Member
Panelists: Gabriela Apiou, PhD, Director, Translational Research Training & Development, MGH RI
Jean-Francois Formela, MD, Atlas Venture, Research Institute Advisory Council Member
Patrick Fortune, PhD, Vice President for Market Sectors, Partners HealthCare
Rajiv Kaul, Fidelity Investments, Research Institute Advisory Council Member

1:55 - 3:00 pm — **Promoting and Sustaining the Clinical Research Mission**
Maurizio Fava, MD, Director, Division of Clinical Research
Mason W. Freeman, MD, Director and Founder, Translational Research Center
David M. Nathan, MD, Program Director, Clinical Research Center

3:00 - 3:15 pm — **Open Discussion**

3:15 - 3:30 pm — **BREAK**

3:30 - 4:00 pm — **SAC Member Session** *(closed)*

4:00 - 4:30 pm — **Executive Debrief** *(Leadership & SAC Members)*

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

Christopher Donna - ***What is the effect of vinegar on metal rust?***
(Michael Duggan, General Academic Pediatrics)

Sheryl Depina Eccles - ***What is the effect of temperature on how well crystals grow?***
(Tanya Behnan, Cancer Center)

Sausha Jones - ***What is the effect of different crystal ingredients on crystal structure?***
(Erin McGivney, Executive Committee on Research)

Ashayla Scott - ***What is the effect of gender on reaction time?***
(Phoebe Finneran & Caroline Harley, Cardiology Research, Center for Genomic Medicine)

8th Grade Students

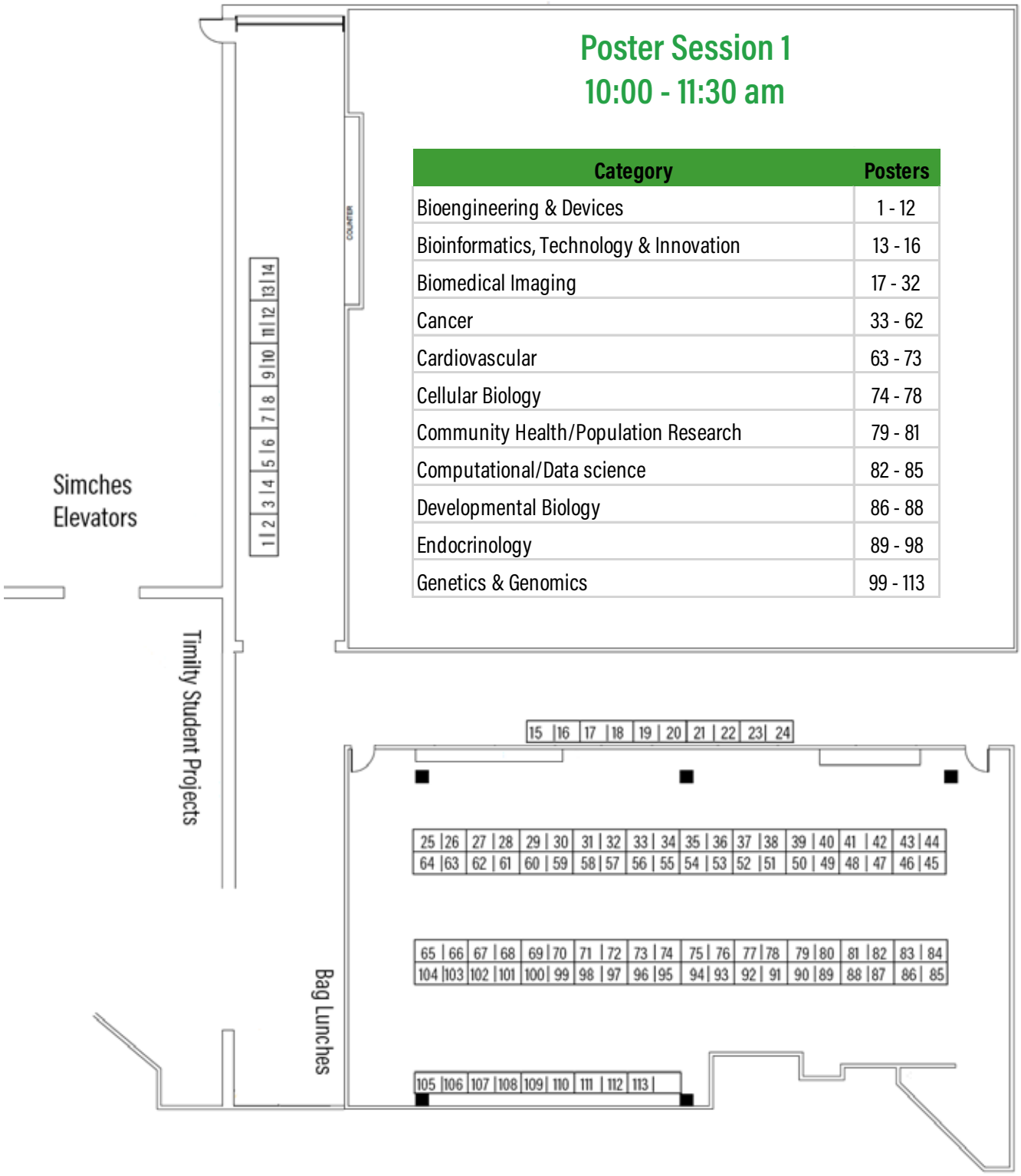
Helina Abrha - ***What are the solubility rates of different painkillers?***
(Wan Yee Leong, Cancer Center)

Randy Luciano Estrella - ***What is the effect of salt on different frozen liquids?***
(Hans Erickson, Neurology)

Mariah Saunders - ***What is the effect of age on heart rate and oxygen levels?***
(Mia Bertalan & Alex Kaplan, Cancer Center)

Angelis Pena Tejeda - ***What affects the consistency of slime?***
(Miranda Lawell & Liz Weyman, Cancer Center)

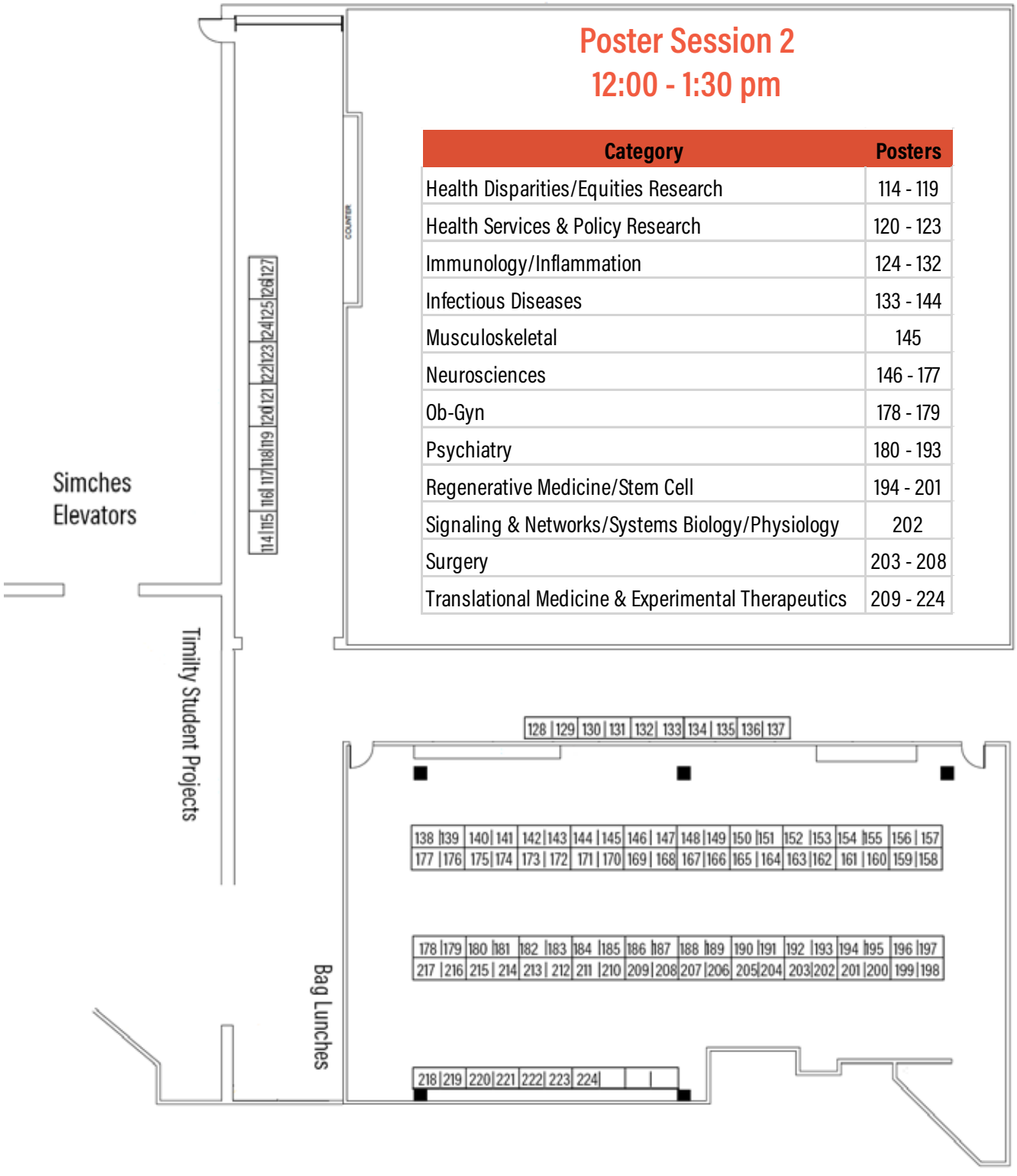
Session 1 Floor Plan



Poster Session 1
10:00 - 11:30 am

Category	Posters
Bioengineering & Devices	1 - 12
Bioinformatics, Technology & Innovation	13 - 16
Biomedical Imaging	17 - 32
Cancer	33 - 62
Cardiovascular	63 - 73
Cellular Biology	74 - 78
Community Health/Population Research	79 - 81
Computational/Data science	82 - 85
Developmental Biology	86 - 88
Endocrinology	89 - 98
Genetics & Genomics	99 - 113

Session 2 Floor Plan



Poster Session 2
12:00 - 1:30 pm

Category	Posters
Health Disparities/Equities Research	114 - 119
Health Services & Policy Research	120 - 123
Immunology/Inflammation	124 - 132
Infectious Diseases	133 - 144
Musculoskeletal	145
Neurosciences	146 - 177
Ob-Gyn	178 - 179
Psychiatry	180 - 193
Regenerative Medicine/Stem Cell	194 - 201
Signaling & Networks/Systems Biology/Physiology	202
Surgery	203 - 208
Translational Medicine & Experimental Therapeutics	209 - 224

Posters

SESSION 1 POSTERS

Presented 10:00 - 11:30 am

Bioengineering & Devices

Bioinformatics, Technology & Innovation

Biomedical Imaging

Posters

Cancer

Posters

Cardiovascular

Posters

Genetics & Genomics

SESSION 2 POSTERS

Presented 12:00 - 1:30 pm

Health Disparities/Equities Research

Health Services & Policy Research

Immunology/Inflammation

Infectious Diseases

Posters

Musculoskeletal

Neurosciences

Ob-Gyn

Posters

Psychiatry

Regenerative Medicine/Stem Cell

Signaling & Networks/Systems Biology/ Physiology

Surgery

Translational Medicine & Experimental Therapeutics

Alphabetical Listing of all Poster Presenters

Bioengineering & Devices

Poster Number 1

Paul Arauz, PhD
Orthopedics, Research Fellow | parauz@mgh.harvard.edu
3-Dimensional In-Vivo Analysis of Gait Symmetry in Bilateral Total Hip Arthroplasty

INVESTIGATORS: P. Arauz, Y. Peng, J. MacAuliffe, Y. Kwon

Three-dimensional (3D) asymmetry has been reported in unilateral total hip arthroplasty (THA) patients during gait. Nevertheless, it is not well understood whether asymmetric hip kinematics during gait persist in bilaterally operated THA patients. The purpose of this study was to compare the in vivo 3D kinematics and component placement between bilateral and unilateral THA patients during gait. Eight bilateral and thirty-three unilateral THA patients were evaluated for both hips during treadmill gait using a validated combination of 3D computer tomography-based modeling and dual fluoroscopic imaging system (DFIS). The in vivo 3D kinematics of the unilateral THA group was first assessed. Then, the magnitudes of kinematics and component placement difference between implanted hips in the bilateral THA group and between the implanted and non-implanted hips in the unilateral THA group were compared. Results showed asymmetric gait kinematics in the unilateral THA group. Although the magnitude of kinematics differences between sides for both the bilateral and unilateral THA groups did not change significantly for hip rotations ($p > 0.05$), the bilaterally operated THA group has significantly lower magnitude of hip gait translation difference. Significant reduction in the magnitude of the acetabular cup adduction, stem adduction, and combine hip anteversion and adduction difference was observed in the bilateral THA group ($p < 0.05$). Our findings demonstrated that despite significant improvements of component placement and reduced magnitude of hip gait translation difference between implanted hips in the bilateral THA group; asymmetric hip kinematic rotations persisted in patients with bilateral THA during gait.

Poster Number 2

Beyza Bulutoglu, PhD
Surgery, Research Fellow | bbulutoglu@mgh.harvard.edu
Elucidation of Drug-Drug Interactions at the Transcriptional Level in Hepatocytes

INVESTIGATORS: B. Bulutoglu, C. Rey Bedon, S. Mert, O. B. Usta, M. Yarmush

More than 75% of the elderly population takes multiple drugs in the United States and adverse drug reactions are one of the leading causes of drug-related deaths. Among these reactions, drug-drug interactions (DDI) arise when one drug alters the metabolism of the other. Moreover, the two drugs can synergistically affect molecular events that result in toxicities. The presented study aims to understand the role of transcription factors in DDIs. Reporter plasmids were constructed possessing the response elements of common transcriptional regulators of cythochrome P450 enzymes, including CYP3A4. Cell lines, e.g. Huh-7, as well as primary hepatocytes were transfected with reporter plasmids bearing the response elements of various transcriptional factors such as pregnane X receptor, reporting on the transcriptional activation of enzymatic activity upon exposure to different drugs. The changes in the transcription of these receptors were monitored via the expression levels of a green fluorescent protein. In addition, a second fluorescent protein was employed as an internal reference for normalization. This protein was included in the same construct, resulting in a dual-promoter reporter plasmid transfected into mammalian cells. This way, different signatures of the regulatory proteins were collected upon exposure to various conditions. The latest results on the role played by transcriptional factors in the regulation of DDIs will be presented.

Poster Number 3

Avilash Cramer, BS
Radiology, Graduate Student | avilash@mit.edu
Static Computed Tomography

INVESTIGATORS: A. K. Cramer, X. Lai, D. Wu, T. Moulton, W. Krull, R. Gupta

Computed tomography (CT) is the clinical standard for diagnosing many emergent medical conditions, such as stroke and traumatic brain injuries. Unfortunately, the size, weight, and expense of CT systems make them inaccessible for patients outside of large trauma centers. We have designed a multiple source x-ray module that would allow for CT scanners to be significantly lighter weight and cheaper, as it has no moving parts. This could expand access to this valuable diagnostic tool to rural and low-income communities, battlefield care, and extended space missions. As part of this system, we present a miniaturized photocathode-based x-ray source, created by depositing a thin film of magnesium on an electron amplifier. When illuminated by a UV LED, this photocathode emits a beam of electrons, with a maximum beam current of up to 500 uA per amplifier. The produced electrons are then accelerated through a high voltage to a tungsten target. These sources individually addressable and can be pulsed rapidly, through electronic control of the LEDs. Seven of these sources comprising a 17.5 degree arc are housed together within a custom vacuum manifold. A full ring of these modules could be used for CT imaging. By turning the sources on and off one after another in series, we are able to demonstrate limited-angle x-ray tomography without any moving parts. With a clinical flat-panel detector, we demonstrate 3D reconstructions of several biological samples.

Poster Number 4

Felix Ellett, PhD
Surgery, Research Fellow | fellett@mgh.harvard.edu
Accurate diagnosis of sepsis from a drop of blood based on neutrophil motility signatures in a microfluidic assay

INVESTIGATORS: F. Ellett, J. Jorgensen, A. L. Marand, Y. M. Liu, M. M. Martinez, V. Stein, K. L. Butler, J. Lee, D. Irimia

Current sepsis diagnostics have limited precision and sepsis is regularly misdiagnosed. Microbiological tests help diagnose sepsis, but are usually too slow to have an impact on timely clinical decision making. Recently, neutrophils, the most numerous white blood cells in circulation, have come under closer scrutiny in the context of sepsis for their high sensitivity to infections. However, measurements of surface markers e.g. CD64, genomic changes, and phenotype alterations have had only marginal effect on improving sepsis diagnosis. Here, we present a microfluidic assay that measures neutrophil spontaneous motility with high precision using one droplet of blood. We applied this assay to patients suspected of sepsis, in two prospective studies, on two independent cohorts of critically ill patients. In the first cohort, we applied machine-learning approaches to develop a scoring system (Sepsis Score) that segregated patients with sepsis from those without sepsis. We found that testing neutrophils in the context of plasma is key for differentiation between septic and non-septic patients, suggesting that the interactions between neutrophils and their blood microenvironment are important for the diagnostic. In the second cohort, we validated the Sepsis Score in a double-blinded, prospective case-control study. Overall, the assay provided sepsis diagnosis with 96.8% sensitivity and 97.6% specificity for the two cohorts studied (n = 42). Future studies will help test the practical utility of the neutrophil assay for the accurate diagnosis and monitoring of sepsis in larger populations of patients at risk.

Poster Number 5

Julian Goldman, MD
Anesthesia, Critical Care and Pain Medicine, Instructor | jmgoldman@mgh.harvard.edu
Building an Advanced Facility for Medical Device Interoperability and Security Research

INVESTIGATORS: J. M. Goldman, D. Arney, D. Guffrey, D. Bagshaw

Since 2004 we have been researching technology, standards, and methods to enable safe, secure interoperability of medical devices for next-generation clinical and research applications. We recently completed building a new laboratory facility for working on medical device interoperability and security. We discuss the design and capabilities of the space, the types of work it facilitates, several research projects it supports, and the myriad constraints that were balanced in designing and building the facility.

Poster Number 6

Mehdi Jorfi, PhD
Surgery, Research Fellow | mjorfi@mgh.harvard.edu
Reconstructing Alzheimer's Amyloid and Tau Pathology in 3D Homogeneous Arrays of Human Neurospheroids

INVESTIGATORS: M. Jorfi, C. D'Aanzo, R. E. Tanzi, D. Y. Kim, D. Irimia

Neurospheroids serve as a widely accepted in vitro platform for disease modeling and drug screening. However, current approaches to recreate neurodegenerative diseases in a dish using neurospheroids rely on mixtures of spheroids that are heterogeneous in size, which limit their applications in basic mechanistic studies and drug screening. Here, we show the in vitro culture of uniformly-sized stem-cell-derived human neurospheroids in large arrays, where they can be monitored for months, and closely recapitulate key hallmarks of familial Alzheimer's disease including pathogenic accumulation of amyloid-β (Aβ) and phosphorylated tau. The three-dimensional (3D) microarray system generates uniform-sized neurospheroids, with less than 1% variability in diameter in a 96-well platform. This performance is key to measuring with unprecedented precision the efficacy and side-effects of Aβ modulating drugs in large scale arrays. We also observed accumulation of amyloid-β and pathogenic phosphorylated tau species after 7-8 week-differentiation in our 3D neurospheroid model of Alzheimer's disease, not in the control 3D spheroids. This accumulation of amyloid-β was blocked by β-secretase inhibitor treatment. To further extend the capability of our array platform and accelerate drug screening of the human neurospheroids for drug discovery, we leveraged microfabrication and 3D printing techniques to develop a 96-well microarray with 1,536 microwells. Using this array, we generated uniformly-sized neurospheroids and treated with various compounds including –secretase inhibitor, –secretase inhibitor, –secretase modulator, Imidazenil, and Methotrexate for various concentrations. We have also confirmed our microarrays can be used for differentiating and modeling disease phenotype using human iPSC-derived neurospheroids.

Poster Number 7

Young Bok (Abraham) Kang, PhD
Surgery, Research Fellow | heavenboki@gmail.com

Metabolic Patterning on a Chip: Towards in vitro Liver Zonation of Primary Rat and Human Hepatocytes

INVESTIGATORS: Y. Kang, J. Eo, S. Mert, M. L. Yarmush, O. B. Usta

An important number of healthy and diseased tissues shows spatial variations in their metabolic capacities across the tissue. The liver is a prime example of such heterogeneity where the gradual changes in various metabolic activities across the liver sinusoid is termed as "zonation" of the liver. Here, we introduce the Metabolic Patterning on a Chip (MPOC) platform capable of dynamically creating metabolic patterns across the length of a microchamber of liver tissue via actively enforced gradients of various metabolic modulators such as hormones and inducers. Using this platform, we were able to create continuous liver tissues of both rat and human origin with gradually changing metabolic activities. The gradients we have created in nitrogen, carbohydrate and xenobiotic metabolisms recapitulated an in vivo like zonation and zonal toxic response. Beyond its application in recapitulation of liver zonation in vitro as we demonstrate here, the MPOC platform can be used and expanded for a variety of purposes including better understanding of heterogeneity in many different tissues during developmental and adult stages.

Poster Number 8

Nathaniel Killian, PhD
Neurosurgery, Research Fellow | nkillian@mgh.harvard.edu

Deep-brain multi-wire electrode array: demonstration as a visual prosthesis

INVESTIGATORS: N. J. Killian, J. S. Pezaris

We have developed a multi-wire electrode array and accompanying implantation system for use as a lateral geniculate nucleus (LGN) prosthesis. The LGN prosthesis will be beneficial for restoration of coarse vision to individuals with blindness resulting from impaired retinogeniculate transmission of light information, which may occur after traumatic injury or in diseases such as glaucoma and macular degeneration. Each electrode array comprises a bundle of up to 128 platinum-iridium microwires mated with miniature connectors and integrated with moving positioning components within a sealed cartridge. A 128-electrode bilateral LGN implant was placed in one monkey (Macaca mulatta). Intraoperative single-neuron recordings were made from the array while the animal was awake and performing a receptive field mapping task. Bundle positioning was fine-tuned with a microdrive to optimize receptive field locations. The connectors were then cemented above the skull. Distinct artificial visual percepts, known as phosphenes, were evoked by activating LGN neurons near the wire tips with charge-balanced voltage pulses. Phosphene characteristics such as location and size in the visual field were determined by tracking changes in the animals' gaze after brief stimulus deliveries. We next examined the animal's perception through patterned stimulation. Phosphenes were simultaneously activated to represent either the letter C or W, artificially presented at a size corresponding to a visual acuity of logMAR 2.2 (20/3200 Snellen). The animal successfully discriminated the artificially presented letters on 60% of trials ($p < 0.05$, binomial test), consistent with our prior studies using a virtual reality simulation.

Bioengineering & Devices

Poster Number 9

Seungwoo Lee, PhD
Neurosurgery, Instructor | lee.seungwoo@mgh.harvard.edu
Implantable micro-coils for precise and reliable neural prostheses

INVESTIGATORS: S. W. Lee, K. Thyagarajan, S. I. Fried

Electrical stimulation via cortically-implanted electrodes has been used to treat a wide range of neurological disorders. Unfortunately, their long-term effectiveness has been limited, largely due to the biological reactions triggered by implantation into cortex as well as the inability of conventional electrodes to selectively activate targeted pyramidal neurons. Recent demonstrations that magnetic stimulation from a micro-coil can selectively activate vertically oriented pyramidal neurons while avoiding horizontal passing axons suggest the possibility that such an approach can help to overcome some of those limitations. Here we describe new micro-coil designs for enhancing selectivity (vertical vs. horizontal) and demonstrate their effectiveness via a series of electrophysiology experiments. A computational model was developed to compare the effectiveness of magnetic stimulation induced by different coil designs. Some of the most promising designs (V vs. W shapes) were fabricated for use in electrophysiological experiments. In vitro patch-clamp recording using mouse brain slices revealed that both V and W-shaped coils could reliably activate layer 5 (L5) pyramidal neurons (PNs) but the V-coils were more effective than the W-coils. We also found that double-loop coils could enhance the strength of stimulation than single-loop coils. In vitro calcium fluorescence imaging using GCaMP6f transgenic mice revealed that the V and W-coils can better confine activation in cortex than conventional electrodes. The enhanced selectivity in stimulation and the reduced susceptibility to encapsulation in cortex suggest that a micro-coil implant could be an attractive alternative to conventional electrode implants for cortical neural prosthetics.

Poster Number 10

Hsing-Ying Lin, PhD
Center for Systems Biology, Research Fellow | hlin17@mgh.harvard.edu
Integrated Magneto-Chemical Sensor for On-Site Food Allergen Detection

INVESTIGATORS: H. Lin, C. Huang, J. Park, D. Pathania, C. M. Castro, A. Fasano, R. Weissleder, H. Lee, Postdoctoral Research Abroad Program, Ministry of Science and Technology, Taiwan

Adverse food reactions, including food allergies, food sensitivities, and autoimmune reaction (e.g., celiac disease) affect 5–15% of the population and remain a considerable public health problem requiring stringent food avoidance and epinephrine availability for emergency events. Avoiding problematic foods is practically difficult, given current reliance on prepared foods and out-of-home meals. In response, we developed a portable, point-of-use detection technology, termed integrated exogenous antigen testing (iEAT). The system consists of a disposable antigen extraction kit coupled with a keychain reader for rapid sensing and internet communication. The device is designed to (i) have analytical capacities comparable to a benchtop system and (ii) be scalable to multichannel readouts. We optimized iEAT to detect five major antigens found in peanuts, hazelnuts, wheat, milk, and eggs. Two strategies were combined: (i) quick allergen extraction through increased temperature and collection on immunomagnetic beads; and (ii) enzymatic reaction to amplify analytical signal. Antigen extraction and detection with iEAT required <10 min and achieved high-detection sensitivities (e.g., 0.1 mg/kg for gluten, lower than regulatory limits of 20 mg/kg). When testing under restaurant conditions, we were able to detect hidden food antigens such as gluten within "gluten-free" food items. Detection results including location, time stamps, food names, and statistics are integrated into personal cloud database for further sharing or analysis. The small size and rapid, simple testing of the iEAT system should help not only consumers but also other key stakeholders such as clinicians, food industries, and regulators to enhance food safety.

Poster Number 11

Jouha Min, PhD
Center for Systems Biology, Research Fellow | jmin7@mgh.harvard.edu
Point-of-Care Sepsis Diagnostics

INVESTIGATORS: J. Min, M. Nothing, B. Coble, J. Park, H. Im, G. Weber, C. M. Castro, F. K. Swirski, R. Weissleder, H. Lee

Sepsis is a life-threatening organ dysfunction syndrome caused by a dysregulated and harmful host reaction to infection, accounting for at least 1/3 of all hospital deaths. A critical unmet need in controlling sepsis is the lack of quantitative, fast tests that produce actionable results in busy clinical settings. Here we aim to develop a new diagnostic platform for rapid, point-of-care (POC) sepsis detection. Termed IBS (integrated Biosensor for Sepsis), the approach leverages our breakthroughs: i) the newly-found pathophysiological role of cytokine interleukin-3 (IL-3) in sepsis, and ii) a hybrid magneto-electrochemical sensor for IL-3 detection. The developed diagnostic platform produces test results within 1 hour from native blood samples, and detects IL-3 at a sensitivity of <10 pg/mL; this performance is 5-times faster and 10-times more sensitive than a current gold standard enzyme-linked immunosorbent assay. Using clinical samples, we show the potential of IL-3 as an early diagnostic biomarker. Compact and fast, the IBS platform can be readily integrated into clinical workflows, enabling timely diagnosis and proactive treatment of sepsis.

Poster Number 12

John Parrish, MD
Dermatology, Professor | jparrish@partners.org
CIMIT's Point of Care Technology Research Center in Primary Care

INVESTIGATORS: J. Parrish, S. Schachter, J. Collins, M. Dempsey, D. Spiliotis, P. Ford-Carleton, CIMIT (Consortia for Improving Medicine with Innovation and Technology)

Primary care providers (PCPs) are at the frontlines of healthcare systems. Increasing numbers of elderly patients and younger patients with chronic diseases and limited access to specialists constrains primary care capacity, limiting access and fragmenting care as patients and families seek out other points of entry into the healthcare system, such as emergency departments, which are often more expensive and less interconnected. The introduction of appropriately-designed point-of-care (POC) technologies into patient-centered primary care environments should increase the capacity of PCPs to care for more patients by empowering patients to maintain better health and/or manage care as well as by eliminating inefficiencies, potentially improving patient outcomes. Although some POC technologies exist, very few are designed to address the rapidly evolving needs and unique challenges of primary care environments.

During five years of funding from NIBIB, CIMIT's POCT Research Center in Primary Care identified unmet needs, translated these needs into RFAs, funded innovative POC solutions, and moved them from concept to commercialization. The total funded portfolio comprised 31 projects at different levels of maturity. By the end of Year 5 (2017), follow-on funding for these projects totaled $54 million (19 times the total of direct costs awarded by the Center). Center-funded teams published 39 papers and filed 16 patents. Here we show that the Center created a dynamic, sustainable national network that identified key unmet needs as well as promising emerging POC technologies and then accelerated their translation into clinical applications for broad impact in primary care medicine.

Poster Number 13

Huidong Chen, BS
Pathology, Graduate Student | huidong.chen@mgh.harvard.edu

STREAM: Single-cell Trajectories Reconstruction, Exploration and Mapping

INVESTIGATORS: H. Chen, J. Y. Hsu, C. A. Lareau, G. L. Bosco, J. Guan, S. Zhou, M. J. Aryee, D. M. Langenau, J. D. Buenrostro, G. Yuan, L. Pinello

With the rapid development of single-cell analysis technologies one important concept that has emerged is that cell states may be "continuous" and modeled as smooth trajectories representing different biological processes such as differentiation or response to stimuli. Several methods have been proposed to reconstruct lineages using single-cell transcriptomes, but no methods have been specifically developed for the recent single-cell chromatin assays such as scATAC-seq. In addition, current methods cannot reliably reconstruct trajectories when multiple branching points are present, do not provide an intuitive and interactive visualization of trajectories, cannot handle large datasets (especially droplet based) and can be used only by experienced bioinformaticians. To fill this gap we are proposing STREAM, a new computational method capable of disentangling complex cellular types with multiple branching points and states using single cell qPCR, scRNA-seq or scATAC-seq data.

Here we show STREAM can accurately detect cellular hierarchies, recover complex developmental trajectories and provide informative and intuitive visualization methods to highlight important genes to define subpopulations. STREAM also has a novel visualization method called "stream plot", a compact and intuitive representation useful to model large datasets that summarizes cellular developmental trajectories, branching points, density of cells and gene expression patterns. In addition, STREAM is the only method that can map new single cells to an inferred tree structure without pooling data and re-computing trajectories making it suitable for perturbation studies or for assigning diseased cells to a normal developmental hierarchy.

Poster Number 14

Kendell Clement, PhD
Pathology, Research Fellow | kclement@mgh.harvard.edu

Ultra-fast quantification of genome editing events for CRISPR-Cas9, Cpf1 and base editors from deep sequencing data

INVESTIGATORS: K. Clement, J. Hsu, M. Cole, M. Canver, D. Bauer, K. Joung, L. Pinello

CRISPResso2 is a software package designed to aid biological researchers in analyzing and visualizing outcomes of genome editing events, and makes substantial improvements on the first version of the software. Experimentalists use genome editing tools such as CRISPR to change the DNA of cells. These changes are read out using next-generation sequencing. CRISPResso2 accepts as input the next-generation sequencing output and allows researchers to determine the efficiency of their genome editing tool, as well as the resulting genome editing outcomes. For example, a researcher can use CRISPResso2 to analyze how effective their experiment was at producing a specific, expected genome modification. In addition, CRISPResso2 visualizes genome editing events in easy-to-understand graphical formats that can be used by researchers in publications.

Poster Number 15

John Collins, PhD
CIMIT | jcollins11@partners.org

The CIMIT Innovation Guidance and Impact Tracking System (GAITS)

INVESTIGATORS: J. Collins, J. A. Parrish, M. K. Dempsey, S. C. Schachter, D. Spiliotis, P. Ford Carleton, M. R. Collins, P. Tessier

CIMIT has been studying the complex journey of innovation in healthcare since its first Clinical Impact Study in 2010. It captured the knowledge gained in CIMIT's 20+ years of experience supporting more than 600 projects and 230 solutions. A primary conclusion was that innovation in healthcare is a learnable, teachable process. The process, which CIMIT calls the Healthcare Innovation Cycle, parallels the Department of Defense's well-established Technology Readiness Levels, with 10 healthcare specific milestones. GAITS guides teams in navigating the cycle by defining core set of deliverables at each milestone in four domains critical to success in healthcare innovation: Clinical, Market/Business, Regulatory/Approvals, and Technology. Deliverables can vary by solution type (e.g. HealthTech, Pharmaceuticals, Health IT, etc.) GAITS provides curated resources (descriptions, videos, templates, examples, etc.) to help teams complete each deliverable. It enables teams to create an innovation record and funders/institutions to track progress of, and measure the impact of their project portfolios.

GAITS has been implemented in CIMIT's CoLab platform; a secure, online tool which supports teams and portfolio managers to increase the likelihood of innovations reaching patient care. CIMIT has formed a consortium of ten leading healthcare innovation organizations across the globe to build on CIMIT's experience of facilitating teams and refine GAITS. Results from consortium members will provide a robust database to study the healthcare innovation process to establish and share best practices. Here we show that CIMIT's first-generation GAITS platform helps teams by codifying, measuring, and establishing best practices in translational research.

Poster Number 16

Nils Fischer, MPH
Cancer Center, Research Associate | nfischer@partners.org

Evaluating medication adherence in patients on oral anti-cancer medications: lessons learned from a two-armed randomized pilot study to evaluate the effect of a mobile health application

INVESTIGATORS: N. Fischer, R. S. Palacholla, S. O. Agboola

Oral chemotherapeutic medications are increasingly used for the treatment of cancer. Their long-term use raises questions about patients' adherence to prescribed regimens. We developed a digital solution, CORA, to promote adherence to oral anti-cancer medications. The objective is to evaluate the effect of CORA on adherence to oral anti-cancer medications. 84 patients were randomized to one of two groups: CORA application and standard care while the control group continued with standard care. Outcomes were evaluated at the end of 12 weeks using data from electronic pill bottle collected continuously throughout the study and the Morisky Medication Adherence Scale (MMAS) was assessed at the enrollment, 6 and 12 weeks. Repeated measures mixed model analysis was used to analyze survey data. Mann-Whitney Test compared median MMAS scores at each study period.

We encountered several challenges s in assessing adherence using the electronic pill bottles. Namely, bottles were difficult to open, complicated with multiple daily dosing regimens, not accommodating the quantity of medications in the bottles, and risking cross-contamination of different medications. In the control group, median MMAS scores were 6 (25-75%: 6-7), 7 (25-75%: 6-7), and 7 (25-75%:6,7) from enrollment, midpoint to closeout, respectively. In the intervention group, median MMAS scores were 6 (25%-75%: 5-7), 7 (25%-75%: 6-7), 7(25%-75%: 6,7). Group differences at all study periods were insignificant ($p=0.90$, $p=0.27$, and $p=0.79$ for the enrollment, midpoint, and closeout, respectively). In conclusion, measuring adherence to oral anti-cancer medications with electronic pill bottles presents several challenges that should be strongly considered in future studies.

Poster Number 17

Iman Aganj, PhD
Radiology, Assistant Professor | iman@nmr.mgh.harvard.edu
Alignment of Multimodal Medical Images through Simultaneous Segmentation

INVESTIGATORS: I. Aganj, B. Fischl

Employing multiple imaging modalities often provides valuable complementary information for clinical and investigational purposes. Computing a spatial correspondence between multimodal images, a.k.a. multimodal image registration, is the key step in combining the information from such images. Since different modalities create images that do not share the same tissue contrast, the alignment of these images can hardly be assessed by a local comparison of their intensities.

In multimodal image registration, the joint histogram of the two images has been widely used to derive global matching measures, such as normalized mutual information, entropy correlation coefficient, and tissue segmentation probability. Histogram computation typically requires an optimized choice of the bin (or kernel) width.

In this work, we introduce a new non-information-theoretical objective function for pairwise multimodal image registration based on simultaneous segmentation. Our underlying assumption is that any improvement in the alignment of two images leads to an improvement in image segmentation from them, hence a lower segmentation error. We propose an efficient algorithm that uses the intensity values of the images to divide the voxels into two classes, while regarding the segmentation error as the registration cost function. Contrary to most existing methods, we do not use the joint histogram or entropy of images or tissue classes. In a comparison with several existing objective functions, we show that our proposed objective function often outperforms competing metrics in registering brain magnetic resonance images with different contrasts, in terms of often converging to the correct (subvoxel-accuracy) solutions, and often resulting in better manual-label matching.

Poster Number 18

Yoonji Baek
Radiology, Research Laboratory Assistant | yjbaek0407@gmail.com
Deep learning of quantum chemistry for molecular targeting prediction

INVESTIGATORS: Y. Baek, H. S. Choi, D. W. Hwang, S. J. Kim, J. H. Lee, M. J. Jo, H. Kang, K. Bao, G. K. Park

The preclinical drug discovery and screening of new chemicals involve the use of live animals, which is a time-consuming, expensive, and unpredictable process. In addition, the sacrifice of research animals and the concerns about animal welfare have raised debates on many bioethical issues. Alternatively, various machine learning techniques have recently been used in chemistry to expect pharmacokinetic properties and biological effects of new chemicals based on the information obtained from traditional structure-activity relationship (SAR). In this study, we applied deep learning to predict molecular behaviors in specific tissues and organs by using quantum chemistry. Since we have systemically organized chemical database of over 550 contrast agents using Instant JChem (ChemAxon, Budapest, Hungary), individual chemical structures were converted into the simplified molecular-input line-entry system (SMILES) format for machine learning and their physiochemical properties (i.e., molecular weight, total polar surface area, hydrogen bond donors/acceptors, acidic/basic pKa, distribution/partition coefficient, and stability) were used to characterize each chemical. On the other hand, the biodistribution and targeting properties of the contrast agents were converted to signal-to-background ratio (SBR) using ImageJ software (NIH, Bethesda, MD) in order to train computer algorithms. Using this systemically developed deep learning system, we could expect in vivo distribution and organ specificity of newly designed contrast agent without sacrificing animals.

Poster Number 19

Ken Chang, BS
Radiology, Graduate Student | ken_chang@dfci.harvard.edu

Distributed Deep Learning Networks Among Institutions for Medical Imaging

INVESTIGATORS: K. Chang, J. Brown, A. Beers, B. Rosen, J. Kalpathy-Cramer

Deep learning has become a promising approach for automated medical diagnoses. When medical data samples are limited, collaboration among multiple institutions is necessary to achieve high algorithm performance. However, sharing patient data often has limitations due to technical, legal, or ethical concerns. In this study, we propose methods of distributing deep learning models as an attractive alternative to sharing patient data. We simulate the distribution of deep learning models across four institutions using various training heuristics and compare the results with a deep learning model trained on centrally hosted patient data. The training heuristics investigated include ensembling single institution models, single weight transfer, and cyclical weight transfer. We evaluated these approaches for image classification in three independent image collections (retinal fundus photos, mammography, and ImageNet). We find that cyclical weight transfer resulted in a performance that was comparable to that of centrally hosted patient data. We also found that there is an improvement in the performance of cyclical weight transfer heuristic with high frequency of weight transfer. We show high model performance can be achieved without centrally hosted data. Distributing deep learning models can effectively utilize data from many institutions as long as the institutions are willing to distribute the model. We show that distributing deep learning models is an effective alternative to sharing patient data. This finding has implications for any collaborative deep learning study.

Poster Number 20

Luke Chao, PhD
Molecular Biology, Assistant Professor | chao@molbio.mgh.harvard.edu

Single particle Cryo-EM studies of mitochondrial inner-membrane dynamics

INVESTIGATORS: L. H. Chao

Mitochondrial membrane dynamics are intimately tied to the organelle's diverse functions. Modulating the ratio of mitochondrial fission and fusion is a regulatory node that directly connects metabolic state with organelle morphology. We present single particle electron cryo-microscopy (Cryo-EM) reconstructions of the inner mitochondrial membrane fusogen OPA1, a dynamin family GTPase mutated in dominant optic atrophy, revealing multiple conformational states. We discuss relationships between these states with the protein's fusogenic conformational rearrangement as well as potential roles for these conformers in the maintenance and rearrangement of cristae. These studies provide a framework for relating conformational change in mitochondrial fusion proteins with their membrane-associated activities and the potential to explain disease mutations with mechanistic detail.

Poster Number 21

Diego dos Santos Ferreira, PhD
Radiology, Research Fellow | ddossantosferreira@mgh.harvard.edu

Molecular MR imaging of liver fibrosis and fibrogenesis is not altered by inflammation in a mouse model of Schistosomiasis

INVESTIGATORS: D. S. Ferreira, N. J. Rotile, G. Arora, R. L. Gieseck III, P. A. Waghorn, C. A. Jones, I. Chen, K. Looby, T. A. Wynn, K. K. Tanabe, P. Caravan, B. C. Fuchs

Non-invasive quantitation of fibrosis remains a largely unmet clinical need. We are developing molecular MRI probes targeted to type I collagen (EP-3533) and to oxidized collagen (Gd-Hyd) to image and quantitatively stage fibrosis and fibrogenesis, respectively. Here we employed two genetically modified mouse models to determine if the presence of underlying inflammation would skew probe readouts. Infection with the helminth Schistosoma mansoni causes either severe liver fibrosis with minimal inflammation in Il10-/-Il12b-/-Il13ra2-/- mice (TAC313) where three negative regulators of pro-fibrotic IL-13 signaling have been deleted or mild liver fibrosis with severe inflammation in Il4rαΔ/Δ mice (4Rδ) which lack a proper Type 2 immune response.

Mice were infected with S. mansoni cercariae and imaged 6-10 weeks later with a small animal MRI. Mice were imaged before and after injection of either Gd-Hyd or EP-3533 and the change in liver-to-muscle contrast-to-noise ratio (ΔCNR) was computed. Following MRI, the liver was collected and quantitatively assessed for fibrosis and inflammation using histological and biochemical measurements. Both probes readily quantified liver fibrosis in S. mansoni infected wild type mice compared to uninfected animals. In transgenic animals, liver fibrosis was significantly increased in TAC313 mice compared to 4Rδ mice ΔCNR was significantly (10-fold, 5-fold) higher in the TAC313 mice with EP-3533 and Gd-Hyd, respectively, verifying the ability of these probes to differentiate fibrosis from inflammation. These results demonstrate the probes' specificity for detecting liver fibrosis independent of inflammation and further highlight their potential as tools for assessing fibrosis of varied etiologies in human clinical trials.

Poster Number 22

David Drew, PhD
Medicine, Research Fellow | dadrew@mgh.harvard.edu

Feasibility, safety, and initial efficacy of cathepsin-activatable fluorescent probes for molecular detection of colorectal and esophageal neoplasia

INVESTIGATORS: D. A. Drew, D. C. Zerjav, M. S. Schuck, M. Jovani, A. D. Joshi, J. Ferrer, J. Misdraji, W. R. Brugge, F. P. Colizzo, D. Forcione, C. Morse, N. S. Nishioka, D. W. Rattner, D. L. Berger, A. J. Bass, C. S. Fuchs, U. Mahmood, A. T. Chan

For colonoscopy, estimates of the miss rate for neoplastic lesions are as high as 22%. For upper endoscopy, areas of high-grade dysplasia (HGD) or intramucosal esophageal adenocarcinoma (AC) are often endoscopically inconspicuous within a background non-dysplastic Barrett's esophagus (BE). Molecular imaging agents that specifically target enzyme pathways upregulated in cancer are a promising strategy to improve detection of gastrointestinal (GI) luminal malignancy. LUM015 is a novel molecular agent activated by tumor-associated cathepsins to release a fluorophore detected in real time with near infrared (IR) light imaging. For colorectal AC (n=6), 3 patients each received LUM015 intravenously at 0.5 or 1.0 mg/kg a mean of 3.5 hours before tumor resection and imaging. Colorectal AC showed significantly brighter signal intensity compared to normal tissue. The 0.5 mg/kg dose resulted in an area under receiver operating characteristic curve (AUC) of 0.72. At a tumor-to-normal (TNR) threshold of 1.2, the sensitivity for AC was 48% with 96% specificity. At 1.0 mg/kg dose, the AUC was improved at 0.86. At a TNR threshold of 1.2, the sensitivity improved to 64% with 87% specificity. For esophageal HGD/AC, 3 patients received a 0.5 mg/kg dose, a mean 2.8 hours before resection and imaging. Signal intensities progressively increased in the sequence of normal esophageal tissue, non-dysplastic BE, BE with HGD, and esophageal AC. For all nine patients, no adverse events were observed. This first-in-human study of GI luminal neoplasia demonstrates that cathepsin-based molecular imaging for the detection of GI cancers is well tolerated and can distinguish tumor areas.

Poster Number 23

Derek Erstad, MD
Surgery, Resident | derstad@partners.org

Collagen Targeted MRI Accurately Measures the Desmoplastic Response to FOLFIRINOX Treatment in a Murine Model of Pancreatic Cancer

INVESTIGATORS: D. J. Erstad, M. Sojoodi, N. J. Rotile, C. Jones, K. A. Graham-O'Regan, C. T. Farrar, D. S. Ferreira, S. Li, S. Ghoshal, G. Aurora, L. Wei, C. Ferrone, M. Lanuti, K. K. Tanabe, P. Caravan, B. C. Fuchs, Institute for Innovation in Imaging, Massachusetts General Hospital

Neoaduvant FOLFIRINOX for PDAC is associated with significant fibrotic response. CT and MRI are unable to distinguish post-treatment fibrotic response from residual tumor, complicating operative planning. We compared a novel type-1 collagen MRI probe, CM- 101, and the standard contrast agent, DOTA, for their abilities to identify FOLFIRINOX-induced fibrosis. C57BL/6 mice were orthotopically implanted with 1x104 Hy15549 PDAC cells. FOLFIRINOX was administered 2x/week via intraperitoneal injection starting on day 10. Mice were imaged on days 17-18 using a 4.7T MRI with Gd-DOTA and Gd-CM101 24h apart.

Compared to untreated tumors, FOLFIRINOX treatment reduced tumor weight (0.52±0.02g vs, 0.43±0.03g, $p = 0.036$) and increased carcinoma-specific necrosis by caspase staining (1.8±1.3% vs. 23.5±2.9%, $p < 0.0001$). FOLFIRINOX treatment increased tumor fibrosis measured by total collagen content (20.7±3.5% vs. 33.9±6.1%, $p < 0.0001$), which was associated with increased peak CM-101 signal-to-noise ratio in enhancing tumor regions. CM-101 MR signal attenuation was delayed in FOLFIRINOX-treated tumors (AUC 86.47±8.65 vs. 94.94±5.36, $p = 0.034$), indicating probe uptake and binding in collagen-dense tissue, which was verified by mass spectrophotometric measurement of CM-101 levels in tumor tissue normalized to muscle (4.7±1.8 vs. 7.2±2.6, $p = 0.014$). In contrast, no treatment-related differences were observed with DOTA imaging (AUC untreated 9.98±5.44 vs. FOLFIRINOX 7.40±1.19, $p = 0.14$). Collagen molecular MRI with CM-101 provides a novel imaging technique that could be used to monitor the fibrotic response to neoadjuvant therapy to assist with patient selection for an operation.

Poster Number 24

Aina Frau-Pascual, PhD
Radiology, Research Fellow | afraupascual@mgh.harvard.edu

Global Quantification of the Structural Brain Connectivity

INVESTIGATORS: A. Frau-Pascual, A. Yendiki, B. Fischl, I. Aganj

Connectomics have proved promising in quantifying and understanding the effects of development and certain diseases on the brain. However, existing literature on the brain functional and structural connectivity is not fully consistent: while several studies have shown functional connectivity to be correlated with structural connectivity, strong functional connections have also been commonly observed between regions with no direct structural connection. Some of this variance has been found to be due to the impact of indirect structural connections, usually not considered in modeling. Such connections have been successfully accounted for via graph theory, although the amount of indirect connections that can be considered is limited.

In this work, we model the brain connectivity globally by exploiting well-studied mathematics of electromagnetism. We assign local anisotropic conductivity values to voxels, which are functions of the diffusion tensors computed from diffusion MRI. Solving the partial differential equations, we then obtain a measure of conductance between each pair of brain regions, computed by considering all diffusion paths between them. Our global approach allows us – without relying on other processing steps such as tractography – to account for direct brain connections, as well as indirect ones that would not be otherwise accounted for by standard techniques.

We will shortly apply this method to the Alzheimer's Disease Neuroimaging Initiative longitudinal diffusion MRI dataset to see how well our global measures of connectivity discriminate different stages of dementia and predict conversion to Alzheimer's disease, while comparing it with the performance of state-of-the-art tractography techniques.

Poster Number 25

Bryan Fuchs, PhD
Surgery, Assistant Professor | bfuchs@mgh.harvard.edu

Molecular Magnetic Resonance Imaging of Collagen Oxidation Accurately Quantifies Fibrogenesis in Multiple Organs

INVESTIGATORS: B. C. Fuchs, P. A. Waghorn, H. H. Chen, L. Wei, L. F. Tapias, C. K. Probst, D. J. Erstad, D. S. Ferreira, I. Chen, M. Mino-Kenudson, G. Y. Lauwers, R. Masia, R. B. Colvin, M. Lanuti, A. M. Tager, K. K. Tanabe, P. Caravan

Fibrosis results from the dysregulation of tissue repair mechanisms affecting major organ systems, leading to chronic extracellular matrix buildup, and progressive, often fatal, organ failure. Current diagnosis relies on invasive biopsies. Noninvasive methods currently cannot distinguish actively progressive fibrogenesis from stable scar, and thus are insensitive to early disease staging and therapeutic response monitoring. Collagen oxidation is a universal signature of active fibrogenesis that precedes collagen crosslinking. Here we describe novel molecular magnetic resonance (MR) probes that target oxidized lysine residues formed by the action of lysyl oxidase on collagen enabling robust detection and staging of pulmonary, hepatic, and renal fibrosis progression and regression. Molecular MRI tracked disease progression in the bleomycin model of pulmonary fibrosis, quantifiable by the 4.3-fold increase in ΔCNR at 1-week, and 6.5-fold increase at 2 weeks post-bleomycin relative to control. Treatment with β-aminopropionitrile, which inhibits lysyl oxidase-mediated collagen oxidation, reduced ΔCNR to control levels. Molecular MRI also tracked disease progression in the carbon tetrachloride (CCl4) model of hepatic fibrosis, quantifiable by the 29-fold increase in ΔCNR at 6 weeks, and 45-fold increase at 12 weeks post-CCl4 relative to control. When CCl4 administration was stopped between 6 and 12 weeks, ΔCNR returned to control levels. Finally, in a model of nephrotoxic serum nephritis (NTN), molecular MRI resulted in a 6.9-fold increase in ΔR1 (probe relaxivity) in the renal cortex of NTN mice compared to control. In conclusion, imaging of collagen oxidation is applicable to diverse fibroproliferative disorders and is currently be translated into clinical trials.

Poster Number 26

Do Won Hwang, PhD
Radiology, Research Fellow | dwhwang@mgh.harvard.edu

In vivo fate re-distribution of the bioengineered exosome dominated by charge controllable NIR agents

INVESTIGATORS: D. Hwang, J. Lee, M. Jo, H. Kang, K. Bao, S. Hu, H. Choi

Modifying physiological behavior of nanomaterials is essential for optimizing target organ specificity and reducing signals from non-specific background in disease modifying therapeutics. The surface engineering of highly bioinert extracellular vesicles (EVs) with chargeable and traceable NIR agents may confer great impact on regulating their distribution, stability, and toxicity for effective disease therapy. In this study, we designed surface engineered-EVs with NIR agents possessing different physicochemical properties by controlling the in vivo physiological fate of EVs. The autologous plasma EVs modified with 4 different charged NIR agents had distinct systemic distribution, showing rapid renal uptake of zwitterionic TG42-labeled EVs with low organ non-specificity, whereas -1 WuA108-EV was found to be excreted by hepatobiliary route with high early liver uptake. In vivo distribution and pharmacokinetics of each NIR-EVs was very similar with its corresponding NIR agents. In addition, unique kinetic profiling of EVs along with lymphatic vessel was markedly found by the intraoperative real time FLARE imaging system, showing the fastest translocation toward primary lymph nodes in the mouse footpad inoculation of -1 WuA108-EV. Based on these findings, EV surface protein engineering may alter in vivo fate of EVs, which provides great opportunities renovating applications of EV therapeutics.

Poster Number 27

Daniel Kim, BS
Radiology, Clinical Research Coordinator | dkim32@mgh.harvard.edu

Predictive Value of Magnetic Resonance Spectroscopic Imaging during Anti-angiogenic Treatment in Recurrent Glioblastoma

INVESTIGATORS: D. Kim, J. Dietrich, J. He, O. Rapalino, Y. Ou, Y. Yen, M. Vangel, O. Andronesi, I. Arrillaga, D. Forst, S. Plotkin, A. Eichler, P. Brastianos, J. Jordan, J. Kalpathy-Cramer, B. Rosen, T. Batchelor, R. Gonzalez, E. Ratai, MGH Cancer Center staffs and Quantitative Tumor Imaging staffs

Glioblastomas are challenging cancers to treat, and long-term favorable clinical outcomes in patients with recurrent glioblastoma continue to be difficult to achieve. One of the main characteristics of glioblastoma is the presence of intratumoral neoangiogenesis, and patients with recurrent glioblastoma are often treated with anti-angiogenic agents such as bevacizumab. Treatment with bevacizumab has shown potential in extending progression-free survival. However, not every patient with recurrent glioblastoma benefits from bevacizumab treatment. Because the use of bevacizumab is frequently associated with substantial reduction in contrast enhancement on T1-weighted magnetic resonance imaging, it is often difficult to distinguish a true favorable tumoral response from pseudo-response using conventional MRI. Therefore, our study examines the use of Magnetic Resonance Spectroscopic Imaging to predict treatment response to anti-angiogenic therapy for recurrent glioblastoma patients. Here we show that early changes at 1-3days, 4 weeks, and 6 weeks in Choline/NAA, Lactate/normal Creatine, and Lactate/NAA can be predictive of 9-months survival outcomes in patients with recurrent glioblastoma treated with bevacizumab. These preliminary findings suggest that early changes in certain metabolic ratios can be useful markers for predicting therapeutic response and overall survival in patients with recurrent glioblastoma treated with bevacizumab.

Poster Number 28

Saeyun Lee, BA
Radiology, Clinical Research Coordinator | slee158@mgh.harvard.edu

Coronary Computed Tomography Angiography to Assess Anti-Atherosclerotic Drug Therapies: A Systematic Review

INVESTIGATORS: S. Lee, M. T. Lu

Change in coronary artery plaque on serial intravascular ultrasound (IVUS) is used to establish the therapeutic effect of drugs to treat coronary atherosclerosis. However, recent improvements in coronary computed tomography angiography (CTA) have led it to become an emerging method for assessing coronary plaque. The major advantage of CTA over IVUS is that it is a noninvasive test, as compared to IVUS which is only possible during invasive coronary angiography. This systematic review will examine completed studies as well as ongoing trials using CTA to determine efficacy of anti-atherosclerotic therapies.

We conducted a systematic review using PubMed and ClinicalTrials.gov to identify studies using CTA to assess drug efficacy in reducing coronary plaque volume. Any drug studies with change in plaque volume measured using CTA as an endpoint were included while any non-English language studies and trials with "unknown" or "not yet recruiting" status on ClinicalTrials.gov were excluded. 17 published studies met the inclusion criteria, 14 of which were prospective and 3 of which were retrospective. 10 out of the 17 studies were published after 1/1/2015. Furthermore, 5 unpublished trials registered on ClinicalTrials.gov met the inclusion criteria. Among these 5 trials, 4 were ongoing and 1 was completed but not yet published. Change in coronary plaque volume on CTA is an emerging modality for following drug treatment effect.

Poster Number 29

Steven Liang, PhD
Radiology, Assistant Professor | liang.steven@mgh.harvard.edu

A surrogate PET biomarker for differentiating brown and white adipose tissue

INVESTIGATORS: T. Shao, Z. Chen, J. Yang, C. R. Ran, S. H. Liang

Brown adipose tissue (BAT) consumes stored lipids and provides non-shivering thermogenesis. Recent studies proposed that enhancing/activating BAT could be a novel therapeutic approach to counteract metabolic diseases, including obesity and type II diabetes. Herein we describe the development of a reversible and peripheral-specific 18F-ligand targeting monoacylglycerol lipase (MAGL), and its application for differentiating brown and white adipose tissue in the lipid network by positron emission tomography (PET).

A novel compound, FEPAD, showed strong binding affinity in vitro to hydrolysis assay and human recombinant MAGL inhibition assay with IC50 values of 77.6 and 23.8 nM, respectively, and excellent target selectivity (> 100 fold) among other major serine hydrolases. 18F-Labeled FEPAD was synthesized in 13% RCY with >99% radiochemical purity and >2 Ci/μmol molar activity. [18F]FEPAD showed characteristic high uptake in BAT (peak value 21.4% %ID/g,) but not in white adipose tissue (WAT, 3.5 %ID/g). Static PET imaging studies showed ~300% and ~200% higher uptake in BAT than that of WAT at 30 and 60 min post injection. Using [18F]FDG as positive control, FEPAD is not sensitive to cold-stimulation at 4°C. Ex vivo validation, including immunohistochemistry (IHC), immunofluorescence (IF) and H&E stains, was also performed to quantify MAGL in adipose tissues, providing the underlying mechanism for differentiating BAT from adipose tissues. We have developed [18F]FEPAD as a surrogate PET biomarker for differentiating BAT from adipose tissues. We expect this work will extend our understanding of the roles of MAGL in vivo and provide a new imaging tool for BAT.

Poster Number 30

Kate Park, MS
Radiology, Graduate Student | gkpark@mgh.harvard.edu

Near-infrared fluorescence imaging for longitudinal cellular trafficking

INVESTIGATORS: G. K. Park, J. H. Lee, H. S. Choi

Longitudinal trafficking of live cells in living organisms is crucial to understand the function, toxicity and therapeutic mechanism of systemically or implanted stem cells in clinical use. The ability to monitor the fate of administered cells for a long period of time using high-resolution in vivo optical imaging techniques would be critical for the development of cell-based therapeutic interventions. To determine the presence of administered cells from the host tissue using in vivo real-time imaging, labeling of the target cells with a nontoxic and stable contrast agent is a prerequisite. However, long-term live cell trafficking is currently limited by the lack of available fluorophores in the near-infrared (NIR) wavelengths with steady optical and physicochemical properties. Here we report, for the first time, the design of fixable cell tracking NIR fluorophores (CTNFs) with high extinction coefficients and quantum yields, excellent cell permeation and retention, and high stability in chemical treatment. We demonstrate the efficient cellular labeling and tracking of CTNFs using real-time intraoperative optical imaging and epifluorescence microscopy to follow the fate of NIR fluorescnet cells from the time of injection into animals to ex vivo single cell analysis after resection of target tissues. Among the tested, CTNF126, a lipophilic cation, diffuses into the cytoplasmic membrane and sequesters inside the lysosomes, which prevent cellular efflux and improve cellular retention. CTNF126 also outperforms all commercially available visible-wavelength fluorophores due to the use of NIR window (650-900 nm).

Poster Number 31

Peng Shao, PhD
Wellman Center for Photomedicine, Research Fellow | pshao1@mgh.harvard.edu

Brillouin ocular analyzer detects biomechanical changes in corneal tissues in the early stages of keratoconus and after corneal crosslinking: clinical study findings

INVESTIGATORS: P. Shao, A. E. Eltony, T. G. Seiler, B. Tavakol, R. Pineda, T. Koller, T. Seiler, S. H. Yun

The biomechanical properties of corneal tissues have been implicated in keratoconus and other ectatic disorders. However, it has been difficult to identify biomechanical changes and abnormalities in patients with sufficient sensitivity and accuracy for disease detection at an early-stage. Here, we demonstrate the diagnostic potential of Brillouin light-scattering microscopy, an imaging modality that measures longitudinal modulus in corneal tissues with high sensitivity and optical spatial resolution. Clinical studies involving over two hundred subjects provided in vivo Brillouin data to analyze interpersonal variability, age-dependence, regional heterogeneity, and bilateral asymmetry among groups with normal corneas, keratoconus corneas of differing severity, and corneas following collagen crosslinking. Our results in vivo provide evidence for focal weakening at the onset of keratoconus and time-dependent increase of corneal stiffness after collagen crosslinking. We introduce promising diagnostic metrics based on spatially-resolved biomechanical measurements.

Poster Number 32

Sehyo Yune, MD, MPH
Radiology, Research Fellow | sehyo.yune@mgh.harvard.edu

Intracranial Hemorrhage Detection and Classification with Radiology Atlas Based on Deep Learning

INVESTIGATORS: S. Yune, H. Lee, M. Kim, D. Y. Ting, M. Mansouri, S. R. Pomerantz, S. M. Kamalian, J. M. Romero, M. H. Lev, R. G. Gonzalez, S. Do

Intracranial hemorrhage (ICH) is a potentially fatal condition that needs immediate attention, but diagnosis of subtle cases can be missed or delayed. There have been interests in using artificial intelligence (AI) to automatically detect ICH, but the black box method in deep learning algorithms has been identified as the biggest challenge for such a system to be accepted by clinicians. In this study, we show the first known automated ICH detection system combined with case-specific radiology atlas using AI. A deep learning algorithm using convolutional neural networks was developed to detect and classify ICH from noncontrast head CT. The system automatically detects ICH showing the area under the curve (AUC) of 0.994 with 97% sensitivity and 97% specificity at the balanced operating point. It classifies intraparenchymal hemorrhage, intraventricular hemorrhage, subdural hemorrhage, epidural hemorrhage, and subarachnoid hemorrhage. The system provides prediction on the presence and the type of ICH and localizes the lesion by using a heat map. By retrieving class activation map, an atlas is created from the training images, composed of the most relevant image features for each test case.
This system allows physicians to learn important radiologic features applied to their cases, while providing preliminary information before a formal radiology report is available. In addition to radiology reports, it can help physicians understand the image findings. This tool can provide informal training for non-radiologist physicians integrated into their clinical duties as well as improved patient safety by immediately detecting cases of ICH.

Poster Number 33

Erik Abels, MS
Neurology, Graduate Student | eabels@mgh.harvard.edu

miRNA21 is functionally transferred within extracellular vesicles from glioma to microglia in vivo

INVESTIGATORS: E. R. Abels, S. L. Maas, E. Tai, D. T. Ting, C. J. Kolsteeg, M. L. Broekman, X. O. Breakefield

Glioblastomas are the most common and lethal adult brain tumors. Glioblastoma cells communicate with surrounding cells by secretion of cytokine and chemokines, and by release of membrane-bound extracellular vesicles (EVs) containing proteins, lipids and RNA. One of the most abundant RNAs is microRNA 21 (miRNA21), which promotes tumor progression by blocking translation of over 8 target mRNAs. We have demonstrated a critical role for miRNA21 in communication between tumor and surrounding cells by examining the extent and functionality of miRNA21 transfer in an in vivo model.
Using mouse glioma cells (GL261) stably transduced with a palmitoylated fluorescent protein, which labels EVs we visualized the uptake of EVs by surrounding cells in the brain. GL261 cells were implanted in the brains of immune compatible mice lacking expression of miRNA21 to monitor the transfer of miRNA21 to microglia and infiltrating myeloid cells. These cells are isolated based on cell-specific markers and extent of EV, uptake followed by transcriptome analysis, including the presence of miRNA21.

Here we show that surrounding normal cells in the brain take up EVs derived from the tumor with delivery of miRNA21 that regulates specific downstream mRNA targets. In addition, EVs isolated from cultured GL261 cells and injected into the brain are taken up by microglia with transfer of miRNA21. Although others have suggested that miRNAs might be transferred from tumor cells to surrounding cells via EVs, this is the first study to show that miRNA21 is functionally transferred from tumor to microglial cells within the brain in vivo.

Poster Number 34

Christian Badr, PhD
Neurology, Assistant Professor | badr.christian@mgh.harvard.edu

Targeting the SCF Ubiquitin Ligase in Glioblastoma

INVESTIGATORS: C. Badr, K. Pinkham, K. Rosiak, A. Hashemiaghdam

Glioblastoma (GBM) is the most malignant and common form of brain tumors with high mortality and resistance to therapy. The presence of glioma stem cells (GSCs) that drive tumor growth and promote recurrence, exacerbates GBM prognosis. Because of its implication in cancer, and its role in regulating several proteins with oncogenic or tumor suppressor properties, we investigated the role of the SKP1–CUL1–F-Box protein (SCF) E3 ubiquitin ligase in GBM and particularly in GSCs. We demonstrate that the SCF complex is essential for maintaining self-renewal and in vivo tumorigenic potential of GSCs. Genetic targeting of CUL1 abrogated tumor formation in mouse orthotopic GSC models. Disrupting the SCF in normal cells did not affect cell viability indicative of its tumor-specific role and its relevance as a therapeutic target. We describe a new role of the SCF scaffold protein, CUL1, in transcriptional regulation of the oncoprotein MYC independently of the ubiquitin ligase function of this complex. Blocking of CUL1 neddylation, a process by which the protein NEDD8 is conjugated to CUL1, depleted GSCs. Additionally, we demonstrate that the inhibition of GSK3 results in CUL1 and MYC downregulation. The combination of the GSK3 inhibitor Tideglusib (blocks CUL1 and MYC expression) with MLN4924 (blocks CUL1 neddylation) showed a potent synergistic effect on GSCs viability. Both compounds are clinically relevant and cross the blood-brain-barrier therefore accessible to brain tumors. In summary, our study depicts a new role of SCF in brain tumors maintenance and growth and identifies a therapeutic strategy to target this complex.

Poster Number 35

Chiara Bellio, PhD
Obstetrics and Gynecology, Research Fellow | cbellio@mgh.harvard.edu

Ovarian cancer stem-like cells persist following olaparib treatment due to differential inherent DNA repair capacity

INVESTIGATORS: C. Bellio, C. DiGloria, K. James, P. Konstantinopoulos, W. B. Growdon, R. Foster, B. R. Rueda

The development of ovarian cancer (OvCa) has been linked to alterations in DNA repair pathways and targeting of these pathways is an effective clinical strategy. The poly (adenosine diphosphate [ADP]–ribose) polymerase inhibitor (PARPi) olaparib is an FDA approved monotherapy for the treatment of recurrent OvCa in patients with germline BRCA mutations. Despite promising response rates, most of the patients develop resistance to olaparib that is poorly understood.
We found that olaparib treatment in vitro and in vivo induced an enrichment of the OvCa stem-like CD133+ and CD117+ cell populations while inducing cell death in the CD133- CD117- non-CSC population. Sphere formation capacity was increased following olaparib treatment in vitro, consistent with the enrichment of stem-like cells. We demonstrated that this olaparib-induced increase is not impacted by BRCA mutation status. We also observed a similar response to the second generation PARPi rucaparib. Interestingly, olaparib induces an accumulation of RAD51 and DMC1 foci and G2/M arrest in CD133+ cells, suggesting activation of homologous recombination (HR) DNA repair pathways in stem-like cells. DMC1 is a meiotic recombinase involved in the initiation of HR and DMC1 gene expression is induced in CD133+ cells following olaparib treatment. Finally, we determined in two separate analyses that CD133+ cells more efficiently repair olaparib-induced DNA damage. These data reveal a potential resistance mechanism to PARPi therapy. The enhanced DNA repair capability of stem-like OvCa cells results in maintenance of their genomic integrity during drug treatment thereby allowing them to survive and drive disease recurrence.

Poster Number 36

Syed Bukhari, PhD
Cancer Center, Research Fellow | bukhari.syed@mgh.harvard.edu

A Poly(A)-Independent Mechanism of MicroRNP-Mediated Translation Activation in quiescent chemoresistant cancer cells

INVESTIGATORS: S. Bukhari, S. S. Truesdell, S. Lee, M. Boukhali, E. Jain, R. Sadreyev, W. Hass, S. Vasudevan

MicroRNAs are well documented as translational repressors. However, under certain cellular condition such as low mTOR 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 low-mTOR, quiescent chemoresistant cells. The mechanism of this translation activation by microRNAs in quiescent chemoresistant cells remains largely unknown. Here we show that upon mTOR inhibition or chemoresistance, microRNA-mediated activation requires short or no poly(A) tails on target mRNAs in chemotherapy treated 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 down-regulation. We show that inhibition of the cap binding deadenylase, poly(A) ribonuclease, PARN, that is active in quiescent state, prevents up-regulation of translation activation. Importantly, we also observed that the interaction of FXR1-associated microRNP with p97/eIF4G2, a paralog of the translation factor eIF4G without PABP-interacting domains, is required for translation activation. This mechanism is required for translation of specific mRNAs and maintenance of the chemo-resistant cells. Taken together, these data reveal a specialized mechanism of microRNA-mediated activation where the FXR1a-associated microRNP targets specific shortened poly(A) mRNAs for p97/eIF4G2 -mediated translation in chemoresistant cells.

Poster Number 37

Huabiao Chen, PhD
Medicine, Instructor | huabiao.chen@mgh.harvard.edu

Immunotherapy for malignant mesothelioma that combines a mesothelin-targeted immune-activating fusion protein and CXCL12/CXCR4 blockade

INVESTIGATORS: B. Li, Y. Zeng, P. Reeves, A. Sluder, J. Gelfand, T. Brauns, M. Poznansky, H. Chen

There is a significant unmet need for new treatment strategies for malignant mesothelioma (MM). Despite relevant advances in many cancer treatment areas, including improvements in diagnosis, staging, and the clinical course of treated patients, MM remains a highly lethal disease. The purpose of this study is to develop a combination immunotherapy for MM, which involves a fusion protein (scFv-MtbHsp70) to target and evoke a cellular immune response to tumor-expressing mesothelin and the blockade of CXCL12/CXCR4 pathway to mobilize cytotoxic effector cells into tumors. The efficacy of the fusion protein, FDA-approved small molecule CXCR4 antagonist AMD3100 (plerixafor), and the combination were evaluated in two syngeneic and orthotopic murine models of MM in immune competent C57BL/6 mice. We found that in both murine mesothelioma models, the fusion protein alone delayed tumor growth and prolonged mouse survival, which was associated with increased tumor infiltration by CD3+CD8+ T cells. Treatment enhanced the cytotoxic function of tumor-specific CD3+CD8+ T cells by evoking dendritic cell activation as well as antigen presentation and cross presentation. AMD3100 alone reduced the proportion of Treg cells in tumors and decreased PD-1 expression on CD3+CD8+ T cells. The combination of the fusion protein and AMD3100 further significantly slowed tumor growth and enhanced mouse survival while augmenting tumor-specific CD8+ T-cell immune responses and abrogating intratumoral immunosuppression. Our findings demonstrated the synergistic antitumor effect of combination of scFv-MtbHsp70 and AMD3100 in treatment of MM in mice. This is a new therapeutic strategy which may significantly prolong survival of patients with this disease.

Poster Number 38

Min-Kyung Choo, PhD
Dermatology, Instructor | choo.minkyung@cbrc2.mgh.harvard.edu

The protein kinase p38alpha regulates epidermal p63 protein stability and stem cell pools during skin tumorigenesis

INVESTIGATORS: M. K. Choo, J. M. Park

Stem cells contribute to tissue regeneration by replenishing injured or aged cells, but also serve as tumor-initiating cells after acquiring mutations. Molecular mechanisms regulating the regenerative and tumorigenic potential of tissue stem cells are not well understood. The protein kinase p38alpha is activated by a variety of physiological cues that signify the threat of tissue damage and neoplastic transformation. We found that mice with keratinocyte-restricted p38alpha deficiency developed skin tumors at an increased rate when subjected to a chemical carcinogenesis protocol, while they exhibited enhanced epidermal regeneration after skin wounding. p38alpha-ablated epidermis harbored greater numbers of cells with stem cell properties such as holocolony formation and label retention (slow cycling). Tumors and the steady-state epidermis from these mice displayed expanded expression of p63, a transcription factor essential for epidermal stem and progenitor cell activity, skin development, and tumorigenesis. Conversely, p38alpha-activating stimuli, such as UVB radiation and anisomycin, induced p63 depletion in keratinocytes. Mechanistically, we discovered that p38alpha directly phosphorylated and thereby destabilized p63 protein. We identified the amino acid residues on p63 that were directly phosphorylated by p38alpha in vivo and in vitro. p63 phosphorylation by p38alpha altered the expression of keratinocyte genes related to stem cell homeostasis, and inflammation. Our findings illustrate a novel tumor suppression mechanism in which stress-activated signaling induces the contraction of tumor-initiating cell pools.

Poster Number 39

Valentine Comaills, PhD
Cancer Center, Research Fellow | vcomaills@mgh.harvard.edu

Proliferation during epithelial to mesenchymal transition induces genomic instability

INVESTIGATORS: V. Comaills, R. Morris, M. Madden, J. LiCausi, M. Boukhali, N. Aceto, D. T. Miyamoto, S. Ramaswamy, W. Haas, L. Zou, D. A. Haber, S. Maheswaran

TGF-β secreted by tumor stroma induces epithelial-to-mesenchymal transition (EMT) in cancer cells, a reversible phenotype linked to cancer progression and drug resistance. However, exposure to stromal signals may also lead to heritable changes in cancer cells, which are poorly understood. We show that epithelial cells failing to undergo proliferation arrest during TGF-β-induced EMT sustain mitotic abnormalities due to failed cytokinesis, resulting in aneuploidy. This genomic instability is associated with the suppression of multiple nuclear envelope proteins implicated in mitotic regulation and is phenocopied by modulating the expression of LaminB1. While TGF-β-induced mitotic defects in proliferating cells are reversible upon its withdrawal, the acquired genomic abnormalities persist, leading to increased tumorigenic phenotypes. In metastatic breast cancer patients, increased mesenchymal marker expression within single circulating tumor cells is correlated with genomic instability. These observations identify a mechanism whereby microenvironment-derived signals trigger heritable genetic changes within cancer cells, contributing to tumor evolution.

Poster Number 40

Meenal Datta, MS
Radiation Oncology, Graduate Student | datta@steele.mgh.harvard.edu

Quantifying solid stress and elastic energy as new measures of tumor mechanopathology

INVESTIGATORS: H. T. Nia, M. Datta, G. Seano, H. Liu, D. Jones, N. Rahbari, J. Incio, V. P. Chauhan, K. Jung, J. D. Martin, V. Askoxylakis, T. P. Padera, D. Fukumura, Y. Boucher, F. J. Hornicek, A. J. Grodzinsky, J. W. Baish , L. L. Munn, R. K. Jain

Elevated solid stress is a mechanopathology observed in both murine and human tumors. Solid stress refers to the solid mechanical forces generated and transmitted by the solid components of tumor tissue: cells and extracellular matrix. The importance of solid stress has been recognized in initial studies: solid stress causes hypoxia, promotes cancer cell invasion, and stimulates tumorigenic pathways. The establishment of robust and objective methods of solid stress in tumors would enable studies on the impact of this abnormal force on tumor progression, metastasis, and resistance to treatment. We have developed three new techniques to rigorously measure and map solid stress that are able to account for heterogeneity in the tumor microenvironment. These methods allow 2-D spatial mapping of solid stress, sensitive estimation of solid stress in small tumors with arbitrary geometry (e.g., metastatic lesions), and in situ quantification via an adapted biopsy punch. Furthermore, the preservation of tissue morphology and structure allows for subsequent histological analyses in matched tumor sections, facilitating quantitative correlations between solid stress and markers of interest. From our application of these methods to a variety of tumor types and host tissues, we were able to make important conclusions about this abnormal force, including that: (i) the magnitude and distribution of solid stress is dictated by both cancer cells and the local host microenvironment, (ii) solid stress – and not stiffness – increases with tumor size, and (iii) the mechanical confinement of a tumor by the surrounding host tissue can significantly contribute to intratumoral solid stress.

Poster Number 41

Renata Fleming, MD, PhD
Neurology, Research Fellow | rfleming2@mgh.harvard.edu

Characterization of Neuropeptide Profile in Glioblastoma Cells and Cancer Stem Cells: Are Neuropeptide a Key Factor for Resistance and Plasticity?

INVESTIGATORS: R. L. Fleming, B. A. Tannous

Gliomas account for the great majority of primary tumors that arise within the brain parenchyma. Glioblastoma (GBM) is the most aggressive type of gliomas. The mainstay of treatment of GBM is surgery, followed by radiation and chemotherapy. Despite this aggressive treatment, the median survival of patients with GBM is <2 years. Recently, GBM molecular profile revealed two predominant subtypes, proneural and mesenchymal. GBM that presents mesenchymal signature is more aggressive and has an increased therapeutic resistance. Plasticity between these two subtypes is observed in tumor recurrence and therapy resistance. Neuropeptides are small protein-like molecules expressed and released by neurons to modulate their communication. Neuropeptides are involved in several biological activities, many of which act as growth factors stimulating cell proliferation and mitogenesis. Since the current standard treatment is unlikely to result in prolonged remission, there is a great effort to better understand the oncobiology of GBM and overcome tumor resistance.

We hypothesized that neuropeptides can also play a role in modulating GBM neural stem-like cells, a subpopulation of cells responsible for tumor initiation, recurrence and resistance, similar to their normal counterparts. Our aim is to characterize the neuropeptide profile of GBM cells (sensitive vs resistant to chemotherapy) and patient-derived GBM stem cells with different molecular subtypes. Our preliminary data show a different neuropeptide profile between sensitive vs resistant GBM cells, as well as proneural vs mesenchymal stem-like cells. These results suggest that neuropeptide may play an important role in tumor resistance and plasticity.

Poster Number 42

Sandro Goruppi, PhD
Dermatology, Instructor | sgoruppi@mgh.harvard.edu

The autophagy regulating kinase ULK3 is critical for convergent control of cancer associated fibroblast activation by CSL and GLI

INVESTIGATORS: S. Goruppi, M. Procopio, A. Clocchiatti, G. Bottoni, V. Neal, G. Dotto

The connection between signaling pathways activating cancer associated fibroblasts (CAFs) remains to be determined. Metabolic alterations linked to autophagy have also been implicated in CAF activation. CSL/RBPJ, a transcriptional repressor that mediates Notch signaling, suppresses gene expression program(s) leading to stromal senescence and CAF activation. While CSL-p53 interactions oversee stromal senescence, the signaling pathway(s) that interplay with CSL in control of CAF activation await discovery. GLI signaling is also deregulated in tumor stroma and contributes to CAF conversion.
We found that conversion of human dermal fibroblasts into CAFs after CSL function loss depends on GLI activation. Notably, conditions inducing autophagy, often found in tumor stroma, can down-regulate CSL levels through a mechanism involving the association with the autophagy adaptor p62/SQSTM1. In turn, decreased CSL up-regulates the expression of the autophagy kinase ULK3, which binds and activates GLI. Increased ULK3 also induces in HDFs autophagy and mitophagy, contributing to their glycolytic metabolic switch, which is however separated from GLI and CAF activation after CSL loss. Analysis of public data set of gene expression indicates that ULK3 levels are elevated in CAF from several tumor types, including prostate, breast and head and neck cancers. Silencing of ULK3 in skin squamous cell carcinoma-derived CAFs suppresses GLI activation and CAF marker expression, and blocks their tumor enhancing properties in vivo. Thus, ULK3 kinase links CSL and GLI involvement in CAF activation and represents an attractive target for stroma-focused anti-cancer intervention.

Poster Number 43

Chunbo He, MS
Obstetrics and Gynecology, Research Technician | che6@mgh.harvard.edu

YAP1-LATS2 negative feedback loop serves as a switch between cellular senescence and malignant transformation

INVESTIGATORS: C. He, X. Lv, C. Huang, B. R. Rueda, J. S. Davis, C. Wang

Our previous studies showed that Yes-associated protein 1 (YAP1) induces tumorigenesis in immortalized ovarian surface epithelial cells and fallopian tube secretory epithelial cells. However, the role of Hippo/YAP1 signaling pathway in primary human ovarian cells is not known. Using primary human ovarian surface epithelial (hOSE) cells and granulosa cells (hGCs) as cellular models, we found ectopic expression of YAP1, the major oncogenic effector of the Hippo pathway, induced cell cycle arrest and cellular senescence in hOSE cells and hGCs. Importantly, LATS2, a major upstream suppressor of YAP1, was elevated in both natural replicative and YAP-induced senescence. Knockout of LATS2 in primary hOSE cells not only diminished natural replicative senescence, but also prevented YAP-induced cellular senescence. Overexpression of LATS2 in primary hOSE cells sped up the progression of natural replicative and YAP-induced senescence. Constitutive activation of YAP1 stimulated the expression of LATS2 in hOSE cells, indicating that YAP1 and LATS2 formed a negative feedback loop to regulate cellular senescence. Intriguingly, constitutive activation of YAP1 in LATS2-deficient hOSE cells induced malignant transformation and tumor formation in a xenograft mouse model. Mechanistic studies showed the ectopic expression of LATS2 in primary hOSE cells increased expression of major components of the DREAM (dimerization partner, RB-Like, E2F and multi-vulval class B) complex, which represses gene expression during cellular quiescence. Our results indicate that disruption of the Hippo pathway and the subsequent loss of the YAP1-LATS2 negative feedback system may switch ovarian cells from YAP-induced senescence to tumorigenesis.

Poster Number 44

Sinyoung Jeong, PhD
Wellman Center for Photomedicine, Research Fellow | sjeong4@mgh.harvard.edu

Highly Sensitive and Reliable Plasmonic Nanoparticle-based Digital Immunoassay as Molecular Diagnostics for Epithelial Ovarian Cancer

INVESTIGATORS: S. Jeong, G. Joshi, N. Nowell, G. Gonzalez, J. Hoballah, P. Krauledat, W. P. Hansen, C. L. Evans

Epithelial ovarian cancer (EOC) is the most lethal gynecologic cancer. It is difficult to detect at the early stage due to lack of specific symptoms and there is a poor understanding of pathogenesis and metastasis of EOC. Interestingly, recent studies indicate that a fraction of shed MUC16 from EOC tumors is bound to the surface of natural killer cells (NK cells), resulting in inhibition of NK cells' anti-tumor function and suppression of immune response. This binding by MUC16 may extend to other leukocyte classes, but occurs on the order of only 10's of molecules per cell, far below the current detection threshold of cytometric techniques. For this purpose, we developed a plasmonic nanoparticle-based, digital immunoassay toolkit that incorporates three key complementary techniques:(1) antibody-conjugated gold plasmonic nanoparticles (PNPs) with target-specificity as an optical probe, (2) darkfield microscopy imaging system to detect the targeted PNPs on the surface of cell, and (3) a highly reliable computational algorithm for automatic counting of the number of the targeted PNPs from individual cells. To demonstrate the feasibility of this toolkit, peripheral blood mononuclear cells (PBMCs) of healthy control and EOC patients were investigated. Consequently, we found significantly high binding of MUC16 on the surface of PBMCs of EOC patients (~40 PNPs/cell) than healthy control (~7 PNPs/cell). These results imply that this approach may have the potential to quantify leukocyte surface bound MUC16 and thereby assist in understanding metastasis of EOC and potentially detecting EOC at earlier stages.

Poster Number 45

Yuanyuan Ji, PhD
Radiology, Associate Professor | yji6@mgh.harvard.edu

Anti-TLR4 antibody conjugated with NIR fluorophores labeling RAW264.7 cells

INVESTIGATORS: Y. Y. Ji, G. Park, H. Kang, G. El Fakhri, H. S. Choi

Circulatory cells have received a considerable interest as new drug delivery vehicles. Especially, targeting macrophages and monocytes has the potential to treat cancers by stimulating the immune system. Anti-TLR4 antibody raised against toll-like receptor 4 (TLR4) is a pathogen recognition receptor that confer functional specificity to macrophages. To investigate the detailed mechanism of action, we have conjugated the anti-TLR4 antibody with a near-infrared (NIR) fluorophore, ZW800-1C. The NHS ester form of ZW800-1C was used to conjugate on the antibody in phosphate-buffered saline (PBS), pH 7.8 for 3 h. The ZW800-1C conjugated anti-TLR4 antibody (ZW-TLR4) was then analyzed the optical properties using the Ocean Optics spectrophotometer. The labeling ratio of NIR fluorophores on an antibody was calculated based on their extinction coefficient, which was found to be about 0.5. Then, the specificity of targeted NIR fluorescent ligand ZW-TLR4 to macrophages was evaluated using mouse RAW264.7 cell lines. Strong NIR fluorescent signals were found in the cytosol of RAW264.7 cells, indicating receptor-mediated endocytosis. Real-time imaging of cellular targeted macrophages will be observed using the intraoperative FLARE imaging system under the 800 nm channel of NIR fluorescence. The present results suggest that anti-TLR4 antibody conjugated with NIR fluorophores could be useful for target-specific diagnosis and even tumor immunotherapy.

Poster Number 46

Zhenyu Ji, PhD
Wellman Center for Photomedicine, Research Scientist | zji@partners.org

Melanoma Lineage Targeting by CDK7 Inhibitor

INVESTIGATORS: Z. Ji, P. Eliades, B. Abraham, C. Christensen, N. Kwiatkowski, D. Miller, B. Miao, R. Kumar, M. Taylor, C. Njauw, T. Zhang, K. Wong, N. Gray, R. Young, H. Tsao

Melanoma is accounted for most skin cancer related deaths. Despite recent progress made on molecular therapy development, targeted therapies usually lead to drug resistance which emerges in months or years. Part of the difficulty in treating melanomas has been attributed to a strong survival program controlled by melanocyte transcription factors such as MITF, which is an example of "lineage dependency." Recently, a covalent CDK7 inhibitor (THZ1) has been shown to potently suppress the growth of various cancers through the depletion of master transcription-regulating oncogenes and the disruption of their attendant super-enhancers. Here we show that melanoma cells are highly sensitive to CDK7 inhibition and that a melanocyte "lineage cluster," whose members are transcriptionally driven by super-enhancers, is also strongly suppressed by THZ1. These results point to CDK7 inhibition as a viable strategy to deprive oncogenic transcription and suppress tumor growth in melanoma.

Poster Number 47

Manol Jovani, MD, MPH
Medicine, Research Fellow | manol.jovani@mail.harvard.edu

A Discovery-based Proteomic Analysis Identifies Circulating Growth Differentiation Factor-15 is Associated with Gastrointestinal and Colorectal Cancer Incidence and Mortality

INVESTIGATORS: M. Jovani, A. D. Joshi, M. G. Larson, D. Levy, R. S. Mehta, J. E. Ho, A. T. Chan

Circulating growth differentiation factor-15 (GDF-15) has been associated with overall and cardiovascular-specific mortality. We aim to study its association with overall, and site-specific cancer incidence and mortality. We measured 85 biomarkers among 5080 participants of the Framingham Heart Study, free of cancer at baseline. We used multivariable-adjusted Cox models. In 12.4 years, we identified 657 incident cancers: 96 gastrointestinal (GI) (59 colorectal cancer,CRC, 37 other) and 561 non-GI (66 lung, 112 breast, 113 prostate and 270 other), and 218 cancer-related deaths: 49 GI-related (18 CRC, 31 other), 142 non-GI related (41 lung, 8 breast, 18 prostate and 75 other),and 34 of unknown primary. Among 85 biomarkers, GDF-15 was the most consistently associated with overall cancer incidence (HR 1.35 per 1-standard deviation increase in GDF-15, 95%CI 1.19, 1.55, p=0.006). Specifically, GDF-15 was associated with GI cancer (HR 2.28, 95%CI 1.64, 3.18, p<0.001), and CRC (HR 2.58, 95%CI 1.70, 3.92, p<0.001) incidence. Further, GDF-15 was associated with overall (HR 2.39, 95% CI 1.91, 2.99, p<0.001), GI (HR 2.67, 95%CI 1.66, 4.29, p=0.005) and non-GI (HR 2.21, 95% CI 1.67, 2.93, p<0.001) cancer deaths. Among GI cancers, GDF-15 was associated with CRC-related death (HR 4.86, 95% CI 2.27, 10.43, p=0.004). We observed no evidence for an association with non-GI and non-CRC GI cancers' incidence, and non-CRC related deaths. GDF-15 was associated with overall, GI, and CRC cancer incidence and cancer-related death. Further investigations of the role of GDF-15 as a potential diagnostic and prognostic factor for cancer are warranted.

Poster Number 48

Homan Kang, PhD
Radiology, Instructor | hkang7@mgh.harvard.edu

Ultrasmall Theranostic Nanocarriers for Gastrointestinal Stromal Tumors

INVESTIGATORS: H. Kang, S. Hu, G. K. Park, Z. Wang, G. El Fakhri, H. Choi

The advances in molecular imaging modalities have innovated the diagnosis and treatment of human disease. However, tumors less than 1 cm in size still remain difficult to localize by conventional means because of the difficulty in specific delivery to the tumor site. Furthermore, high nonspecific uptake in the major organs and persistent background retention result in a low tumor-to-background ratio (TBR). Here, we report that ultrasmall (< 5.5 nm) zwitterionic nanocarriers (a.k.a. H-Dots) can travel systemically the whole body through the bloodstream without nonspecific tissue uptake, and then eventually clear out to urine. The basic design of H-dots is composed of 3 specific functional domains: 1) a targeting cavity to deliver specific anticancer drugs to the tumor site, 2) a charge balancing domain to minimize nonspecific uptake, and 3) an imaging domain to track the process of tumor targeting. In this study, we designed H-Dots to target gastrointestinal stromal tumor (GIST) with providing high TBR, to deliver imatinib anticancer drug to mutated KIT receptors selectively, and to treat unreachable GIST efficiently. Moreover, H-Dots enabled to monitor the drug delivery, targetability, pharmacokinetics, and therapeutic efficacy in GIST-bearing xenograft mice and genetically engineered tumor models. More importantly, imatinib-loaded H-Dots exhibited lower uptake into the immune system, improved tumor selectivity, and higher tumor suppression compared to free imatinib. These precisely designed H-Dots could be used as a promising theranostic nanoplatform that potentially reduce the side effects of conventional chemotherapies.

Poster Number 49

Siang-Boon Koh, PhD
Cancer Center, Research Fellow | sbkoh@mgh.harvard.edu

Chemoresistance in triple-negative breast cancer is mediated via a therapeutically actionable YAP/RASAL2 pathway

INVESTIGATORS: S. B. Koh, S. J. Isakoff, K. Ross, K. Matissek, L. He, A. Schultz, A. Langenbucher, M. S. Lawrence, L. W. Ellisen

Triple negative breast cancer (TNBC) is one of the most therapeutically intractable human malignancies, owing to genetic heterogeneity and a lack of druggable targets. The primary systemic treatment option for TNBC patients remains cytotoxic chemotherapies. While much work has focused on identifying TNBC patients who will respond to conventional chemotherapies, this effort does not address those who are unlikely to respond and therefore have the poorest clinical outcomes. We sought to identify rational therapeutic options for treatment-refractory TNBC patients through transcriptomic, proteomic and biochemical approaches. Here we show that chemoresistance in both TNBC human tumors and cell lines is mediated by hyperactive YAP, a transcriptional cofactor which positively regulates RASAL2. Overexpression of RASAL2 in TNBC cells induces a mesenchymal phenotype associated with chemoresistance. Conversely, these cells are sensitive to clinical MEK inhibitors, showing attenuated activation of survival pathways following inhibitor exposure. Together, our findings provide potential biomarkers and an actionable therapeutic approach for refractory TNBC patients.

Poster Number 50

Sooncheol Lee, PhD
Cancer Center, Research Fellow | slee91@mgh.harvard.edu

A post-transcriptional program of chemoresistance by AU-rich elements/TTP in cancer quiescence

INVESTIGATORS: S. Lee, S. S. Truesdell, S. I. Bukhari, Y. Kato, M. Lawrence, R. Gandhi, D. A. Sweetser, W. Hass, S. Vasudevan

Quiescent cells are transient cell-cycle-arrested subpopulations. Quiescent cancer cells are clinically important because they are resistant to most chemotherapy drugs and lead to cancer recurrence. While quiescent leukemic cells were studied transcriptionally, their unique translation profile and post-transcriptional mechanisms that control their proteome are unknown. Here, we found that AU-rich element (ARE)-containing mRNAs like TNFα are stabilized and translated by phosphorylation of tristetraprolin (TTP) via the p38 MAPK/MK2 signaling pathway, although overall protein synthesis is repressed. Surprisingly, pharmacological inhibition of this signaling pathway and TNFα with Pirfenidone and LY2228820 prior to AraC treatment—called PLA therapy—was very effective in killing leukemic cells. It eliminated at least 80% of chemoresistant cells in multiple AML cell lines and primary AML cells. Furthermore, it inhibited tumor growth in an AML mouse model in vivo. These studies reveal the significance of post-transcriptional regulation of ARE mRNAs-mediated chemoresistance, and developed a new combination therapy against chemo-survival.

Poster Number 51

Xiangmin Lv, PhD
Obstetrics and Gynecology, Research Fellow | xlv@mgh.harvard.edu

Reprogramming of Ovarian Granulosa Cells by Yes-Associated Protein 1 Leads to Development of Mesenchymal Type of High Grade Ovarian Carcinoma with Serous Features

INVESTIGATORS: X. Lv, C. He, C. Huang, B. R. Rueda, J. S. Davis, C. Wang

Recent cancer genomic studies identified a mesenchymal type of high grade serous ovarian carcinoma (mHGSOC) with the poorest prognosis among ovarian cancers. The cell-of-origin of this new subtype of ovarian cancer is unknown. While pursuing studies to understand the role of the Hippo signaling pathway in ovarian granulosa cell physiology and pathology, we unexpectedly found that ovarian granulosa cells (GCs) with overactive Yap signaling develop a phenotype resembling mHGSOC. YAP1, the major effector of the Hippo pathway, is predominantly localized to nuclei of proliferative GCs, but to the cytoplasm of terminally-differentiated GCs. Ectopic expression of constitutively active YAP1 (YAPS127A) induced de-differentiation and reprogramming of luteinized GCs. Importantly, expression of YAPS127A in less-differentiated human GCs induced tumors in a xenograft mouse model. Intriguingly, these tumors resembled human HGSOC morphologically, histologically, and genetically. The tumors were accompanied by accumulation of ascites in the peritoneal cavity. Highly proliferative tumor cells invaded into gastrointestinal system, omentum, visceral fat tissue, diaphragm, liver and pancreas. Deletion of the BRCA1 gene in these tumor cells promoted further tumor development and progression and significantly reduced survival rate. In addition, these tumors have high expression of N-cadherin but low expression of CA125 and E-cadherin, which are key molecular characteristics of mHGSOC. In conclusion, our results suggest YAP1 plays a critical role in regulating granulosa cell proliferation and differentiation. Over-activation of YAP1 can result in de-differentiation and re-programming of ovarian GCs, leading to development of mesenchymal subtype of HGSOC.

Poster Number 52

Hai Dang Nguyen, PhD
Cancer Center, Research Fellow | dnguyen22@mgh.harvard.edu

Spliceosome Mutations in Myelodysplastic Syndrome Induce R Loop-Associated Sensitivity to ATR Inhibitor

INVESTIGATORS: H. D. Nguyen, W. Y. Leong, W. Li, M. J. Walter, L. Zou, T. A. Graubert

Heterozygous somatic mutations in spliceosome genes (e.g., U2AF1, SF3B1, ZRSR2, and SRSF2) occur over 50% of myelodysplastic syndrome (MDS) patients. These mutations occur early in disease development, suggesting that they contribute to MDS pathogenesis and may represent a unique genetic vulnerability for targeted therapy. Bone marrow cells from MDS patients and genetically-engineered mouse models carrying spliceosome gene mutations (U2AF1-S34F/Y or Q157P/Q; SF3B1-K700E; SRSF2-P95H) display RNA splicing abnormalities, suggesting that cells harboring spliceosome gene mutations affect normal splicing programs that may contribute to MDS pathogenesis. Intriguingly, expressing different spliceosome mutations in cells caused distinct aberrant splicing patterns, raising an important question as to whether a common mechanism underlies MDS pathogenesis in the setting of different spliceosome mutations.

Here, we show that RNA splicing perturbation by expression of the U2AF1-S34F mutant caused accumulation of R loops, a transcription intermediate containing RNA:DNA hybrids and displaced single-stranded DNA, and elicited an ATR response. ATR inhibitors (ATRi) induced DNA damage and cell death in U2AF1-S34F-expressing cells, and these effects of ATRi were enhanced by splicing modulating compounds. Moreover, ATRi-induced DNA damage was suppressed by overexpression of RNaseH1, an enzyme that specifically removes the RNA in RNA:DNA hybrids, suggesting that the ATRi sensitivity of U2AF1-S34F-expressing cells arises from R loops. Taken together, our results highlight that ATR may represent a novel therapeutic target in MDS patients carrying spliceosome gene mutations.

Poster Number 53

Juhyun Oh, PhD
Center for Systems Biology, Research Fellow | joh11@mgh.harvard.edu
Aging immune system controls tumor growth in mice

INVESTIGATORS: J. Oh, A. Magnuson, D. Mathis, C. Benoist, M. Pittet, R. Weissleder

Cancer is a disease of aging. Currently, an estimated of 50% of all cancers and 70% of cancer deaths occurs in the elderly (≥65 years), and these percentages are projected to rise even higher due to increasing number of older adults. Although aging is a leading risk factor, cancer has been modeled and interrogated in young animals. Studying cancer in context of aging will provide a new avenue for diagnostic/therapeutic strategies optimized for older cancer patients.

Immunotherapy has emerged as a potent treatment for many cancer types, by harnessing the patient's immune system to fight cancer. It has been well described that immune system is affected by aging (e.g., declined T lymphocyte function and T cell repertoire, and reduced responsiveness to vaccination); however, it is unclear how aging immune system in elderly patients would affect the outcome of cancer immunotherapy.

In this study, we assessed multiple tumor-infiltrating immune cell populations of young and aged hosts, to investigate how aging affects the anti-tumor immune response. Using MC38 and B16 tumor models, we found out that tumor growth is delayed in aged hosts as compared to young hosts, largely due to increased infiltration of cytotoxic T cells. Also, we examined our hypothesis that compromised immunosuppressive function of intra-tumoral regulatory T-cells in aged mice entails elevated cytotoxic T cell infiltration. Further study for better understanding of age-related changes in tumor-infiltrating immune cells will provide means to optimize cancer immunotherapy in the population most at risk for cancers.

Poster Number 54

Ethel Pereira, PhD
Radiation Oncology, Research Fellow | epereira@steele.mgh.harvard.edu
Lymph node metastasis in solid tumors: A marker or driver of disease progression?

INVESTIGATORS: E. R. Pereira, D. Kedrin, G. Seano, O. Gautier, E. F. Meijer, D. Jones, S. M. Chin, S. Kitahara, E. M. Bouta, J. Chang, E. Beech, H. S. Jeong, M. C. Carroll, A. G. Taghian, T. P. Padera

The presence of lymph node metastasis in patients with solid tumors is associated with poorer prognosis and recommendation for systemic therapy. However, whether tumor cells exit the lymph node and contribute to distant metastases remains controversial. Evidence shows that treating disease in the lymph node improves survival in some patients. In this study, we used syngeneic murine cell lines representing melanoma and breast cancer that spontaneously metastasize to the lymph node. We engineered these cells to express Dendra2, a photoconvertable protein, to determine if tumor cells from the lymph node are able to seed distant organs.

Here we show that spontaneous lymph node metastasis photoconverted in the node can exit the organ, enter the blood circulation and seed the lung. Cancer cells could take two possible routes to exit the node and spread systemically. We hypothesized that nodal metastases directly invade lymph node blood vessels, as opposed to draining through the efferent lymphatic vessel. Intravital imaging and immunohistochemical analysis of metastatic lymph nodes revealed 23±2% of isolated cancer cells were within 5μm of a blood vessel, compared to only 11±1% using a predictive model of randomly distributed cells in the lymph node. Further, 6±2% of the cancer cells were inside the lumen of blood vessels and having cell centroids more than 3μm from the blood vessel endothelium. Together, our data show for the first time that in spontaneous breast and melanoma mouse models, tumor cells in the lymph node can invade blood vessels, exit the node and colonize the lung.

Poster Number 55

Shuxi Qiao, PhD
Cancer Center, Research Fellow | sqiao1@mgh.harvard.edu

Rewired lipid metabolism drives metastasis in Ras-driven tumors

INVESTIGATORS: S. Qiao, D. Salunke, M. Dennis, X. Song, C. B. Clish, E. Zaganjo, M. Haigis, N. Bardeesy, L. W. Ellisen

KRAS is one of the most commonly mutated oncogene in human cancer. Metabolic reprogramming such as enhanced glycolysis is a hallmark of RAS-driven tumors. However, there is a substantial unmet need to exploit the metabolic vulnerabilities of RAS driven-tumors that in turn can be exploited for therapeutic benefits. Here we uncover a novel metabolic network that is fundamental to the metastatic progression of Ras-driven tumors with genetic loss of REDD1, an essential regulator controlling mTOR activity, redox status, autophagy and glycolytic reprogramming. By developing genetically engineered mouse models, we consistently observe increased tumor burden and frequent hematogenous metastasis in REDD1 deficient mice in the context of KRAS activation, which is not observed with its WT counterparts in vivo. By developing primary pancreatic epithelial cells and employing transcriptional and metabolic analyses, we discover that oncogenic cooperation between REDD1 loss and KRAS activation achieves metabolic robustness through bypassing de novo lipogenesis and selectively scavenging phospholipid species that is coupled to active generation of antioxidants and energy through enhanced fatty acid oxidation. Correspondingly, REDD1 deficiency sensitizes cells and tumors to inhibition of reduced glutathione synthesis and lipid uptake using small molecule drugs. Strikingly, we find that REDD1 is deregulated in RAS mutant human cancers including pancreatic ductal adenocarcinoma (PDAC) and lung adenocarcinoma (LUAD) and is associated with decreased overall survival. Thus, we define a distinct hypermetabolic state that support the metastatic progression of REDD1 deficient Ras-driven tumors, which reveals therapeutic vulnerabilities.

Poster Number 56

Xiying Qu, PhD
Medicine, Research Fellow | xqu@mgh.harvard.edu

Combination of mesothelin-targeted immune-activating fusion protein and anti-PD-L1 augments antitumor immunity and prolongs survival in murine model of ovarian cancer

INVESTIGATORS: X. Qu, Y. Zeng, P. Reeves, A. Sluder, J. Gelfand, T. Brauns, M. Poznansky, H. Chen

Ovarian cancer is a life-threatening tumor in women as its diagnosis often occurs at a late stage. Although immunotherapy as an adjuvant to surgery and chemotherapy has been broadly investigated in ovarian cancer as a means of reducing tumor recurrence and improving survival, there remains a significant unmet need for combinatorial strategies to enhance the antitumor immune response. The purpose of this study was to develop a novel combination immunotherapy for ovarian cancer, utilizing our novel mesothelin-targeted immune-activating fusion protein (VIC-008) to target and generate a cellular immune response to tumor-expressing mesothelin in conjunction with blockade of the PD-1/PD-L1 checkpoint pathway to restore the function of cytotoxic T cells in order to enhance cancer control and prolong survival. The efficacies of the VIC-008, αPD-L1, and the combination were evaluated in an intraperitoneal ovarian tumor model in immunocompetent C57BL/6 mice. Our results showed that VIC-008, αPD-L1 or combination treatment delayed tumor growth. The combination treatment resulted in the greatest prolongation in survival, followed by αPD-L1 treatment and then VIC-008 treatment. Through activating dendritic cells and enhancing antigen presentation and cross-presentation, VIC-008 augments antitumor CD8+ T cell responses and facilitates generation of memory T cells and reduction of regulatory T cells when combined with PD-1/PD-L1 blockade. Our findings demonstrate for the first time a mechanistic rationale for combining MSLN-targeted immune-activating fusion protein VIC-008 and an immune checkpoint inhibitor αPD-L1 in treatment of ovarian cancer in mice, positioning this combination therapy as a potential promising new immunotherapeutic approach for ovarian cancer.

Poster Number 57

Srinivas Vinod Saladi, PhD
Cancer Center, Instructor | ssaladi@partners.org

Defining SWI/SNF complex Mediated Immune Reprogramming in Squamous Cell Carcinoma

INVESTIGATORS: S. V. Saladi, M. Karaayvaz, P. R. Tata, M. Lawrence, J. Rajagopal, L. W. Ellisen

Squamous cell carcinoma (SCC) remains among the most treatment-refractory of human cancers, reflecting in part a limited understanding of the key pathways and mechanisms that drive this disease. The p53-related transcription factor p63 is a central tumor maintenance factor subject to overexpression and/or genomic amplification in a high fraction of SCCs. Loss-of-function mutations in SWI/SNF chromatin remodeling subunit genes are observed in many cancers, but an oncogenic role for SWI/SNF is not well established. We reveal that ACTL6A, encoding a SWI/SNF subunit linked to stem and progenitor cell function, is frequently co-amplified and highly expressed together with p63 in head and neck squamous cell carcinoma (HNSCC). ACTL6A and p63 physically interact and cooperatively control a transcriptional program that promotes proliferation and suppresses differentiation, in part through activation of the Hippo-YAP pathway via regulators including WWC1. Consequently, loss of ACTL6A or p63 in tumor cells induces YAP phosphorylation and inactivation, associated with growth arrest and terminal differentiation, all phenocopied by WWC1 overexpression. In vivo, ectopic ACTLC6A/p63 expression promotes tumorigenesis, while ACTL6A expression and YAP activation are highly correlated in primary HNSCC and predict poor patient survival. More recently, we show the major cell-autonomous pathway repressed by ACTL6A is the interferon response in vitro, while we find ACTL6A amplification in HNSCC is associated with immunologically "cold" tumors despite a high mutation burden in vivo. Collectively, these findings reveal that ACTL6A orchestrates a tumor cell-intrinsic mechanism of immune evasion and define ACTL6A as a potent driver of human SCC.

Poster Number 58

Sheng Sun, PhD
Cancer Center, Research Fellow | ssun9@mgh.harvard.edu

Expressed Gene Fusions as Frequent and Actionable Drivers of Poor Outcomes in Hormone Receptor Positive Breast Cancer

INVESTIGATORS: S. Sun, K. J. Matissek, M. L. Onozato, Z. Zheng, M. Tavallai, A. Schultz, J. Lee, K. Patel, K. Jerevall , S. V. Saladi , A. Macleay, J. St. Louis, N. Horick , D. M. Finkelstein, L. P. Le, A. Bardia, P. E. Goss , D. C. Sgroi, A. J. Iafrate, L. W. Ellisen

The majority of breast cancers are hormone-receptor positive (HR+), and therapies that antagonize hormonal signaling via these receptors remain the safest and most effective treatments. Resistance to primary hormonal therapy is a major cause of metastatic relapse. However, the mechanisms that lead to hormonal therapy resistance remain poorly understood. We employed a powerful and clinically-focused diagnostic approach, Anchored Multiplexed PCR (AMP) followed by next generation sequencing (NGS), to identify novel and prevalent expressed genetic rearrangements involving driver genes in breast cancer including PIK3CA, AKT3, RAF1 and ESR1. Here we show that novel gene fusions are identified in 14% of patients with advanced HR+ breast cancer. Most strikingly, these gene fusions correlate with dramatically shortened overall survival. Correspondingly, these fusions were uncommon (<5%) among the patients with non-metastatic HR+ breast cancer. Functional studies demonstrate that the novel kinase fusions induce activated phosphoprotein signaling, hormonal therapy resistance, hormonal-independent proliferation and aggressive disease course. Also strikingly, our in-vivo studies show that combination of estrogen withdrawal and the CDK4/6 inhibitor could overcome the therapeutic resistance conferred by the kinase fusions. These findings suggest that the novel fusions that we have identified are potentially actionable drivers in HR+ breast cancer.

Poster Number 59

Hadi Tavakoli Nia, PhD
Radiation Oncology, Research Fellow | htnia@steele.mgh.harvard.edu

Neurological dysfunction caused by brain tumor-generated solid stress is reversed by lithium

INVESTIGATORS: G. Seano, H. Tavakoli Nia, K. Emblem, M. Datta, J. Ren, J. Kloepper, S. Krishnan, M. Ghosh, M. Pinho, V. Askoxylakis, G. Ferraro, L. Riedemann, E. Gerstner, T. Batchelor, P. Wen, N. Lin, A. J. Grodzinsky, D. Fukumura, P. Huang, J. Baish, T. Padera, L. Munn, R. K. Jain

The compression of brain tissue by a tumor mass is believed to be a major cause of the clinical symptoms seen in patients. However, the biological consequences of these physical stresses on the brain tissue are unknown. Using clinical imaging and preclinical studies, we discovered that a subgroup of primary and metastatic brain tumors, classified as nodular based on the growth pattern, exert solid stress on the surrounding brain tissue, leading to a decrease in local vascular perfusion, as well as neuronal death and impaired function. We demonstrated a causal link between solid stress and neurological dysfunction, by applying and removing cerebral compression, mimicking the mechanics of tumor growth and surgical resection respectively. Finally, we showed that treatment with lithium reduced solid stress-induced neuronal death and improved motor coordination in mice. Our results indicate that brain tumor-generated solid stress impairs neurological function in patients and show lithium as a potential therapeutic intervention to counter these effects.

Poster Number 60

Zhidong Wang, PhD
Radiology, Associate Professor | wzhidong@mgh.harvard.edu

CD117-targeted SCF800 contrast agents for gastrointestinal stromal tumors

INVESTIGATORS: Z. D. Wang, D. W. Hwang, S. Hu, G. El Fakhri, H. S. Choi

Surgical resection is the main treatment for most gastrointestinal stromal tumors (GIST), but surgery appears to be difficult for metastatic or recurrent GIST patients due to the risks of positive margins. Stem cell factor (SCF) is a CD117 ligand and can specifically serve as a molecular cell surface target for the detection of GIST during surgical operation when conjugated with a contrast agent. In this study, ZW800-1C, zwitterionic near-infrared (NIR) fluorophore, was conjugated on SCF and used for highly sensitive, rapid, and non-radioactive imaging of GIST. Bioconjugation of SCF was performed using the NHS ester form of ZW800-1C in phosphate-buffered saline, pH 7.8 for 3 h. The labeling ratio, calculated by the extinction coefficient of SCF at 280 nm and ZW800-1C at 760 nm, was found to be about 0.5. ZW800-1C conjugated SCF (SCF800) was then used to target CD117 receptors in GIST-T1(receptor-positive) and GIST-5R (receptor-negative) cell lines. We confirmed the specific targeting of SCF800 on the surface of GIST-T1 cells using the NIR fluorescence microscopy, while negligible fluorescence signals were observed in receptor-compromised GIST-5R cells. Intraoperative imaging and image-guided tumor resection will be followed in GIST-bearing xenograft mice and genetically engineered animal models. This result suggests that SCF targeting using NIR fluorescence imaging is an effective approach for early diagnosis of GIST and intraoperative tumor imaging.

Poster Number 61

Su Wu, PhD
Cancer Center, Research Fellow | swu16@mgh.harvard.edu

SREBP1-driven Lipogenic Gene Expression Controls Cell Survival in Melanoma

INVESTIGATORS: S. Wu, A. M. Näär, TCGA, ENCODE

Pressing clinical needs demand a novel therapeutic target for treatment of metastatic melanoma. Gold-standard BRAF inhibitor (BRAFi) treatments initially succeed in patients with BRAFV600E mutation but tumors inevitably acquire drug resistance and relapse. Elevated de novo fatty acid biosynthesis (lipogenesis) is a hallmark phenotype of many cancers that supports rapid and unchecked cell proliferation. We show that melanoma exhibits markedly elevated lipogenic gene expression that correlates with poor prognosis in the Cancer Genome Atlas (TCGA) database. We find that the sterol regulatory element-binding protein 1 (SREBP1) functions in melanoma as a master regulator of lipid metabolism via transcriptional regulation of lipogenic gene expression, and we correlate that regulation with ChIP-seq analysis of SREBP1 binding sites. Further, we observe that lipogenic gene activity is a survival trait exhibited in BRAFi-resistant melanoma. We present antisense oligonucleotides (ASOs) as proof-of-principle agents that are more potent than (yet similarly specific as) siRNA for SREBP1 inhibition. Mechanistically, SREBP1 ASOs diminish RNA pol II and histone markers for actively transcribed chromatin at the lipogenic gene promoters. They act across multiple lipogenic genes synergistically. Independently, these ASOs decrease viability of both proliferative and quiescent melanoma cells. As adjuvants, they overcome the resistance mechanisms of melanoma to BRAFi treatment. Our findings implicate SREBP1 as a promising target for less-toxic and more-effective cancer therapies.

Poster Number 62

Chuan Yan, PhD
Pathology, Research Fellow | cyan1@mgh.harvard.edu

Imaging tumor heterogeneity and therapy responses at single cell resolution using human xenografts into immune deficient zebrafish

INVESTIGATORS: C. Yan, D. C. Brunson, N. A. Iftimia, K. M. McCarthy, Q. Tang, M. Ligorio, G. P. Nielson, A. J. Iafrate, D. M. Langenau

Cell transplantation of human cells into immunocompromised mice has increased our understanding of cancer. However, mice are expensive and engraftment is often difficult to visualize directly. Here, we have used xenotransplantation into optically clear Casper-strain prkdc-/-, il2rga-/- mutant zebrafish. These fish lack T, B and NK cells and robustly engraft human melanoma, rhabdomyosarcoma (RMS), and breast cancer when raised at 37C. Building on the optical clarity of our model, we next dynamically visualized human cancer cell heterogeneity in vivo at single cell resolution. In these experiments, we used a lenti-virally delivered photoconvertible H2B-Dendra fluorescent protein to label 3-5 RMS cells within the tumor to track proliferation rates and migration of human RMS cells. From this work, we identified three functionally distinct populations: persister, migratory and proliferative RMS cells. Remarkably, we found that migratory cells seldom divide over the seven days of imaging, providing exciting new cellular mechanisms that may account for metastatic progression and relapse after long latency. Finally, we used our xenograft fish models to identify a clinically relevant combination of PARP inhibitor and DNA damaging agent Temozolomide that effectively killed human RMS cells in vivo. Studies using the four-color FUCCI cell cycle reporter and single-cell, live imaging showed that combination therapy caused a G2 cell cycle arrest followed by cell death. In total, our work has optimized the zebrafish xenograft model for use in evaluating therapy responses and dynamically imaging cell behaviors at single cell resolution, opening new avenues for study in a wide array of human cancers.

Poster Number 63

Raza Alvi, MD
Radiology, Research Fellow | ralvi@mgh.harvard.edu

Implantable cardioverter defibrillators among persons living with HIV

INVESTIGATORS: R. M. Alvi, A. M. Neilan, N. Tariq, M. Awadalla, C. P. Mulligan, V. Triant, M. V. Zanni, T. G. Neilan

Persons living with HIV (PLHIV) are an increased risk of sudden cardiac death (SCD). Implantable cardioverter defibrillator (ICD) are indicated in the primary and secondary prevention of SCD. However, there are no data characterizing ICD use among PLHIV. From a heart failure (HF) registry of 2,308, we identified 326 (14%) with an ICD. We compared baseline characteristics between PLHIV and controls. The primary outcome was ICD discharge. Within PLHIV, we assessed the association between traditional and HIV-specific parameters with ICD discharge. The secondary outcome was the effect of ICD discharge on cardiovascular (CV) mortality and 30-day HF readmission. There were 59 with HIV and 267 HIV-uninfected controls with ICD's. Of the PLHIV, 81% were on ART and the mean CD4 count was 208±192 cells/mm3. As compared to controls, PLHIV with ICD had a higher prevalence of hyperlipidemia, CAD and cocaine use; the ICD insertion indication was similar. In follow-up, PLHIV had a trend toward a higher ICD firing rate compared to un-infected controls (39% vs 28%, p=0.087). Among PLHIV, CAD, cocaine use, low LVEF and lower CD4 count were independent predictors of ICD firing. Among PLHIV, ICD firing was associated with increased CV mortality and 30-day HF readmission rates (78% vs 39%, p=0.003 and 87% vs 52%, p=0.01, respectively). There is a trend toward more ICD discharge among PLHIV. Cocaine use is independent predictor of an ICD discharge in HIV and ICD discharge in HIV is associated with a two-fold increased risk of CV mortality.

Poster Number 64

Krishna Aragam, MD, MS
Center for Genomic Medicine, Clinical Research Fellow | karagam@partners.org

Clinical Characteristics and Treatment Patterns by Strata of Genetic Risk for Coronary Artery Disease in the Partners Healthcare Biobank

INVESTIGATORS: K. G. Aragam, M. Chaffin, A. Cagan, G. Hindy, A. V. Khera, L. C. Weng, S. A. Lubitz, J. W. Smoller, E. W. Karlson, S. Kathiresan, P. Natarajan, Partners Healthcare Biobank

High polygenic risk for coronary artery disease (CAD) has been independently associated with increased prevalent and incident CAD in several prospective cohorts. However, accruing data suggest that polygenic risk for CAD is modifiable through a favorable lifestyle and by statin therapy. The clinical characteristics and treatment patterns of patients at high polygenic risk for CAD have yet to be defined in a contemporary, hospital-based cohort. Here, we applied a polygenic risk score (PRS) for CAD comprised of 50 single nucleotide polymorphisms to ~14,000 participants with available genotypic and phenotypic data in the Partners Biobank and confirmed the association of the CAD PRS with increased risk for prevalent CAD (OR = 1.32 per standard deviation increase in PRS; CI = 1.26-1.39). Furthermore, we demonstrated that known cardiovascular risk factors do not comprehensively identify individuals at high polygenic CAD risk (top quintile of PRS, N = 2,805). Among individuals at high polygenic risk for CAD, 35% were classified as low-intermediate risk by conventional clinical criteria (American College of Cardiology/American Heart Association Pooled Cohort Equation for atherosclerotic cardiovascular disease risk), and 48% lacked a statin prescription within the Partners Healthcare system. Our findings suggest that polygenic CAD risk is not captured adequately by standard clinical parameters and that a treatment gap exists in the healthcare setting for primary prevention of CAD, which may be addressed through ascertainment of genetic risk.

Poster Number 65

Eric Grabowski, MD
Pediatrics, Associate Professor | egrabowski@mgh.harvard.edu

Differentiation of Patients with Type 1 von Willebrand Disease (vWD) and with Symptomatic Low Normal von Willebrand Factor (vWF) from those with Asymptomatic Low vWF, Using a Very High Shear Rate, High Image Resolution Blood Flow Chamber

INVESTIGATORS: E. F. Grabowski, E. M. Van Cott, L. Bornikova, D. C. Boyle

Accurate diagnosis of von Willebrand Disease (vWD) remains the greatest challenge in vWD today: Up to half of patients are underdiagnosed or overdiagnosed. We studied 24 patients (mean 17.9 yrs) with "probable type 1 vWD", diagnosed according to the 2007 NHLBI guidelines, and 12 healthy controls (mean 18.0 yrs). Blood, collected into a plastic tube containing enoxaparin, a monoclonal antibody directed against platelet GPIIb (Tab), and an ALEXA 555-conjugated secondary antibody, was drawn at a high shear rate of 4,000 sec-1 through a microfluidic flow chamber allowing for real-time epifluorescence digital video-microscopy of platelets interacting with a microfibrillar collagen substrate with 1 µm resolution. This permitted us to take advantage of a flow-dependent weakness in the vWF-platelet GPIbα bond found at such a high shear rate. We quantified percent area (PA) covered by adherent platelet aggregates, and volume of platelet aggregates (V) vs. time. All Group I patients had rates of increase in PA that were below the 2.5th percentile of controls, and had bleeding scores based on the ISTH bleeding assessment tool > 3 (range 3 to 8) if ≥ age 12. Group II (8) had rates of PA similar to controls, and, if the vWF low at time of study, ISTH bleeding scores ≤ 4 (range zero to 4). Therefore, our blood flow system readily distinguished all 16 patients with "true" type 1 vWD from 12 normal controls, while another 8 with low levels of the vWF but who were relatively asymptomatic were similar to controls.

Poster Number 66

Seyed Mohammadreza Hosseini, MD
Medicine, Research Fellow | shosseini@mgh.harvard.edu

Trends in Admission Rates for and Economic Burden of Atrial Fibrillation Emergency Department Visits in the United States from 2007–2014

INVESTIGATORS: S. M. Hosseini, G. Rozen, M. I. Kadaan, Y. Biton, E. K. Heist, M. Vangel, M. C. Mansour, J. N. Ruskin, The Harvard Catalyst Biostatistical Consulting Program

Atrial fibrillation (AF) is an increasingly prevalent public health problem and one of the most common causes of Emergency Department (ED) visits and hospital admissions. In a repeated cross-sectional study of data from the Nationwide Emergency Department Sample (NEDS) database, we identified a weighted total of 3,886,520 adults (95% CI: 3,736,118–4,036,922) with a principal diagnosis of AF who visited ED between 2007-2014. Annual ED visits for AF increased by 23.5% from 411,406 in 2007 (95% CI: 389,819–432,993) to 537,801 (95% CI: 506,747–568,855) in 2014 (Ptrend= 0.008). Patient demographics remained consistent with an average age of 69-70 years and slight female predominance (51%-53%) throughout the study period. Hospital admission rates were stable at approximately 70% between 2007-2010, after which they gradually declined to 62% in 2014 (Ptrend=0.017). Despite the decline in hospital admission rates, AF hospitalizations increased from 288,225 in 2007 to 333,570 in 2014 due to the increase in total annual ED visits. At the same time, the total adjusted annual charges for admitted AF patients increased by 37% from $7.39 billion in 2007 (1.12% of the "national bill") to $10.1 billion in 2014 (1.22% of the "national bill"). This study demonstrates that annual ED visits and admissions for AF increased significantly from 2007-2014, despite a reduction in admission rates. These data emphasize the need for implementation of strategies aimed at improving the management of AF in the community and at the ED level to reduce hospital admissions and the economic burden of AF.

Poster Number 67

Dongjian Hu, BS
Medicine, Graduate Student | dhu6@mgh.harvard.edu

Metabolic Maturation of Human Pluripotent Stem Cell Derived Cardiomyocytes by Inhibition of Hif1α and LDHA

INVESTIGATORS: D. Hu, A. Linders, A. Yamak, C. Correia, J. D. Kijlstra, A. Garakani, P. van der Meer, M. Serra, P. Alves, I. J. Domian

Human Pluripotent Stem Cell-Derived Cardiomyocytes (hPSC-CMs) are a readily available, robustly reproducible and physiologically appropriate human cell source for cardiac disease modeling, drug discovery, and toxicity screenings in vitro. However, 133 like adult myocardial cells in vivo, hPSC-CMs cultured in vitro maintain an immature metabolic phenotype where majority of ATP is produced through aerobic glycolysis instead of oxidative phosphorylation. Little is known about the underlying signaling pathways controlling hPSC-CMs' metabolic and functional maturation. We aim to define the molecular pathways controlling CMs' metabolic pathway selections and improve CM metabolic and functional maturation. We found CMs cultured in the presence of glucose utilize mostly aerobic glycolysis whereas CMs cultured in the absence of glucose depend on oxidative phosphorylation for energy productions. Hypoxia-inducible factor 1-alpha (Hif1α) and its target lactate dehydrogenase A (LDHA) were aberrantly upregulated in CMs cultured in the presence of glucose and corrected by glucose deprivation. We show that chemical inhibition of this pathway results in an appropriate metabolic shift from aerobic glycolysis to oxidative phosphorylation. This metabolic shift was accompanied by an increase in mitochondrial content and cellular ATP levels. Likewise, functional gene expressions, sarcomere length and contractility were improved by Hif1α/LDHA inhibition. These findings provide key insight into molecular control of hPSC-CMs' metabolism and may be used to generate more physiologically mature CMs for drug screening, disease modeling and therapeutic purposes.

Poster Number 68

James Rhee, MD, DPhil
Anesthesia, Critical Care and Pain Medicine, Instructor | jrhee@partners.org

Regulation of Cardiac Inflammation and Remodeling after Myocardial Infarction by Family with sequence similarity 3D (FAM3D)

INVESTIGATORS: J. Rhee, D. Zlotoff, A. Yeri, C. Xiao, V. Chaudhari, J. Roh, F. Damilano, X. Liu, R. Shah, M. Silverman, R. Kwong, S. Das, A. Rosenzweig

After myocardial infarction (MI), patients are at high risk for developing heart failure (HF), which carries high morbidity and mortality despite medical therapy. While large infarct size and reduced left ventricular ejection fraction at the time of MI are good predictors of eventual HF, a significant number of patients with initially small infarcts or preserved function go on to progressive dilation and failure. Biomarkers that help prognosticate HF could be used to risk-stratify and manage post-MI patients, and reveal new therapeutic targets. Recent studies have highlighted the important role of inflammation in remodeling. Optimal or "reverse" remodeling of the myocardium after injury is dependent on appropriate sequential activation and inactivation of the immune response.

Here, we have matched patients on initial infarct size 4 weeks after MI, and then stratified them as either "Good" or "Poor" remodelers based on improvement or worsening in left ventricular function six months later. Plasma samples from these patients at the 4 week timepoint were analyzed by a sophisticated aptamer-based proteomics screen. The highest scoring candidate by fold change (and second highest by statistical significance) was a chemokine called Family with sequence similarity (FAM) 3D. While thought to regulate neutrophils and monocytes, its role in cardiovascular biology is completely unknown. Our data clearly show that FAM3D is dynamically regulated by IRI, directly in proportion to the degree of cardiac injury. When overexpressed, it robustly limits acute infarct size via immune cell suppression. FAM3D likely serves as a critical link between cardiac injury, inflammation and remodeling.

Poster Number 69

Crystal Tan, MD, MS
Anesthesia, Critical Care and Pain Medicine, Clinical Research Fellow | ctan4@partners.org

Rescue Echo for Non-Cardiac Surgeries: An Interim Analysis of an Institutional Initiative

INVESTIGATORS: C. Tan, G. Staudt, K. Shelton

Intraoperative transesophageal echocardiography (TEE) is recommended for patients undergoing non-cardiac surgery who encounter life-threatening hemodynamic compromise, hypotension, or hypoxia. In May 2015, a Rescue Echo Service was created at MGH to facilitate access to TEE in non-cardiac operating rooms. The Rescue Echo Service is activated by either a pager or a voice-controlled, wearable Vocera badge to alert a team including a cardiac anesthesia attending, fellow, technician, and nurse who respond to provide a rapid intraoperative assessment.

The first two years of data included 139 rescue TEEs. Of these, 52% were performed on patients who were actively unstable or had potential to become unstable based on known medical history. Another 47% were performed for monitoring purposes. Evaluative Rescue Echoes tended to occur in older patients and patients ASA class 3 and higher. The most common problem was unexplained hypotension, followed by cardiac arrest. The most common echo findings were a normal exam, followed by hypovolemia. The most common intervention was disposition to the ICU, followed by case cancellation. The frequency of Rescue Echoes has tripled in the two years since the service was founded. Intraoperative TEE can be a valuable tool to rapidly and confidently assess patients experiencing hemodynamic instability during non-cardiac surgery. We hope to streamline the process from recognizing an intraoperative event to acquisition and interpretation of useful images and intervention. Future research will include continued tracking of Rescue Echo demographics and outcomes as well as clinician utilization and satisfaction surveys.

Poster Number 70

Nicolas Thibodeau-Jarry, MD, MMSc
Medicine, Clinical Research Fellow | nthibodeau-jarry@mgh.harvard.edu

Using Simulation to Teach Transthoracic Echocardiography to Cardiology Fellows: A Study Using the Mastery Learning Concept

INVESTIGATORS: N. Thibodeau-Jarry, D. Bamira, M. H. Picard

Learning transthoracic echocardiography (TTE) can be challenging. Mastery learning (ML) is a method of education that relies on feedback and mastery of a portion of a topic before moving on to the next portion. This pilot project tested the feasibility of training cardiology fellows in TTE through ML with the use of simulation. Six participants were recruited. None of them had received any formal training in TTE. The complete TTE examination was broken into six "learning units" (LUs). After a baseline assessment, participants were shown how to demonstrate all structures and measurements in the LU. They were then asked to try to demonstrate them, until they could get a perfect score three consecutive times. After having mastered the six LUs, they were asked to perform a complete TTE on the simulator.

The time required for the participants to complete the study varied from 5 hours 45 minutes to 9 hours 30 minutes. Participants were able to master each LU with a mean number of attempts varying from less than four attempts for LU six to six attempts for LU one. Three participants were able to obtain a perfect score on the complete TTE three consecutive times on their first three attempts. The three other participants required four attempts. The time required for the participants to complete the TTE on their last attempt varied from 16 to 33.5 minutes. Using simulation and the concept of ML may improve the efficiency of teaching TTE.

Poster Number 71

Abir Yamak, PhD
Center for Regenerative Medicine, Research Fellow | fyamak@mgh.harvard.edu

Asb2-dependent proteolysis is important for mammalian cardiac development and disease

INVESTIGATORS: A. Yamak, D. Hu, J. W. Buikema, C. Moog-Lutz, P. G. Lutz, I. J. Domian

Congenital heart diseases account for 25% of birth defects and are major risk factors for adult cardiovascular problems; thus, the need to understand different regulators of cardiac formation. The Ubiquitin-Proteasome System (UPS) is important in controlling protein turnover during organ development but its role in the heart remains ambiguous. We've identified specificity subunit of ubiquitin-mediated proteolysis (Asb2) as being specific for cardiac myogenic lineage. Asb2 regulates hematopoiesis and skeletal myogenesis through targeting filamins (FlnA, B and C), actin-binding proteins important for cytoskeleton stabilization. Our present study reveals marked Asb2 enrichment in myocardial progenitor cells and cardiomyocytes. To investigate Asb2 role and UPS dependent proteolysis in heart development, we generated two cardiac-specific murine knockouts (KO) deleting Asb2 in early cardiomyocyte progenitors and anterior heart field progenitors, respectively. Both KOs are embryonic lethal with pericardial edema. We used tissue clarifying and confocal microscopy to define the morphological defects of Asb2-null hearts. Moreover, we found that FlnA is overexpressed in Asb2-null hearts and its deletion therein partially rescues lethality. Using transcriptomic analysis on Asb2-null e9.5 hearts, we identified novel potential Asb2 targets in the heart. Finally, to understand Asb2 role in differentiation and function of human cardiomyocytes, we used CRISPR/Cas9 genome editing to generate Asb2-null human embryonic stem cells.

Collectively, our study provides novel mechanistic understanding of UPS proteasome role in cardiac development and disease pathogenesis. Given recent interests in both the UPS and the cytoskeleton as therapeutic targets, our study provides an innovative platform for the development of pharmacotherapy for cardiac disease.

Poster Number 72

Brandon Yates, MS
Physical Medicine and Rehabilitation Services, Research Fellow | byates@partners.org

The Influence of Dehydration on Executive Function Task Following Endurance Exercise in Middle-Age and Older Adults

INVESTIGATORS: B. A. Yates

Dehydration attenuates exercise and cognitive function performance in younger adults, but less is known about the impact of dehydration on the aging population. Middle-age and older adults often display a blunted thirst perception, which makes them vulnerable to dehydration and subsequently may reduce the cognitive health-related benefits of exercise. The purpose is to examine the impact that pre-exercise hydration status has on executive function task following endurance exercise within a middle-age and older adult cohort. This field study was conducted at a mass participation cycling event in Wichita Falls, Texas. Forty-nine recreational cyclists (55±6 y, range 44-70y) were enrolled following informed consent and were retrospectively separated into 2 groups (euhydrated, EUH; dehydrated, DEH) based on pre-exercise hydration status. Cyclists completed the Trail Making Test (TMT) prior to and immediately following the event.

After the HHH 164-km endurance cycling event, there was a significant improvement (i.e., faster completion time) in the TMT noted in the EUH group (pre vs post, 83±24 vs 71±18 s; $p<0.05$) but not the DEH group (pre vs post, 86±26 vs 79±22 s; $p>0.05$). Further analysis showed the Cohen d effect sizes to be moderate (0.5) in the EUH and small (0.2) in the DEH group. In this prospective study, dehydration negatively impacted an executive function task (TMT) in middle-age and older adults following an acute bout of prolonged, moderate-intensity endurance exercise. This suggests that older adults should adopt adequate drinking behaviors to reduce cognitive fatigue and potentially enhance the cognitive benefits of regular exercise participation.

Poster Number 73

Long Zhao, PhD
Medicine, Instructor | lzhao@cvrc.mgh.harvard.edu

Endocardial Notch signaling supports cardiomyocyte proliferation by inhibiting Wnt activity during zebrafish heart regeneration

INVESTIGATORS: L. Zhao, R. Ben-Yair, C. E. Burns, C. G. Burns

Zebrafish can achieve near complete regeneration of amputated or injured myocardium through the proliferation of spared cardiomyocytes (CM). Previously, we reported that Notch receptor expression becomes activated specifically in endocardium and epicardium, but not myocardium, after amputation of the zebrafish ventricular apex. In addition, we demonstrated that global inhibition of Notch signaling inhibits heart regeneration by compromising CM proliferation. Based on these observations, we hypothesize that Notch signaling, induced by injury in the endocardium and epicardium, activates the production of an unidentified paracrine signal that stimulates regenerative CM proliferation. Here, we report that suppression of Notch signaling specifically in endothelial/endocardial cells using a novel transgenic system profoundly decreases CM proliferation, impairs cardiac regeneration and induces scar formation at the amputation site. RNA-sequencing and quantitative PCR analyses reveal that Notch inhibition decreases the expression of two genes, wif1 and notum1b, which are induced in the endocardium following injury and encode secreted inhibitors of Wnt signaling. These data bring up the possibility that endocardial Notch signaling is required for CM proliferation by dampening Wnt activity, which is inherently inhibitory for CM proliferation. Consistent with this hypothesis, pharmacological hyperactivation of Wnt signaling dramatically decreases CM proliferation and blocks heart regeneration. In summary, our results demonstrate that endocardial Notch signaling is required for CM proliferation likely by stimulating the endocardial secretion of Wnt inhibitors. Additional studies designed to further decipher the role of Notch signaling in zebrafish heart regeneration will inform therapeutic strategies for redirecting or repurposing activated Notch for clinical benefit in humans.

Poster Number 74

Peter Chindavong, BS
Center for Genomic Medicine, Research Technician | pchindavong@mgh.harvard.edu

Reduced Representation Phosphosignature Profiling in Schizophrenia Patient-Derived Stem Cell Models

INVESTIGATORS: P. Chindavong, K. C. DeRuff, S. B. Egri, M. Papanastasiou, W. N. Zhao, S. D. Sheridan, R. H. Perlis, S. A. Carr, J. D. Jaffe, S. J. Haggarty, Psychiatric & Neurodevelopmental Genetics Unit

Human induced pluripotent stem cell (iPSC)-derived neural progenitor cells (NPCs) provide an ex vivo model to study cellular mechanisms in complex brain disorders such as schizophrenia (SCZ). We subjected two SCZ and two healthy control iPSC-derived NPC lines to 10 chemical perturbations. A targeted mass spectrometry-based assay that profiles a reduced representation of the phosphoproteome, termed P100, was used to analyze phospho-signaling in response to chemical perturbations. Our results show P100 is sensitive to differences in perturbations, cell lines, and disease states and shows reproducibility between replicates. P100 is thus a promising assay to further understand the biological underpinnings of neuropsychiatric disorders and contribute towards novel experimental therapeutics. Future directions include incorporating epigenetic and transcriptomic data into our results, and subjecting drug-perturbed neurons differentiated from SCZ patient-derived iPSC lines to P100 profiling.

Poster Number 75

Shuo Jiang, PhD
Molecular Biology, Research Fellow | sjiang@molbio.mgh.harvard.edu

A primary cilium architect: How a non-motile kinesin finds microtubule ends

INVESTIGATORS: S. Jiang, E. Wilson-Kubalek, N. Mani, R. Milligan, R. Subramanian

The primary cilium is a solitary microtubule-based organelle on the surface of most vertebrate cells. Proper organization of cilia architecture is critical for Hedgehog (Hh) signal transduction. The conserved non-motile kinesin Kif7 localizes to the cilium tip during ciliogenesis and is required for microtubule organization. However, the biochemical mechanism by which a non-motile kinesin can localize to the cilium tip is unknown. Here we applied TIRF-microscopy based in vitro reconstitutions and cryo-EM to define the Kif7-microtubule interaction. Our results show the localization of Kif7 to the growing ends of microtubules through a specific high affinity interaction with GTP-tubulin, unusual for a kinesin. Interestingly, we find that the kinesin's ATP hydrolysis cycle does not confer specificity but instead contributes to the recycling of the enzyme. Based on these observations, we also hypothesize a feedback between the kinesin ATPase and tubulin GTPase cycle such that hydrolysis is inhibited and end recognition is sustained. Furthermore, our Cryo-EM based reconstruction reveal an unusual kinesin footprint on microtubules. Our results demonstrate how a non-motile kinesin can localize to microtubule tips without motility by distinguishing the GTP form of tubulin at growing microtubule ends. This provides an initial mechanism for understanding cilia organization and how structural mis-regulation leads to developmental diseases.

Poster Number 76

Wondong Kim, PhD
Medicine, Research Fellow | wkim11@mgh.harvard.edu

Polyunsaturated Fatty Acid Desaturase-Mediated NAD+ Recycling Permits Ongoing Glycolysis and Cell Proliferation

INVESTIGATORS: W. Kim, A. Deik, J. C. Florez, S. B. Jacobs, C. B. Clish, E. P. Rhee

The delta-5 desaturase (D5D) and delta-6 desaturase (D6D), encoded by FADS1 and FADS2 respectively, are required for the synthesis of highly unsaturated fatty acids (HUFAs). Genetic variants associated with FADS1 and FADS2 expression are associated with HUFA content in circulating lipids, as well as fasting glucose, height and weight, and risk of colon and laryngeal cancer. Why genetic variation in HUFA synthesis has such a broad impact on human metabolic and proliferative phenotypes is unknown. Here, we show that HUFA synthesis is a mechanism for cytosolic NAD+ recycling that permits ongoing glycolysis. Consistent with this, lowering the cytosolic NAD+/NADH ratio via inhibition of mitochondrial respiration increased D5D and D6D activity in vitro and in vivo. Conversely, increasing the cytosolic NAD+/NADH ratio via expression of an NADH oxidase or activation of lactate dehydrogenase decreased D5D and D6D activity. Increasing D5D and D6D expression increased cytosolic NAD+/NADH, whereas reduced expression or inhibition of D5D and D6D decreased cytosolic NAD+/NADH and increased lactate production. Inhibition of D5D and D6D also reduced cell proliferation, an effect that was reversed by boosting cytosolic NAD+ but not with HUFA end-product supplementation. Finally, we show that the type 2 diabetes risk haplotype in SLC16A11, which reduces pyruvate transport, also increases D5D and D6D activity, demonstrating the relevance of our findings to human disease. These results outline a novel link between glycolysis and HUFA desaturation mediated by cytosolic NAD+, and provide new insight on how D5D and D6D activity may impact glycemic, growth, and cancer traits in humans.

Poster Number 77

Eunjeong Kwon, PhD
Dermatology, Instructor | ekwon@mgh.harvard.edu

The RNA binding proteomes in epidermal progenitor homeostasis

INVESTIGATORS: E. Kwon, K. Todorova, J. Wang, M. Hentze, A. Mandinova

Past research has demonstrated that regulation of gene expression at the transcriptional level can decisively alter cell fate of stem cells. However, cellular contents of mRNAs are sometimes not equivalent to proteins which are the functional units of cells. It is increasingly realized that post-transcriptional and translational regulation of gene expression are also fundamental. RNA-binding proteins (RBPs), main keys in posttranscriptional regulation, exert a broad range of biological functions. To explore the scope of RBPs in epidermal cells to maintain a balance in tissue homeostasis, we determined the in vivo RBP repertoire of the human keratinocytes in proliferating and in differentiated cells by using interactome capture. We identified 445 proteins and 391 proteins as enriched RBPs by biochemical and statistical criteria from different epidermal cell condition and uncovered RBPs involved in mitochondria metabolism related pathway were dominants in differentiated cells. To verify the function of mitochondrial metabolism in this differentiation process, metabolic profiling analysis was carried out and showed mitochondrial function controls the commitment to epidermal cell differentiation. RBDmap identified the regions of RNA contact with the RBPs, especially, using MDH2 (mitochondrial malate dehydrogenase 2) a key Kreb cycle component, we found RNA binding effect on its enzyme activity. Our study provides a new paradigm to understand the epidermal cell homeostasis in posttranscriptional regulation with RNA regulatory protein in mitochondria.

Poster Number 78

Sithara Wijeratne, PhD
Molecular Biology, Research Fellow | wijeratne@molbio.mgh.harvard.edu

Geometry of antiparallel microtubule bundles regulates relative sliding and stalling by PRC1 and Kif4A

INVESTIGATORS: S. S. Wijeratne, R. Subramanian

Motor and non-motor crosslinking proteins play critical roles in determining the size and stability of microtubule-based architectures. Currently, we have a limited understanding of how geometrical properties of microtubule arrays, in turn, regulate the output of crosslinking proteins. Here we investigate this problem in the context of microtubule sliding by two interacting proteins: the non-motor crosslinker PRC1 and the kinesin Kif4A. The collective activity of PRC1 and Kif4A also results in their accumulation at microtubule plus-ends ('end-tag'). Sliding stalls when the end-tags on antiparallel microtubules collide, forming a stable overlap. Interestingly, we find that structural properties of the initial array regulate PRC1-Kif4A mediated microtubule organization. First, sliding velocity scales with initial microtubule-overlap length. Second, the width of the final overlap scales with microtubule lengths. Our analyses reveal how micron-scale geometrical features of antiparallel microtubules can regulate the activity of nanometer-sized proteins to define the structure and mechanics of microtubule-based architectures.

Poster Number 79

Rebecca Hock, PhD
Psychiatry, Research Fellow | rhock@mgh.harvard.edu

Childhood maltreatment in Barbados predicts personality pathology in the next generation

INVESTIGATORS: R. S. Hock, C. P. Bryce, P. T. Costa, J. R. Galler

Evidence suggests a link between childhood maltreatment (abuse and/or neglect) and personality pathology in adulthood. However, studies on personality pathology in the next generation are limited, particularly in populations outside of the US. Using data from the 50-year longitudinal Barbados Nutrition Study, we assessed the relationship between parental (G1) childhood maltreatment history, using the Childhood Trauma Questionnaire (CTQ-SF) and their offspring's (G2; mean age=21.22, N=110) NEO-PI-R-derived personality disorder (PD) scores. We also examined two potential mediators of this relationship: G1 depressive symptoms and G2 childhood maltreatment history. In repeated measures mixed regression models adjusting for childhood ecology, we found that G1 maltreatment history was associated with Borderline, Histrionic, and Narcissistic PD in G2 (all $p<0.05$). Parent depressive symptoms, measured using the Zung scale, appear to partially mediate between parent CTQ and offspring Borderline PD (0.43 ± 0.14 to 0.36 ± 0.14). Self-reports of G2 emotional abuse partially mediated the relationship between parental maltreatment history and offspring Borderline (0.43 ± 0.14 to 0.39 ± 0.13). In this study, the mental health consequences of parental exposure to childhood maltreatment present in the next generation. Parent depressive symptoms and G2 exposure to emotional abuse both appear to partially mediate these relationships. Future studies should investigate potential biological mechanisms.

Poster Number 80

Jasreena Nijjar, MD
Pediatrics, Clinical Research Fellow | jknijjar@mgh.harvard.edu

Exposure to Food Marketing Among Young Children in Mumbai and Its Association with Their Diet: A Qualitative Assessment

INVESTIGATORS: J. Nijjar, A. Revette, K. Viswanath

Systematic reviews of evidence mostly from high-income countries support that television advertisements influence children's diet-related behavior. However, information is scarce regarding children's exposure to food marketing and its impact on their diet related behavior in low middle-income countries. We conducted a qualitative study in Mumbai, India, with the aim of gaining deeper insights into parent-child dyad's exposure to food advertising and its influence on children's diet related behavior. The interviews and focus group discussions were conducted with children aged 7-12 years and their mothers (n=34) purposively enrolled from low to middle-income communities in the Mumbai urban area. Analysis revealed that children in Mumbai are frequently exposed to food marketing through various platforms. Children reported mostly learning about food products from TV advertisements while they themselves or others in the family were watching television. Seeing products displayed at local vendors was also frequently quoted. Packaged foods and aerated drinks seemed to be the easiest advertisements for children to remember, with health messaging, celebrities, and audiovisual elements being the most consequential. Health messaging emerged as a key factor, both parents and children reported on a foods' ability to affect height, brainpower, and strength. Parents reported on the omnipresence of advertisements, children demanding items seen and this often leading to conflict. India has a significant food-advertising market and an increasing burden of pediatric obesity. Given the significant role food marketing plays in determining childhood dietary patterns, understanding how media can be tapped to prevent NCDs among children is timely and important.

Poster Number 81

Nicolas Oreskovic, MD, MPH
Pediatrics, Assistant Professor | noreskovic@mgh.harvard.edu

Walking Routes to Promote Physical Activity in Children with Autism

INVESTIGATORS: N. M. Oreskovic, A. M. Neumeyer, M. P. Duggan, K. A. Kuhlthau

Children with autism are at increased risk for overweight/obesity and face a variety of challenges with achieving the recommended levels of physical activity. Walking is an achievable form of physical activity for children with autism, and prior work has shown that the use of walking routes can increase daily physical activity in children and adolescents who are overweight/obese. The objective is to test the feasibility and preliminary efficacy of using walking routes as a novel approach for increasing physical activity among children with autism. Twelve overweight/obese children with autism ages 6-17 provided 3 separate weeks of physical activity data, using accelerometers. Families were counseled on using walking routes to increase physical activity after collecting the child's baseline (T1) physical activity. Physical activity data were collected for one week after counseling (T2), and again for one week three months later (T3). Changes in moderate-to-vigorous physical activity (MVPA) and sedentary time are assessed. Eight children have completed the study to date. Mean age was 11.9 years. MVPA has increased after counseling (Δ MVPA=+2.9, range=-12 - 32) and remained elevated at 3 months (Δ MVPA=+ 13.9, range=-11 - 133). Sedentary time has decreased (Δ sedentary time=-12.7, range=-141 - 44) and remained lower at 3 months (Δ sedentary time=-19.5, range=-146 - 147). Counseling on walking routes may provide a means for increasing MVPA and decreasing sedentary time in children with autism, representing a novel approach to increasing physical activity in this high-risk population. These findings should be corroborated in larger controlled studies.

Poster Number 82

James Brown, PhD
Radiology, Research Fellow | jbrown97@mgh.harvard.edu

Diagnosis, monitoring and risk: automated diagnosis of retinopathy of prematurity using deep learning

INVESTIGATORS: J. M. Brown, A. Beers, K. Chang, J. Kalpathy-Cramer, Imaging and Informatics in Retinopathy of Prematurity (i-ROP)

Retinopathy of prematurity (ROP) is one of the leading causes of childhood blindness worldwide. Although most cases are mild and resolve without intervention, some cases can progress to severe disease and require prompt treatment. Abnormal vascularization of the retina is characterized by increased tortuosity and dilation of the retinal vessels, also known as 'plus disease'. Timely and accurate diagnosis of plus disease is critical, as it is the most important prognostic indicator of treatment-requiring (type 1) ROP. As neonatal intensive care improves, the incidence of ROP is predicted to increase. This poses a significant public health problem, as there is already an unmet need for clinicians with adequate training and expertise in ROP diagnosis. Furthermore, inter-rater disagreement on plus disease diagnosis has been widely documented, even among experts. Therefore, there is a strong impetus to develop objective means of diagnosing the disease. Here, we present an algorithm based on deep convolutional neural networks (CNNs) that can diagnose plus disease automatically from retinal photographs with an area under the receiver operating characteristic curve (AUROC) of 0.99. The algorithm also compares favorably in terms of agreement with a 'reference standard diagnosis', outperforming seven out of eight ROP experts. We have also devised a severity score using our algorithm which is sensitive to changes over multiple patient visits, and can be incorporated into risk models for type 1 ROP with an AUROC of 0.93. We are currently looking to develop an affordable point-of-care diagnostic tool for use in low-resource settings.

Poster Number 83

Parastou Eslami, PhD
Radiology, Research Fellow | peslami1@mgh.harvard.edu

Quantifying Coronary Artery Calcium via Radiomics and Machine Learning: A Framingham Heart Study

INVESTIGATORS: P. Eslami, U. Hoffmann, C. Parmer, B. Foldyna, H. Aerts, M. Lu, R. Zeleznik, A. Ivanov, J. Scholtz

Clinicians and Radiologists use relatively few metrics to quantify images. Presence of coronary artery calcium (CAC) is indicative of coronary artery disease and has been proven to add prognostic value in asymptomatic patients. Recently, Radiomics and machine learning (ML) have been introduced as powerful tools to convert imaging data into quantitative information in tumors. Here we show application of Radiomics and ML to quantify CAC in prediction of cardiovascular events (CVD) in the community-based Framingham Heart Study. Radiomics was used to extract features from CAC in segmented coronary CT images. First CT (2002-2005) and independent second CT (2008-2010) were used as training and validation cohorts, respectively. Summary statistics was used to create six meta features for each patient where highly-correlated features (R=0.95) were eliminated. Top 20 features were selected implementing minimum redundancy maximum relevance algorithm and random forest classification methods was used to create the model. Final Radiomics score (RS) was calculated as the average of probabilities of each meta feature.

In this study 2120 features were extracted in in 624 images in training (mean age 58.7; 36% female) and 498 of images in validation (mean age 60.25; 49.40% female) cohort. In the validation cohort, the area under curve (AUC) for CAC score was calculated to be AUC = 0.69 whereas RS and Combined Score (RS + CAC) had an incremental AUC = 0.70 with P=0.43 and AUC=0.73 with P=0.17, respectively. Radiomics is a novel approach to quantify coronary calcium and can be used to predict CVD.

Poster Number 84

Yun Peng, PhD
Orthopaedics, Research Fellow | ypeng5@mgh.harvard.edu

In-vivo Quantification of Polyethylene Wear Using Subject-Specific Kinematics-Coupled Finite Element Analysis

INVESTIGATORS: Y. Peng, P. Arauz, S. An, Y. Kwon

Polyethylene remains the most commonly used bearing surface material in current total hip arthroplasty (THA). Periprosthetic osteolysis caused by polyethylene wear debris remains the major challenge to implant longevity and patient health. Factors associated with an increased component wear remains unclear. Computational modeling using patient-specific data could provide useful insight into this phenomenon; however, patient-specific THA wear model remains lacking. Using a computational model with patient-specific kinematics and component orientations, we aimed to characterize the variations of polyethylene wear patterns among 48 THA and identified key factors influencing polyethylene wear.

We found strong patient variations in wear indicators, with an averaged volumetric wear rate of 54.1 (13.9) mm3/year ranging from 21.9 to 86.3 mm3/year and linear wear rate of 0.20 (0.08) mm/year ranging from 0.07 to 0.60 mm/year. Multiple linear regression analyses identified an increased axial range of motion as the leading cause to increases in both linear and volumetric wear rates, possibly because of an enhanced multidirectional sliding pattern that led to increased cross-shear ratio and friction. Surprisingly, component orientations were not significant contributors to either wear rate indicator, but showed strong connections to the peak linear wear locations. This is the first study to quantify polyethylene wear using comprehensive patient-specific data. Strong patient variations in wear rates were observed. Axial rotation was identified as a key factor to wear rates because of its intimate relation to the multidirectional sliding and therefore cross-shear ratio. Acetabular component orientations were significant predictors only to wear locations but not wear rates.

Poster Number 85

Umit Sami, MS
Partners Healthcare System, Systems Engineer | udsami@partners.org

Neuromorphic Chip Design for Deep Learning Applications in Genomics

INVESTIGATORS: U. D. Sami

The invention we describe in this document is a brain inspired chip with neuro-transistors, or Neural Processing Unit (NPU), capable of processing and storing information very similarly to how human brain uses neurons to make synaptic connections with other neurons to transmit information. The NPU chip is enclosed inside a device and can be plugged into any ordinary computer system as a module through its peripheral and networking interface making it a pluggable computational unit of arbitrary scale. The combination of neural spiking, routing and search algorithms, linear scalability, and built-in memory features of our NPU chip make it highly energy efficient, reconfigurable, and most importantly extremely fast for certain type of applications that require real-time updating of data at massive scales within multi-layer structures, among other things. One domain of applications in life sciences and healthcare that depend on such computational power, flexibility and efficiency is deep learning – a sub field of Artificial Intelligence and Machine learning. In particular we believe medical image processing with deep learning to classify cancer tumors as well as their growth rate and location, DNA sequencing with deep learning to identify variant calling with applications in precision medicine, and genomic data encryption with deep learning models for securing patients genomic information are very promising medical applications that our proposed NPU chip research can be used as a core enabling AI/ML technology.

Poster Number 86

Duygu Saatcioglu, PhD
Surgery, Research Fellow | hsaatcioglu@mgh.harvard.edu
Exogenous MIS dysregulates uterine cell differentiation

INVESTIGATORS: H. D. Saatcioglu, M. Kano, L. H. Zhang, N. Nagykery, R. Suliman, J. Hsu, K. James, D. Wang, M. E. Sabatini, G. Gao, P. K. Donahoe, D. Pepin

Mullerian Inhibiting Substance Receptor-2 (MISR2) is expressed in the mesenchyme of the bipotential Mullerian duct during embryonal development. Secretion of MIS (Anti-Mullerian Hormone), the ligand of MISR2, by the embryonic testes causes regression of the Mullerian duct in male embryos. However, role of MISR2 in neonatal uterine development has not been studied before. In females, the mesenchymal cells of the Mullerian duct give rise to the two main layers of the uterus after birth: endometrium and myometrium, both of which play crucial roles in menstrual cycle, pregnancy, and labor.

We confirmed that MISR2 expression is high and restricted to the mesenchymal cells of the uteri during development. Neonatal rats with supraphysiological levels of the MIS on postnatal day 1 had smaller uteri (n>3, p=0.0047, day 15), with disorganized myometrial layers and underdeveloped endometrial stromal layer (n>2, days 6 and 10). We further characterized the differentially expressed genes of the developing uteri in response to MIS by single-cell RNA-sequencing with droplet-based microfluidics (InDrop). Our results uncover new set of genes that are regulated by MIS: 1) Dormant mesenchymal genes only present in the MIS treated uteri (BAMBI, HDAC4, MISR2, SMAD6), 2) Early differentiating endometrial stroma genes which are not present in the treated mesenchyme (VCAN, COL3A, BMP7). These previously unidentified gene signatures reveal differentially expressed genes during uterine development and the effect of MIS on early uterine cells to inhibit mesenchymal differentiation.

Poster Number 87

Parul Sarwal, MBBS
Surgery, Research Fellow | psarwal@mgh.harvard.edu
Alveolar type II cells are regulated by SLIT3 in postnatal lung culture

INVESTIGATORS: P. Sarwal, P. Bhayani, M. Elango, C. M. Kelleher, F. A. High, P. K. Donahoe, M. Longoni, M. Loscertales

We aim to identify the mechanism of lung hypoplasia in congenital diaphragmatic hernia (CDH), it being a cause of mortality despite surgical repair. Based on studies in animal models and a large human cohort, we hypothesize that SLIT3 and its receptors play a role in alveolarization during lung development. Lungs from four-day-old mice were inflated and cultured with the SLIT3-neutralizing antibody. Morphometric and quantitative analyses were performed on light microscopy and immunofluorescence images of the explants. mRNA levels in organ culture and cell-based assays were measured by qPCR. Statistical analysis was done using paired t-test (significance level $p<0.05$).

We observed that Slit3 expression in mice peaks postnatally during alveolarization. SLIT3 and its receptor, ROBO1, were found to be expressed on alveolar type II cells (ATII cells) upon co-staining with prosurfactant protein C (pSPC). Explants treated with the SLIT3-neutralizing antibody had focal thickening of the interstitium on histology. A significant increase in pSPC+ ATII cells was observed by immunofluorescence and confirmed by Sftpc qPCR. Matrix metalloproteinases (Mmp2, Mmp9), downstream effectors in the SLIT/ROBO pathway, were increased in the treated explants on qPCR, suggesting extracellular matrix remodeling. We propose a role for SLIT3 in the autocrine regulation of proliferation and/or differentiation of ATII cells during alveolarization in mice. ATII cells produce surfactant and transdifferentiate into alveolar type I cells. Since an increase in ATII cells with concomitant surfactant deficiency has been reported in CDH patients, the mechanism behind this increase and the functionality of the surfactant protein are further studies in progress.

Poster Number 88

Michka Sharpe, BS
Center for Regenerative Medicine, Graduate Student | michkasharpe@fas.harvard.edu
Deciphering the role of reptin in driving zebrafish cardiomyocyte proliferation

INVESTIGATORS: M. G. Sharpe, J. M. González-Rosa, C. E. Burns, C. G. Burns

In contrast to the adult mammalian heart, the zebrafish heart is able to regenerate lost myocardium through robust proliferation of pre-existing cardiomyocytes. However, the molecular mechanisms that drive zebrafish cardiomyocyte proliferation remain largely unknown. A previous ENU genetic screen identified a gain of function mutation in the reptin locus (ruvbl2lik/lik) that resulted in cardiac hyperplasia. In order to determine whether reptin loss of function (ruvbl2-/-) mutants exhibit the reciprocal phenotype, we used genetic approaches to label cardiomyocyte nuclei and found that ruvbl2-/- mutants have significantly fewer cardiomyocytes compared to wild-type siblings at 72 hours post-fertilization (hpf). Additionally, we found that this phenotype was associated with the complete absence of cardiomyocyte proliferation from 48 to 72 hpf, the time in which cardiomyocytes in the zebrafish heart tube begin to proliferate. Interestingly, overexpression of Reptin specifically in cardiomyocytes is not sufficient to rescue this proliferative defect. However, heat-shock inducible overexpression of Reptin in all tissues rescues cardiomyocyte proliferation in ruvbl2-/- embryos. To identify the cell type in which Reptin is required to induce cardiomyocyte proliferation, we have designed innovative genetic strategies to perform conditional deletion of reptin in different cardiac tissues. Tissue specific overexpression of Reptin in the endocardium was sufficient to partially rescue proliferation of cardiomyocytes, demonstrating a non-cell autonomous effect in driving cardiomyocyte proliferation. Our study aims to elucidate the mechanisms by which Reptin drives cardiomyocyte proliferation in order to provide therapeutic insights into stimulating the adult mammalian heart to proliferate after injury.

Poster Number 89

Ana Aulinas, MD
Medicine, Research Fellow | aaulinasmaso@mgh.harvard.edu

Oxytocin response to a peripheral angiotensin II infusion among healthy individuals

INVESTIGATORS: A. Aulinas, S. Srinivasa, T. O'Malley, P. Maehler, S. Grinspoon, E. A. Lawson

Oxytocin (OT) regulates sodium homeostasis. In rodents, OT secretion is stimulated by central administration of AngiotensinII (AngII) and induces natriuresis. In humans, pressor doses of peripheral AngII infusions increased peripheral OT levels. To improve our understanding of OT physiology, we measured basal OT levels under low and liberal sodium controlled conditions and following a peripheral AngII infusion at subpressor doses, both for the first time in humans. We hypothesized that OT levels would be higher during liberal vs. low sodium conditions and would increase in response to AngII. 10 healthy subjects (70% males, age 52±7 years) were recruited. Subjects underwent separate graded AngII infusions (30' sequential intervals at subpressor doses of 0.3, 1.0, 3.0 ng/kg/min) intravenously each preceded by a 6 day standardized low or liberal sodium diet. Fasting serum OT levels were measured.

Basal OT levels did not differ after low vs. liberal sodium diets. Following the 90' AngII infusion, AngII levels and mean arterial pressure increased as expected, whereas OT levels (1499±300 vs. 1152±374 pg/mL, P<0.001 and 1663±676 vs. 1095±262 pg/mL, P=0.03) decreased during low and liberal sodium conditions, respectively. The percent change in OT following AngII did not differ by sodium controlled conditions (-25±15% vs. -28±21% low vs. liberal sodium conditions, P>0.99). Sodium controlled conditions did not affect OT levels. AngII infusion at subpressor doses suppressed OT independent of sodium conditions. Our data are contrary to effects of pressor doses of AngII on OT in humans and its known natriuretic function in animals.

Poster Number 90

Kendra Becker, MS
Psychiatry, Clinical Research Fellow | krbecker@mgh.harvard.edu

Differing Release of Ghrelin and BDNF Around a Standardized Meal in Girls and Young Women with Low-Weight Avoidant/Restrictive Food Intake Disorder Compared to Anorexia Nervosa

INVESTIGATORS: K. R. Becker, C. J. Mancuso, E. A. Asanza, F. Plessow, A. M. Izquierdo, M. Slattery, O. B. Wons, R. L. Pulumo, K. T. Eddy, J. J. Thomas, M. Misra, E. A. Lawson

Avoidant/restrictive food intake disorder (ARFID) is characterized by failure to meet nutritional needs in an absence of the weight/shape concerns that typify anorexia nervosa (AN). Accumulating evidence suggests that appetite regulating hormones are altered in AN, but there are no published data on these hormones in ARFID. We investigated levels of orexigenic ghrelin and anorexigenic BDNF in ARFID, AN and healthy controls (HC). 66 adolescent females (34 typical and atypical AN, 6 ARFID, 26 HC; age: mean±SD = 18.1±3.2 years) underwent blood draws for total ghrelin and BDNF fasting and 30, 60, and 120 minutes after a 400 kcal standardized mixed meal.

Individuals with ARFID were at a lower percent median BMI (73.3%mBMI±4.1) than HC (101.8%mBMI±16.7; p<.001) and AN (85.7%mBMI±10.9; p<.05). AN were also at a lower %mBMI than HC (p<.001).Total ghrelin levels decreased at each time point after the meal in AN and HC and at 60 minutes post-meal in ARFID (p<.05). In AN compared to HC, ghrelin levels were elevated during the fasting state and each post-meal time point (p<.05). Fasting ghrelin levels were higher in AN than ARFID (p=.02), who did not differ from HC. While BDNF levels did not differ between AN and HC at any time point, fasting BDNF levels were higher in ARFID compared to AN and HC (p<.05) and remained higher than HC at 30 minutes post-meal (p=.04). Low-weight females with AN and ARFID demonstrated distinct patterns of ghrelin and BDNF release that may contribute to restrictive eating behaviors.

Poster Number 91

WuQiang Fan, MD, PhD
Medicine, Clinical Research Fellow | wfan2@mgh.harvard.edu

The dynamics of circulating oxytocin after a standard meal in young healthy females

INVESTIGATORS: W. Fan, C. J. Mancuso, F. Plessow, A. Aulinas, A. Izquierdo, M. Slattery, K. T. Eddy, M. Misra, E. A. Lawson

Oxytocin(OXT) acts via central and peripheral pathways to reduce food intake and body weight. While intranasal OXT acutely reduces caloric consumption in humans, it is unknown whether nutritional intake modulates endogenous OXT levels. After an overnight fast, twenty females (age: 13.5 to 21.6 years; mean ± SEM: 18.4±0.4 years. % ideal BMI: 90.7-119.8 %; 102.1±1.8%) consumed a standard 400 kcal mixed breakfast meal. Blood samples were obtained before and 30, 60, and 120 min after the meal. Circulating levels of OXT, PYY, CCK, and BDNF were measured at each time point. OXT levels increased after the meal with a peak at 60 min (+19.5±7.2%, p=0.019). The change in OXT levels at 60 min was negatively associated with change in CCK levels at 60 min (r = -0.64, p = 0.004) and positively associated with change in PYY levels at 60 min (r=0.49, p=0.04). The change in OXT levels at 120 min were also positively associated with change in PYY levels at 120 min (r= 0.54, p = 0.014). There was also a negative association between OXT area under the curve (AUC) and BDNF AUC (r= - 0.63, p = 0.006). Circulating OXT levels increase in response to a standardized mixed meal in healthy young females with a peak at 60 minutes, consistent with the known anorexigenic effects of this hormone. Dynamic associations between postprandial OXT levels and those of other anorexigenic hormones are demonstrated.

Poster Number 92

Lindsay Fourman, MD
Medicine, Clinical Research Fellow | lfourman@partners.org

Unexplained Infertility Is Associated with High-Normal Thyroid-Stimulating Hormone

INVESTIGATORS: L. T. Fourman, T. Orouji Jokar, H. Lee, K. Mentzinger, P. K. Fazeli

Unexplained infertility, the inability to conceive after 12 months of unprotected intercourse with no diagnosed etiology, affects 10-30% of infertile couples. Understanding mechanisms that underlie unexplained infertility may lead to less invasive and costly treatments. Whereas abnormalities in thyroid-stimulating hormone (TSH) and prolactin (PRL) are known to cause infertility, whether unexplained infertility is associated with TSH or PRL within the reference range has not been well-established. We hypothesized that women with unexplained infertility would have higher TSH and PRL compared to infertile women with a similarly normal evaluation but whose partners were severely oligospermic/azoospermic. We utilized the Partners HealthCare System Research Patient Data Registry to identify women 18-39 years with unexplained (n=187) or severe male factor (n=52) infertility. All women had a normal infertility evaluation including TSH and PRL within the reference range. Infertility was considered unexplained if one semen analysis was normal and due to severe male factor if sperm concentration was <1 million/mL on two occasions. TSH was higher among women with unexplained versus severe male factor infertility (1.95 [1.54,2.61] vs. 1.66 [1.25,2.17] mIU/L, P=0.003), which remained significant upon adjusting for age, BMI, and smoking. Nearly twice as many women with unexplained infertility had TSH ≥2.5 mIU/L (upper-half of normal range) compared to male factor controls. PRL did not differ between groups. Women with unexplained infertility had higher TSH compared to controls. Prospective studies are needed to ascertain whether treatment of high-normal TSH in women with unexplained infertility would reduce time to conception.

Poster Number 93

Joel Habener, MD
Medicine, Professor | jhabener@partners.org

GLP-1-Derived Nona- and Penta-Peptides Inhibit Cytochrome C Peroxidase Activity and Reduce Obesity-Related Mitochondrial Oxidative Stress

INVESTIGATORS: V. S. Guzzetta, C. R. Ertz, V. Stanojevic, K. McManus, A. Khatri, E. Tomas, J. F. Habener

Excessive energy intake during obesity, causes cellular oxidative stress resulting in metabolic disorders such as diabetes, cardiovascular disease, and fatty liver disease. It is known that Glucagon-like peptide-1 (GLP-1), an intestinal hormone of 30 amino acids, can augment glucose-dependent insulin secretion and effectively treat type 2 diabetes. We have discovered both a penta-and a nona-peptide, cleaved from the C-terminus of GLP-1 by an endogenous endopeptidase that appear to penetrate cell membranes, home in to mitochondria, and modulate energy metabolism. We have shown that the peptides curtail weight gain, increase basal energy expenditure and reduce fat mass in diet-induced obese mice by increasing fatty acid oxidation; however, the molecular mechanism of action remains unknown. One proposed target is Cytochrome-c, (Cyto-c) a component of the electron transport chain involved in promoting oxidative phosphorylation (OXPHOS). In normally functioning mitochondria Cyto-c facilitates electron flow and ATP synthesis during OXPHOS. Obesity causes energy overload in mitochondria, and increased generation of H2O2 and free radicals, which combine with the phospholipid, cardiolipin, to transform Cyto-c into a peroxidase that impairs OXPHOS. Here we demonstrate that our peptides reduce peroxide-mediated oxidative stress (ROS levels) in INS-1 pancreatic beta cells. We also show that our peptides inhibit cardiolipin-dependent Cyto-c peroxidase activity. This evidence suggests that our peptides can stabilize healthy Cyto-c conformation and prevent peroxidase formation. Our findings indicate that peptide treatment increases mitochondrial efficiency under oxidative stress, and hold promise as a treatment for obesity and its related metabolic disorders.

Poster Number 94

Kanakaraju Kaliannan, MD
Medicine, Instructor | kkaliannan@mgh.harvard.edu

Gut microbiome mediates sex differences in the metabolic syndrome

INVESTIGATORS: K. Kaliannan, R. Ruairi, K. Murphy, C. Stanton, C. Kang, B. Wang, L. Hao, A. K. Bhan, J. X. Kang, APC Microbiome Institute, University College Cork, Cork, Ireland

Mechanisms underlying sex differences in the metabolic syndrome (MS) remain obscure. With high-throughput 16s rRNA gene sequencing analysis and predicted metagenomics, we discovered that alterations in the gut microbiome (e.g. decreased Proteobacteria and Firmicutes to Bacteroidetes ratio, increased Bifidobacterium to Enterobacteriaceae ratio and Akkermansia and decreased lipopolysaccharide biosynthesis) of female and 17β-estradiol treated male and ovariectomized female C57BL/6 mice is associated with lower susceptibility to develop metabolic endotoxemia (ME), low-grade chronic inflammation (LGCI) and MS. Importantly, male mice fecal microbiota-transplant transferred the MS to female mice and antibiotics treatment eliminated sex differences in the MS, suggesting the causative role of the gut microbiome. Estrogen like compounds (e.g. isoflavones) produced similar microbiome modulating effects of estrogens and reversed MS in the male mice. Intestinal alkaline Phosphatase (IAP), a major gut microbiota-modifying factor, is elevated by estrogens and isoflavones and IAP inhibition induced ME and LCGI in female mice. We discovered a novel microbiome-based mechanism that explain sex differences in the MS and may provide new therapeutic targets for MS prevention in males and postmenopausal women.

Poster Number 95

Jordi Merino, PhD
Center for Genomic Medicine, Clinical Research Fellow | jmerino@mgh.harvard.edu

Dietary Fat Quality and Genetic Risk of Type 2 Diabetes

INVESTIGATORS: J. Merino, M. Guasch-Ferre, C. Ellervik, H. S. Dashti, C. E. Smith, T. O. Kilpelainen, D. I. Chasman, J. C. Florez, Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Nutrition Working Group

Type 2 diabetes (T2D) is a complex disease driven by lifestyle and genetic factors. The extent to which dietary fat quality may modify T2D genetic burden on the incidence of T2D is unknown. We used Cox proportional-hazards models to calculate adjusted hazard ratios (HRs) for T2D among 103,206 participants of European descent from 15 prospective cohort studies. T2D genetic risk profile was characterized by a 68-variant genetic risk score (GRS) weighted by published effect sizes. Diet was recorded using validated cohort-specific dietary assessment tools. During a median follow-up of 12 years, 20,451 participants developed T2D. The relative risk of T2D was 1.68 (95% confidence interval [CI], 1.62 to 1.74) per increment of 10 risk alleles. Increasing monounsaturated fat intake in place of carbohydrates was associated with a higher risk of T2D (HR 1.08; 95% CI, 1.02 to 1.15 per 5% of energy), while increasing polyunsaturated or omega-3 fat in place of carbohydrates was associated with a lower risk of T2D (HR 0.92; 95% CI, 0.85 to 1.00 per 5% of energy and HR 0.95; 95% CI, 0.92 to 0.99 per increment of 1g/d, respectively). There were no significant interactions between dietary fat and GRS on T2D risk. Genetic risk and subtypes of dietary fat are independently associated with T2D incidence. Our findings do not support tailoring dietary fat quality recommendations to individual T2D genetic risk profiles for primary prevention of T2D. Healthy dietary fat quality exert benefit across the spectrum of T2D genetic risk.

Poster Number 96

Madhusmita Misra, MD, MPH
Pediatrics, Professor | mmisra@mgh.harvard.edu

Physiologic Transdermal Estrogen Replacement Improves Bone Density and Geometry in Oligo-amenorrheic Athletes Compared to a Combined Oral Contraceptive or No Estrogen

INVESTIGATORS: K. E. Ackerman, V. Singhal, C. Baskaran, M. Slattery, K. J. Campoverde Reyes, A. Toth, K. T. Eddy, M. L. Bouxsein, H. Lee, A. Klibanski, M. Misra

Normal-weight oligo-amenorrheic athletes are at risk for low bone mineral density (BMD) and impaired bone microarchitecture (BMA). Data are lacking regarding the impact of estrogen administration on BMD and BMA in this population. Our objective was to determine the impact of estrogen administration via transdermal versus oral routes on BMD and BMA in normal-weight oligo-amenorrheic athletes engaged in weight-bearing activity. 121 oligo-amenorrheic athletes 14-25 years old were randomized to (i) a 100 mcg 17- β estradiol transdermal patch with cyclic progesterone (PATCH), or (ii) a 30 mcg ethinyl estradiol pill with 0.15 mg desogestrel (PILL), or (iii) no estrogen/progesterone (NONE). All received calcium and vitamin D. Areal BMD was assessed at the spine, femoral neck and whole body using DXA at baseline, 6 and 12-months. BMA was assessed using high resolution peripheral quantitative CT (HRpQCT) at the ultradistal tibia and radius at baseline and 12-months. We adjusted for age, height, race and ethnicity in all analyses. Groups did not differ for age, BMI or BMD Z-scores at baseline. Mean age and BMI were 19.9±0.2 years and 20.7±0.2 kg/m2. For the intent to treat analysis, spine and femoral neck BMD Z-scores increased in PATCH vs. PILL ($p=0.011$ and $p=0.021$) and NONE ($p=0.021$ and $p=0.033$) groups, and hip BMD Z-scores increased in PATCH vs. PILL group ($p=0.018$). PATCH compared with the PILL group demonstrated improvement in several BMA parameters. Transdermal estradiol improves BMD and bone geometry in young oligo-amenorrheic athletes, particularly compared to the ethinyl estradiol containing pills.

Poster Number 97

Melanie Schorr, MD
Medicine, Instructor | mschorr1@partners.org

High Prevalence of Impaired Skeletal Integrity in Men with Anorexia Nervosa, Atypical Anorexia Nervosa and ARFID

INVESTIGATORS: M. Schorr, A. Drabkin, A. Watters, E. Meenaghan, T. M. Holmes, M. Misra, K. T. Eddy, A. Klibanski, P. Mehler, K. K. Miller

Few data are available regarding bone loss in men with AN, atypical AN (ATYP) (cognitive symptoms without low weight) or avoidant/restrictive food intake disorder (ARFID) (restrictive eating without cognitive symptoms). We studied 55 men, 18-63y, with AN (n=26), ARFID (n=11) or ATYP (n=18). Body composition, hip and spine bone density (BMD), and hip structural analysis section modulus (HSA IT Z) were assessed by DXA. Mean age (29±2y) and disease duration (7±1y) were comparable among groups. Mean BMI was similar in AN and ARFID and higher in ATYP (14.7±0.4 vs 15.3±0.4 vs 20.6±0.5kg/m2, p<0.0001), as were lowest past BMI, %fat mass, and appendicular lean mass (ALM). Vitamin D deficiency was present in 24 vs 0 vs 18% of AN vs ARFID vs ATYP (p=NS). Prevalence of BMD Z-score <-2 was 66 vs 18 vs 33% in AN vs ARFID vs ATYP (p<0.05). Mean BMD Z-scores and IT Z were lower in AN, but not ARFID, than ATYP (p<0.05). In the cohort, BMI, lowest BMI and ALM were positively, and disease duration negatively, associated with BMD Z-scores and IT Z. Men with vitamin D deficiency, compared to without, had lower BMD Z-scores and IT Z (p<0.05). Conclusions: Men with AN are at risk for impaired skeletal integrity. Men with ARFID (despite comparably low weight) and atypical AN (who are higher weight) are at lower, but significant, risk. Low BMI and long disease duration increase risk, as do low muscle mass and vitamin D deficiency. DXA screening should be considered in these men.

Poster Number 98

Stephanie Seminara, MD
Medicine, Professor | sseminara@mgh.harvard.edu

PNPLA6 Mutations in a Patient with a Neurodegenerative/Hypogonadal Syndrome: Intact but Enfeebled Gonadotropin Secretion and a Possible Hypothalamic Defect

INVESTIGATORS: I. R. McDonald, L. E. Honrubia Sanchez, L. D. Faulkner, J. D. Schmahmann, C. D. Stephen, M. F. Lippincott, L. Plummer, S. B. Seminara

Biallelic mutations in PNPLA6 have been associated with a neurodegenerative spectrum including hypogonadotropic hypogonadism (IHH), cerebellar ataxia, and retinal dystrophy (Boucher-Neuhäuser syndrome [BNS]). As such, biallelic variants in PNPLA6 appear to be fully penetrant but differentially expressed. The sub-phenotype of IHH is thought to be secondary to impaired LH release from pituitary gonadotrophs. Whole exome sequencing was performed on probands with IHH (n=414), 39 of whom had additional neurologic abnormalities. Detailed neuroendocrine characterization, including stimulation with kisspeptin and GnRH, was performed on a subgroup of these individuals. A female proband presented with ataxia, IHH, and mild retinal dystrophy. Brain MRI revealed marked volume loss of the bilateral cerebellum, middle cerebellar peduncles and upper brainstem. Whole exome sequencing revealed biallelic rare variants in PNPLA6 (NM_0066111:c.[3729+5G>A];[1749G>C]). RT-PCR studies revealed the c.3729+5G>A variant leads to disrupted splicing and a loss of 54 amino acids in the protein product; in silico modeling predicted the c.1749G>C variant to be deleterious. Neuropeptide stimulation with q10 minute blood sampling revealed (1) no spontaneous LH pulsatility, (2) an absence of a kisspeptin-induced-GnRH-induced LH response, but (3) a positive GnRH-induced LH response, albeit abnormal in morphology. Despite the variable expressivity associated with PNPLA6 genetic variation, this proband exhibited the classic triad of BNS. She mounted an intact but impaired GnRH-induced LH response, which has yet to be reported in a patient with biallelic PNPLA6 mutations. Furthermore, the absent response to kisspeptin stimulation suggests the possibility of a hypothalamic component underlying her hypogonadism.

Poster Number 99

Chia-Yen Chen, ScD
Psychiatry, Research Fellow | chiayen.chen@mgh.harvard.edu

Genetic validation of bipolar disorder identified by automated phenotyping using electronic health records

INVESTIGATORS: C. Chen, P. Lee, V. Castro, J. Minnier, A. W. Charney, E. A. Stahl, D. M. Ruderfer, S. N. Murphy, V. Gainer, T. Cai, I. Jones, C. Pato, M. Pato, M. Landen, P. Sklar, R. H. Perlis, J. W. Smoller, International Cohort Collection for Bipolar Disorder

Bipolar disorder (BD) is a heritable mood disorder characterized by episodes of mania and depression. Although genomewide association studies (GWAS) have identified genetic loci contributing to BD risk, sample size has become a rate-limiting obstacle to genetic discovery. Electronic health records (EHRs) represent a vast but relatively untapped resource for high-throughput phenotyping. As part of the International Cohort Collection for Bipolar Disorder (ICCBD), we previously validated EHR-based algorithms for BD against in-person diagnostic interviews. Here, we establish the genetic validity of EHR-based BD. Phenotyping algorithms were derived from structured and narrative text in the Partners Healthcare system comprising more than 4.6 million patients over 20 years. Genomewide genotype data for 3,330 BD cases and 3,952 controls of European ancestry were used to estimate SNP-based heritability (h2g) and genetic correlation (rg) between EHR-based phenotype and traditionally ascertained BD cases in GWAS by the ICCBD and Psychiatric Genomics Consortium (PGC). We showed that the estimated h2g was comparable between BD identified by natural language processing (NLP)-based algorithm and observed by the ICCBD+PGCBD (0.24, p=0.015 vs. 0.23, p=3.17E-80). The estimated h2g were lower for codified data-based algorithms (from 0.13 to 0.00). The rg between ICCBD+PGCBD and the EHR-based cases were high for NLP and codified data-based algorithms (ranged from 1.00 to 0.66). These results provide the first genetic validation of EHR-based phenotyping for BD and suggest that this approach identifies cases that are highly genetically correlated with those traditionally ascertained. High-throughput phenotyping using EHRs represents a viable method for accelerating psychiatric genetic research.

Poster Number 100

Samuel Ching
Center for Genomic Medicine, Undergraduate Student | srching@mgh.harvard.edu

Modeling Familial Schwannomatosis using CRISPR/Cas9 Gene Editing in Human Schwann Cells

INVESTIGATORS: S. R. Ching, R. J. Drake, J. T. Jordan, S. R. Plotkin, J. A. Walker

Schwannomatosis (MIM 162091) is a late-onset tumor predisposition syndrome, distinct from Neurofibromatosis types 1 and 2, that has only recently been recognized. It is a rare disorder, affecting only around 1 in 40,000 individuals and is characterized by multiple cutaneous schwannomas, which are often associated with intractable pain. Constitutional inactivating variants in two genes, SMARCB1 and LZTR1 have been reported, while NF2 mutations are somatically acquired in schwannomas. Evidence suggests that a common route to schwannoma formation in familial cases involves three events: SMARCB1 or LZTR1 germline mutation (event 1), inactivation of the NF2 allele located on the same chromosome as the mutant SMARCB1 or LZTR1 allele (event 2), followed by simultaneous loss of both wild-type SMARCB1 and NF2 alleles by loss of heterozygosity (event 3). The SMARCB1 protein is a core subunit of the multi-protein SWI/SNF chromatin-remodeling complexes. To investigate the molecular and cellular consequences that underlie schwannoma formation, we have used multiplex CRISPR/Cas9 gene editing using human Schwann cell lines to introduce mutations in NF2 and SMARCB1. In particular, we have focused our attention on modeling a specific germline mutation (*82C>T) in the 3'UTR of SMARCB1. We present evidence that this mutation leads to reduced expression of the SMARCB1 transcript, possibly by destabilizing the mRNA. We are also examining whether germline missense mutations within the coding region of SMARCB1 alter protein-protein interactions

Poster Number 101

Katherine Crawford, BS
Center for Genomic Medicine, Research Technician | kcrawford2@mgh.harvard.edu

The Cerebrovascular Disease Knowledge Portal: An Open Access Data Resource to Accelerate Genomic Discoveries in Stroke

INVESTIGATORS: K. Crawford, C. Kourkoulis, L. Miyares, N. Burtt, J. Flannick, J. Hall, J. Rosand, G. Falcone

High throughput genotyping technologies have revolutionized genomic data; these new technologies have been potentiated by the creation of large collaboration networks that allowed the assembly of sample sizes previously unimaginable. Open data access is the final barrier to capitalizing fully on the opportunities available. Consequently, the International Stroke Genetics Consortium (ISGC) in partnership with the American Heart Association (AHA) created the Cerebrovascular Disease Knowledge Portal (CDKP), a comprehensive web-based resource to explore and access genetic and phenotypic data related to cerebrovascular diseases. We show how the CDKP seeks to democratize access to genomic data and potentiate stroke genomics research by providing open access to genetic and phenotypic data on patients from around the world. Within the CDKP, data is aggregated, integrated, and harmonized according to a standardized pipeline. Any investigator working with stroke genomic data can deposit their data or use available data. The CDKP houses both summary level data and individual level data, to meet different regulatory and analytical needs. The CDKP offers three main tools: (1) a graphical user interphase that allows the exploration of stroke genomics information through integrated web-based tools for analysis and data visualization; (2) a repository of summary statistics produced by published studies in the field; and (3) a repository of individual level data, accessible through a secure informatic working space provided by the AHA Platform for Precision Medicine. The CDKP aims to advance the ISGC's goal of liberal data sharing of stroke genomic and related data. The CDKP is available here: http://www.cerebrovascularportal.org/

Poster Number 102

Hassan Dashti, PhD
Center for Genomic Medicine, Research Fellow | hassan.dashti@mgh.harvard.edu

Genome-wide association analysis identifies over 75 genetic loci associated with sleep duration in UK Biobank participants

INVESTIGATORS: H. S. Dashti, S. Jones, J. M. Lane, H. Wang, Y. Song, K. Patel, S. Gill, D. Gottlieb, H. Tiemeier, D. Ray, T. M. Frayling, M. K. Rutter, M. N. Weedon, R. Saxena

Sleep disturbances have negative consequences on health. Mechanisms regulating sleep and underlying the link to diseases remain poorly understood. Genome-wide association studies (GWAS) have thus far identified PAX8 and VRK2 signals associated with sleep duration. We performed GWAS of self-reported sleep duration (n=446,118) and separately for short (<7 hours) and long (>8 hours) sleep using linear/logistic regression adjusting for age, sex, principal components of ancestry, and genotyping array of ~11m imputed variants in participants of European ancestry in the UK Biobank.

Trait heritability was 9.8% for sleep duration. We identified 78, 27 and 8 independent genome-wide significant ($P<5 \times 10^{-8}$) loci for sleep duration, short and long sleep durations, respectively. New associations implicated genes FTO, DRD2, GNAO1, among others. Pathway analysis indicates enrichment of genes involved in brain development, long term depression and neurotransmission. A combined genetic-risk score of 78 SNPs was associated with sleep duration in independent GWAS of adults [beta(se)=0.64(0.06) mins/allele; P=1.2E-25] and children [beta(se)=0.15(0.07) mins/allele; P=0.03]. However, strong genome-wide genetic overlap was only observed between our current GWAS and the GWAS in adults (P<0.001), suggesting different mechanisms regulating sleep across the lifespan. Mechanisms underlying short and long sleep durations were suggested to be partially distinct since there was only minor overlap among significant hits and moderate pairwise genetic correlation (rg=-0.283). Genetic correlations indicated shared biological links between sleep duration and psychiatric, cognitive, and metabolic traits. The present findings expand our understanding of the genetic architecture of sleep and the shared genetics links with disease traits.

Poster Number 103

Anne-Katrin Giese, MD
Neurology, Research Fellow | agiese@mgh.harvard.edu

Genetics of Acute Ischemic Lesion Volume: The MRI-Genetics Interface Exploration (MRI-GENIE) Study

INVESTIGATORS: A. K. Giese, H. Xu, M. D. Schirmer, K. L. Donahue, A. V. Dalca, B. J. Gaynor, J. W. Cole, P. F. McArdle, J. P. Broderick, J. Jimenez-Conde, C. Jern, B. M. Kissela, D. O. Kleindorfer, R. Lemmens, A. Lindgren, T. Rundek, R. L. Sacco, R. Schmidt, P. Sharma, A. Slowik, V. Thijs, D. Woo, B. B. Worrall, S. J. Kittner, J. F. Meschia, J. Rosand, P. Golland, B. D. Mitchell, O. Wu, N. S. Rost

Genome-wide association (GWA) studies have contributed substantially to the understanding of complex vascular traits including acute ischemic stroke (AIS); however, genetic determinants of acute cerebral ischemia remain to be elucidated. The objective is to investigate the genetic architecture of cerebral infarct lesion burden in patients with AIS. Twelve sites contributed 3,301 AIS subjects with acute MRI and genome-wide genotyping. In a preliminary analysis of 657 subjects of European ancestry, acute AIS lesions were semi-automatedly outlined on diffusion-weighted images (DWI). Standard quality control measures were performed per single nucleotide polymorphism (SNP) and per subject. Natural log-transformed DWI volume was used for GWA analysis with allelic dosage per SNP, age, sex and principal components 1-5 of genetic ancestry as covariates. GWA testing for DWI volume in 7.7 million SNPs yielded no signal crossing the Bonferroni-corrected genome-wide significance threshold of p<5*10-8. However, several loci passed the nominal significance threshold of p<1*10-6, including a locus on chromosome 2 in the SPATA3-AS1 (spermatogenesis associated 3 antisense RNA 1) gene (lead SNP: rs2368999, MAF=36%, p=8.4*10-7) and an intronic locus on chromosome 10 in the ATRNL1 (Attractin like 1) gene (lead SNP: rs592284, MAF=10%, p=9.1*10-7). In this first-to-date, preliminary GWAS of DWI volume in AIS patients, we identified several new, nominally significant loci including a locus in ATRNL1, a gene previously linked to carotid plaque burden. Further analyses of the MRI-GENIE cohort to replicate these findings, to include stroke subtype specific analyses, and to increase the overall statistical power of the study are ongoing.

Poster Number 104

Frances High, MD, PhD
Pediatrics, Instructor | fhigh@partners.org

Systematic analysis of copy number variation associated with congenital diaphragmatic hernia

INVESTIGATORS: F. A. High, Q. Zhu, C. Zhang, E. Cerveira, M. Russell, M. Longoni, M. Ryan, A. Mil-Homens, L. Bellfy, C. Coletti, P. Bhayani, R. Hila, C. Lee, P. K. Donahoe

Congenital diaphragmatic hernia (CDH), characterized by malformation of the diaphragm and hypoplasia of the lungs, is one of the most common and severe birth defects and is associated with high mortality rates and long-term morbidity among survivors. There is a growing body of evidence demonstrating that genetic factors contribute to many cases of CDH, although the pathogenesis remains largely elusive. Single nucleotide polymorphisms (SNPs) have been studied in recent whole exome sequencing (WES) efforts but larger copy number variants (CNVs) have not been systematically studied on a large scale at the genome level. In this study, to capture CNVs within CDH candidate regions in a cost-effective manner, we developed and tested a targeted array comparative genomic hybridization (aCGH) platform to identify CNVs within 140 candidate regions across 196 patients with CDH and 987 normal control samples. As a result, we identified 6 significant CNVs from this patient-control study. Three of these CNVs were found in multiple patients with CDH, but not in any controls, increasing the likelihood that they are disease-causing. These CDH-specific CNVs reveal high-priority candidate genes including HLX, LHX1, and HNF1B. The remaining 3 CNVs were found in multiple patients and a low frequency in controls, but were significantly enriched in patients with CDH. The candidate genes within these predicted disease-causing CNVs form functional networks with other known CDH genes and play putative roles in both DNA binding/transcription regulation and embryonic organ development. Further functional studies will increase our understanding of their contribution to the pathogenesis of CDH.

Poster Number 105

David Keefe, BA
Medicine, Clinical Research Coordinator | dlkeefe@mgh.harvard.edu

Loss of function mutations in TCF12 cause autosomal dominant Kallmann Syndrome and reveal network-level interactions between causal loci

INVESTIGATORS: D. L. Keefe Jr, R. Balasubramanian, E. E. Davis, N. Katsanis, W. F. Crowley

Kallmann Syndrome (KS) is a rare Mendelian phenotype defined by the co-occurrence of gonadotropin-releasing hormone (GnRH) deficiency with anosmia. While ~20 genes are known to cause KS, the genetic etiology in >50% remains unknown. To identify novel molecular causes of KS, we initiated a systematic genetic interrogation of 300 KS families using whole exome sequencing. Rare sequence variants (RSVs) (<0.01 in gnomAD database) were analyzed under autosomal recessive (AR) or dominant (AD) inheritance models. 9 KS families with autosomal dominant loss-of-function mutations in TCF12 were identified. TCF12 encodes a basic-helix-loop-helix (bHLH) transcription factor has been implicated in syndromic craniosynostosis. TCF12 carriers displayed incomplete penetrance for KS. The KS cohort was significantly enriched for TCF12 LOF RSVs compared to gnomAD using Fischer's exact test (P= 2.62 E-14). Loss of tcf12 in zebrafish larvae shortened crainial terminal nerve length and perturbed GnRH neuronal patterning consistent with aberrant GnRH neuronal migration alongside concomitant attenuation of the expression of several genes associated with KS. This effect was rescued using human TCF12 mRNA. Human genetic data and in vivo zebrafish functional validation nominate LOF mutations in TCF12 as a novel cause of autosomal dominant KS. The incomplete penetrance of the TCF12 LOF mutations reiterate the established oligogenic basis of KS suggesting that identification of additional modifiers will aid in understanding the complex architecture of KS in humans. These findings uncover a critical role for the bHLH transcription factors in the ontogeny of the GnRH neuron and human reproduction.

Poster Number 106

Kimberly Keefe, MD
Medicine, Clinical Research Fellow | kwkeefe@partners.org

Missense mutations in SOX2 contribute to non-syndromic forms of isolated GnRH deficiency revealing a differential sensitivity for SOX2 in GnRH vs. olfactory neurogenesis

INVESTIGATORS: K. W. Keefe, M. I. Stamou, D. L. Keefe Jr, L. Plummer, W. F. Crowley Jr, R. Balasubramanian

Mutations in the SOX2 gene, encoding a transcription factor critical for embryonic/neural stem cells causes a syndromic form of Isolated GnRH Deficiency (IGD) with anophthalmia/microphthalmia, neurocognitive delay and epilepsy. However, the role of SOX2 mutations in IGD patients without ocular/neurological phenotypes (i.e. non-syndromic IGD) is not known. Whole exome sequencing data from 800 IGD patients was examined for SOX2 rare sequence variants (RSV): Minor Allele Frequency <0.1% in GnoMAD; predicted deleterious by at least one bioinfomatic variant prediction program (Polyphen, SIFT and/or Combined Annotation Dependent Depletion [CADD]) and confirmed by Sanger sequencing. Clinical charts and patient questionnaires were reviewed for phenotypic evaluation. SOX2 RSVs were identified in 7 IGD patients: 5 missense mutations - 3 novel & 1 de novo; 1 novel de novo frameshift; and 1 novel de novo nonsense mutation, of whom 5 were normosmic & 2 hyposmic. While SOX2 frameshift/nonsense RSVs caused syndromic IGD phenotypes, SOX2 missense RSVs resulted in non-syndromic IGD without ocular/neurological phenotypes. One SOX2+ IGD patient underwent reversal of his IGD later in adulthood. This largest and first comprehensive analysis of SOX2+ RSVs in IGD reveals that SOX2 missense alleles contribute to 0.6% of non-syndromic IGD, with a predominant normosmic phenotype vs. Kallmann (hyposmic/anosmic) syndrome, suggesting that SOX2 signaling may be more critical for GnRH neurons than for olfactory neurogenesis. A spontaneous reversal of IGD in a SOX2+ IGD individual suggests that SOX2 signaling, which is clearly critical for early GnRH neurogenesis, may be dispensable in postnatal GnRH neurons.

Poster Number 107

Benjamin Kleinstiver, PhD
Pathology, Instructor | bkleinstiver@mgh.harvard.edu

An Improved CRISPR-Cpf1 Nuclease Platform Enhances Genome and Epigenome Editing

INVESTIGATORS: B. P. Kleinstiver, A. A. Sousa, R. T. Walton, M. M. Welch, J. K. Joung

Genome editing nucleases have been widely adopted for research applications as they enable the permanent sequence-specific modification of genes. CRISPR-Cpf1 nucleases possess various distinct and advantageous properties relative to the commonly used Cas9. We have previously shown that the Cpf1 ortholog from Acidaminococcus sp. BV3L6 (AsCpf1) can robustly function for genome editing in a variety of human cell types, and that it possesses high genome-wide specificity. However, wild-type AsCpf1 is restricted to targeting sites that encode a T-rich protospacer adjacent motif (PAM) of the form TTTV (where V is any nucleotide other than T), substantially limiting targeting relative to the NGG PAM utilized by SpCas9.

To improve the targeting range of CRISPR-Cpf1 nucleases, we performed rational mutagenesis screens to engineer AsCpf1 variants capable of recognizing an extended range of PAM sequences. These engineered Cpf1 PAM variants have activity equivalent or better than their wild-type counterparts on canonical TTTN PAM sequences, and importantly, also show robust activity on sites with PAMs of the form NTTV, TATV, and TTCV, sequences that are not efficiently cleaved by wild-type Cpf1. These variants expand the targeting range of Cpf1 by more than three-fold, effectively eliminating the major limitation that precludes broad adoption and use of Cpf1 nucleases. Collectively, our results demonstrate that both natural and engineered forms of CRISPR-Cpf1 nucleases are robust and useful enzymes, and that our Cpf1 PAM variants expand targeting range. These findings should encourage broader implementation of these genome editing reagents for both research and therapeutic applications.

Poster Number 108

Anna Kutateladze, BS
Medicine, Graduate Student | akutateladze@mgh.harvard.edu

Differential Sensitivity to Sox10 Missense Mutations in Kallmann Syndrome Vs. Waardenburg Syndrome

INVESTIGATORS: A. A. Kutateladze, M. Stamou, L. Plummer, D. L. Keefe Jr, W. F. Crowley Jr, R. Balasubramanian

Isolated Gonadotropin Releasing Hormone Deficiency (IGD) is a rare Mendelian disorder that presents either as Kallmann syndrome (KS) or as normosmic IGD (nIGD). SOX10 mutations, previously known to cause Waardenburg syndrome (WS), have recently been shown to cause KS. We hypothesized that the allelic spectrum of SOX10 mutations in KS will differ from those previously reported in WS. Whole exome sequencing data from 800 patients with IGD (382 KS/418 nIGD) were examined for rare sequence variants (RSVs) in SOX10. WS-associated SOX10 RSVs were obtained from the Leiden Open Variation Database. RSVs in SOX10 were identified in 11 IGD patients - 10 with KS (6M:4F) & 1 male with nIGD. These mutations included: 1 novel de novo frameshift, 1 novel inherited frameshift and 9 [7 novel] inherited missense RSVs. WS-like features were identified in 5/11 IGD patients. Compared to WS, missense SOX10 RSVs were significantly enriched in IGD (81.8% in IGD vs. 27.1% in WS; p=0.000509). In contrast, WS was enriched for loss-of-function mutations. Although most SOX10 missense (6/9) RSVs in IGD were localized to the HMG domain of the SOX10 protein, the proportion of HMG domain mutations did not differ between IGD and WS. SOX10 missense RSVs are significantly enriched in IGD compared to WS. Most SOX10 RSVs (6/11) occur in KS individuals lacking WS-like features. These findings provide yet another example of how mutations in a single gene can give rise to pleiotropic phenotypes based on the differential pathogenic sensitivity to missense vs. inactivating mutations.

Poster Number 109

Brittany Leger, BS
Center for Genomic Medicine, Research Technician | bsleger@mgh.havard.edu

A Drosophila Screen to Identify Conserved Genes and Pathways Regulating Sleep

INVESTIGATORS: B. Leger, S. Gill, R. Saxena, J. Walker

Sleep disturbances affect >50M US adults, and contribute to chronic disease burden, productivity loss, and cause comorbidities. While environmental factors contribute notably to self-reported sleep disturbances, abnormal sleep behaviors have also been shown to be heritable. Our current understanding of sleep is that all organisms with a brain require sleep, so we hypothesized that mechanisms of sleep are conserved across phyla from humans to fruit flies. Identifying genetic regulators of sleep will help understand its function, define molecular mechanisms linking sleep to disease, and spur development of new therapies to modulate sleep. Recently, human GWASes in the UK Biobank cohort have identified genomic loci correlated with abnormal sleep phenotypes. To facilitate the identification of the causal genes under GWAS peaks, determine directionality of effects and refine phenotype classes, we are screening Drosophila orthologs of genes on human GWAS haplotypes associated with atypical sleep phenotypes. Flies with neuronally-restricted knockdown of genes were monitored using the Trikinetics Drosophila Activity Monitoring (DAM), and analyzed in R for markers of abnormal sleep behavior. We have already identified several conserved genes for sleep and circadian behavior. We propose mechanisms through which they might regulate sleep. To complement the genetic approach, we are also screening a curated collection of small molecules for effects on sleep behaviors when fed to the flies. Our chemical biological approach will clarify protein targets and provide leads for human therapeutics. In addition, these can be to perturb sleep in non-model organisms.

Poster Number 110

Sandro Marini, MD
Center for Genomic Medicine, Research Fellow | smarini1@mgh.harvard.edu

Comparison of genetic and self-identified ancestry in modeling intracerebral hemorrhage risk

INVESTIGATORS: S. Marini, U. K. Lena, L. M. Crawford, C. J. Moomaw, F. D. Testai, S. J. Kittner, M. L. James, D. Woo, C. D. Langefeld, J. Rosand, C. D. Anderson, ERICH investigators

We sought to determine whether a small pool of ancestry-informative DNA markers (AIMs) improves modeling of intracerebral hemorrhage (ICH) risk in heterogeneous populations, compared with self-identified race/ethnicity (SIRE) alone. We genotyped 15 preselected AIMs to perform principal component (PC) analysis in the ERICH study (a multi-center case-control study of ICH in whites, blacks, and Hispanics). We used multivariate logistic regression and tests for independent samples to compare associations for genetic ancestry and SIRE with ICH-associated vascular risk factors (VRFs). We then compared the performance of models for ICH risk that included AIMs and SIRE alone. Among 4,935 subjects, 34.7% were non-Hispanic black, 35.1% non-Hispanic white, and 30.2% Hispanic by SIRE. In stratified analysis of these SIRE groups, AIM-defined ancestry was strongly associated with all eight VRFs analyzed ($p < 0.001$). Within each SIRE group, regression of AIM-derived PCs against VRFs confirmed independent associations of AIMs across at least two race/ethnic groups for seven VRFs. Akaike information criterion (6294 vs. 6286) and likelihood ratio test ($p < 0.001$) confirmed that genetic ancestry defined by AIMs significantly improved ICH risk modeling over SIRE alone. Genetically-defined ancestry provides valuable risk exposure information that is not captured by SIRE alone. Particularly among Hispanics and blacks, inclusion of AIMs adds value over self-reported ancestry in controlling for genetic and environmental exposures that influence risk of ICH. Additional studies across other populations and risk exposures are needed to confirm and extend these findings

Poster Number 111

Christopher Moran, MD
Pediatrics, Assistant Professor | cjmoran@mgh.harvard.edu

Genetic Variation Affects C-Reactive Protein Elevations in Crohn's Disease

INVESTIGATORS: C. J. Moran, J. L. Kaplan, H. S. Winter

C-reactive protein (CRP) is a serum marker that is used to measure disease activity in Crohn's disease (CD). However, a subset of CD patients has a normal CRP during flares. In rheumatoid arthritis and lupus, genetic variants can restrict CRP elevations during flares. This study sought to determine if common CRP genetic variants affect CRP values during active CD. Subjects with CD who participated in the Partners Healthcare BioBank were genotyped for five common CRP genetic variants (rs2794520, rs3122012, rs3093077, rs2808635, and rs1800947). Medical records were reviewed to determine disease activity and the highest CRP value during active CD. CRP values during active infection or malignancy at the time of the test were excluded. CRP values were compared by genotype using Mann-Whitney test. The study included 199 subjects with active CD (21 to 86 years of age). Subjects with the rs2794520 TT genotype had a lower CRP than subjects with CC genotype (58.3 mg/L vs 28.4 mg/L, p=0.008). Subjects with rs1800947 CG genotype had a lower CRP than those with CC genotype (54.3 mg/L vs 22.4 mg/L, p<0.0001). 41.6% of TT subjects had a normal CRP compared to 24.1% of CT subjects and 16.5% of CC subjects (p=0.041). This study demonstrates that rs2794520 and rs1800947 are associated with a restriction of CRP elevations during active CD. While CRP is typically a reliable biomarker in CD, there is a subset of CD patients with a genetically-determined restriction of CRP in whom other disease markers should be utilized.

Poster Number 112

Tarjinder Singh, PhD
Center for Genomic Medicine, Research Fellow | tsingh@broadinstitute.org

The meta-analysis of rare coding variants in the whole-exomes sequences of 25,000 cases and 50,000 controls implicates individual risk genes for schizophrenia

INVESTIGATORS: T. Singh, B. M. Neale, M. J. Daly, on behalf of the SCHEMA consortium

Schizophrenia, a debilitating psychiatric disorder affecting nearly 1% of the general population, has been demonstrated to have a substantial genetic component. While over a hundred common risk loci of small effect have been implicated, analyses of rare protein-coding variants have had limited success in identifying individual genes, presumably owing to power limitations. Here, we present the Schizophrenia Exome Sequencing Meta-Analysis (SCHEMA) Consortium, a large global collaboration to aggregate, generate, and analyze high-throughput sequencing data of schizophrenia to advance gene discovery. Our study actively recruited from diverse global populations, and includes individuals of European, Latin American, East Asian, Ashkenazi Jewish, and African American ancestry. To date, we have sequenced and processed the whole exomes of 25,000 cases and 50,000 matched controls using a standardized protocol, yielding one of the largest sequencing data sets of a complex trait to date.

We first show that schizophrenia cases carry a substantial excess of rare damaging variants in genes demonstrated to be under strong selection, with a notable enrichment in genes for broader neurodevelopmental disorders. We present the first gene-based burden results from our exome meta-analysis, and implicate at least three individual genes in which ultra-rare damaging coding variants confer substantial risk for schizophrenia. Finally, we present a new results browser that allows for easy viewing of identified variants and gene-based results. In summary, we introduce the largest multi-center effort to aggregate sequencing data of a psychiatric trait, and the initial results from the harmonization and analysis of over 75,000 exomes.

Poster Number 113

Rachita Yadav, PhD
Center for Genomic Medicine, Research Fellow | ryadav1@mgh.harvard.edu

Dissecting the Causal Mechanism of X-Linked Dystonia-Parkinsonism by Integrating Genome and Transcriptome Assembly

INVESTIGATORS: R. Yadav, T. Aneichyk, W. T. Hendriks, D. Shin, D. Gao, C. A. Vaine, R. L. Collins, A. Domingo, B. Currall, A. Stortchevoi, T. Multhaupt-Buell, E. B. Penney, L. Cruz, J. Dhakal, H. Brand, C. Hanscom, C. Antolik, M. Dy, A. Ragavendran, J. Underwood, C. Klein, U. Müller, K. Wilhelmsen, P. Acuna, D. Jaffe, N. Sharma, X. O. Breakefield, L. J. Ozelius, D. C. Bragg, M. E. Talkowski, CCXDP

X-linked Dystonia Parkinsonism (XDP) is an adult-onset neurodegenerative disorder indigenous to the Philippines and exhibits features of both dystonia and parkinsonism. Conventional genetic approaches have linked XDP to a 410 kb founder haplotype that included five single nucleotide variations known as Disease Specific Changes (DSCs), 2627 bp sine-VNTR-Alu (SVA) retrotransposon and 48bp deletion. Still, the causal variant and pathogenic mechanism have remained unknown. We integrated de novo genome and transcriptome assembly using multiple emerging long-read, short-read, and linked-read genomics technologies and layered analyses to capture the complete genetic architecture of XDP. We characterized genome and transcriptome variation using DNA from 792 probands and unaffected individuals as well as RNA from fibroblasts (n=46), iPSC-derived neural stem cells (NSCs) and induced neurons (n=24). These analyses identified 47 shared variants among probands and five recombination events that narrowed the causal locus to the TAF1 gene. Transcriptome assembly revealed a striking expression signature involving aberrant splicing and partial intron retention (IR) proximal to XDP-specific SVA insertion within intron 32 of TAF1 in XDP probands that was never observed in controls. Canonical TAF1 transcripts were significantly reduced in XDP probands proportional to the IR, and both the aberrant splicing and TAF1 expression signatures were rescued following CRISPR/Cas9 excision of the SVA in patient-derived NSCs. These data implicate aberrant splicing as a consequence of a noncoding SVA insertion into TAF1 as a pathogenic mechanism in XDP that can be ameliorated with CRISPR/Cas9 manipulation, and propose a potential roadmap for reference-free assembly for other unsolved Mendelian disorders.

Poster Number 114

Utibe Essien, MD
Medicine, Research Fellow | uessien@partners.org

Race, Ethnicity, and Use of Non-Vitamin K Antagonist Oral Anticoagulants in Patients with Atrial Fibrillation: A National Study

INVESTIGATORS: U. Essien, L. R. Jackson, D. Holmes, J. P. Piccinni, D. E. Singer, ORBIT-AF II Investigators

Blacks and Hispanics are less likely than whites to use oral anticoagulants (OAC) for atrial fibrillation (AF). Little is known about differences in non-vitamin K antagonist oral anticoagulant (NOAC) use for AF by race/ethnicity. Within the ORBIT-AF II registry, we evaluated overall OAC and NOAC use by race/ethnicity using logistic regression, adjusting for clinical and socioeconomic factors. The cohort included 11,100 whites, 646 blacks and 671 Hispanics with AF. Overall, OAC use was high (89%), predominantly NOACs (75%). After adjusting for clinical features, fewer blacks received any OAC versus whites, adjusted odds ratio, aOR, 0.75, (95% CI: 0.56, 0.99), and fewer received NOACs, aOR 0.63 (95% CI: 0.49-0.83). After further controlling for socioeconomic factors, there was a trend towards lower OAC use in blacks (aOR 0.78 [95% CI: 0.59-1.04]); NOAC use remained lower in blacks using OAC (aOR 0.74 [95% CI: 0.56-0.97]). There was no significant white-Hispanic difference in OAC or NOAC use. Among warfarin users, median time in therapeutic range was lower in blacks (57.1%) and Hispanics (51.7%) than whites (67.1%, p<.0001) and blacks and Hispanics treated with NOACs were less likely to receive appropriate dosing than whites (15.5% vs. 18.1% vs. 12.6% respectively, p=0.01). After controlling for clinical and socioeconomic factors, blacks with AF were less likely than whites and Hispanics to receive NOACs. When treated, the quality of OAC and NOAC use was poorer in blacks and Hispanics. Identifying modifiable causes of these disparities should improve overall quality of care in AF.

Poster Number 115

Sara Kalkhoran, MD
Medicine, Instructor | skalkhoran@mgh.harvard.edu

Cigarette Smoking and Quitting-Related Factors among US Adult Health Center Patients with Serious Mental Illness

INVESTIGATORS: S. Kalkhoran, A. N. Thorndike, N. A. Rigotti, V. C. Fung, T. P. Baggett

The prevalence of smoking among US adults with low socioeconomic status and serious mental illness (SMI) is 1.5- to 3-fold higher than in the general US population and is a major contributor to the shorter life expectancy of those with SMI. We aimed to determine the prevalence of cigarette smoking and factors associated with current smoking and quitting among US adults with and without SMI who received care at federally funded health centers in 2014 using data from the Health Center Patient Survey.

Here we found that prevalence of ever smoking was 68% in adults with SMI and 41% in adults without SMI (p<0.001). Current smoking was also higher among those with SMI (48% vs. 22%, p<0.001). Adults with SMI were more likely to be living below the federal poverty level than adults without SMI (71% vs 52%, p<0.001) and reported higher rates of smoking-related diseases such as COPD (15% vs 5%, p<0.001) and cardiovascular disease (19% vs 10%, p=0.003). Contrary to many clinicians' perceptions that smokers with SMI do not want to quit, smokers with SMI were more likely to have made a quit attempt and were equally likely to have plans to quit than smokers without SMI. This could reflect both higher motivation to quit and more difficulty in succeeding. Health center clinicians can help reduce the mortality gap in patients with SMI by providing their patients who smoke with evidence-based smoking cessation treatment that extends beyond advice to quit.

Poster Number 116

Julie Levison, MD
Medicine, Assistant Professor | jlevison@partners.org

HIV Testing Outcomes in a Multi-National Cohort of Latino Migrants with Substance Use and Mental Health Problems

INVESTIGATORS: J. H. Levison, Y. Wang, S. Markle, L. Fuentes, D. Mejia, L. Albarracin, L. Cellerino, M. Alegria

Globally, individuals with substance use disorders and migrants are at high risk for undiagnosed HIV and are key populations to achieve the United Nation's 90-90-90 targets. High levels of mistrust of systems, social isolation, and "double discrimination" of HIV infection and migrant status delay diagnosis and access to treatment in migrants in the US and Europe. Our objectives were: 1) to identify acceptance of HIV/sexually transmitted infection (STI) testing in Latino migrants in Boston, Madrid, and Barcelona with co-occurring mental health and substance use problems 2) to assess predictors of HIV/STI testing in this population.

Poster Number 117

Claire McGlave, BA
Medicine, Clinical Research Coordinator | cmcglave@mgh.harvard.edu

Smokefree TXT for homeless smokers: A pilot RCT with a mixed methods analysis

INVESTIGATORS: C. C. McGlave, G. Kruse, A. Yaqubi, Y. Chang, N. Rigotti, T. Baggett, Boston Healthcare for the Homeless Program

Homeless smokers want to quit smoking but face barriers to doing so. Mobile phone possession is common among homeless people, and a short message service (SMS) could provide homeless smokers with virtual support to manage smoking cessation. We aimed to evaluate the effect of a stop-smoking SMS program on smoking abstinence among homeless smokers. We conducted a pilot RCT that used qualitative interviews to explain our quantitative results. Control and SMS arm participants were offered nicotine patches, in-person counseling, and a mobile phone. Additionally, SMS arm participants were offered SmokefreeTXT, a free service from the NCI that sends 1-5 SMS messages daily and offers on-demand tips. The primary outcome was smoking abstinence (measured by exhaled CO) assessed 14 times over 8 weeks. The secondary outcomes were use and satisfaction with SmokefreeTXT, assessed by surveys and interviews; and mobile phone retention, assessed by self-report.

Smoking abstinence did not differ significantly between arms. 88% of SMS arm participants enrolled in SmokefreeTXT; of these, 32% responded to ≥1 interactive prompts sent by the program. Exit surveys indicated satisfaction with SmokefreeTXT, but qualitative interviews revealed that participants felt the messages were "impersonal" and "overwhelming". 40% of SMS arm participants retained their study-supplied mobile phone for the 8-week duration of the trial. SmokefreeTXT, when added to NRT and in-person counseling, did not improve smoking abstinence in this RCT of homeless smokers. SMS interventions for homeless smokers may need to be better tailored to their unique needs and combined with efforts to promote mobile phone retention.

Poster Number 118

Alexander Tsai, MD
Psychiatry, Assistant Professor | actsai@partners.org

Spillover Effects of Police Killings on the Mental Health of African-Americans in the General U.S. Population

INVESTIGATORS: J. Bor, A. S. Venkataramani, D. R. Williams, A. C. Tsai

Police kill over 300 African-Americans each year in the U.S. These events may have spillover effects on the mental health of people not directly affected. We estimated difference-in-differences regression models -- adjusting for state-month, month-year, and interview day fixed effects, as well as age, gender, and educational attainment -- to estimate the causal impact of police killings of unarmed African-Americans on self-reported mental health of other African-Americans in the U.S. general population. We combined novel data on police killings with individual-level data from the nationally representative 2013-2015 U.S. Behavioral Risk Factor Surveillance Surveys. We additionally assessed the timing of effects, the specificity of the effects to African-Americans, and the robustness of our findings. Nearly half of the 103,710 African-American respondents were exposed to one or more police killings of unarmed African-Americans in their state of residence in the quarter prior to the survey. Each additional police killing of an unarmed African-American was associated with 0.14 additional poor mental health days (95% CI, 0.07-0.22; p<0.001) among African-American respondents. The largest effects on mental health occurred in the 1-2 months after exposure, with no effects found for respondents interviewed before police killings (falsification test). The estimated mental health impacts were not observed among White respondents and resulted only from police killings of unarmed African-Americans (not unarmed Whites or armed African-Americans). Police killings of unarmed African-Americans have adverse spillover effects on mental health among African-American adults in the general population.

Poster Number 119

Erica Warner, MPH, ScD
Medicine, Assistant Professor | ewarner@mgh.harvard.edu

Race, obesity, tumor subtype and breast cancer survival in a pooled analysis of four Alliance clinical trials

INVESTIGATORS: E. Warner, K. V. Ballman, C. Strand, A. H. Partridge, Alliance for Clinical Trials in Oncology

Previous studies have demonstrated poorer survival of Black women with breast cancer, with the strongest associations among estrogen receptor positive (ER) subtypes. We assessed whether race/ethnicity was associated with survival among women with breast cancer and further investigated whether BMI modifies race-survival associations. 10,011 women enrolled in one of four adjuvant chemotherapy trials: CALGB 9741, CALGB 49907, CALGB 40101, or NCCTG N983 were included. Cox proportional hazards regression was used to compare overall survival among Non-Hispanic (NH) black (n=928), Hispanic (n=437), and other race participants (n=408) to Whites (n=8,238) overall, and stratified by subtype defined by ER and HER2. We assessed interaction between race and BMI. In age-adjusted models, NH-Black patients were 35% more likely to die compared to Whites (hazard ratio [HR] 1.35, 95% CI 1.16-1.57). With adjustment for clinical characteristics this association became non-significant (HR: 1.10, 95% CI 0.92-1.30). When stratified by ER/HER2 status, there was no survival difference between black and white women for ER+/HER2- (HR: 0.98, 95% CI 0.71-1.35), ER-/HER2+ (HR: 1.16, 95% CI 0.82-1.65), or ER-/HER2-tumors (HR: 1.03, 95% CI 0.75-1.42), but Blacks were at 55% higher risk of death for ER+/HER2+ tumors (HR: 1.55, 95% CI 1.03-2.33). Overall our results were null, with the exception of higher observed mortality among black women with ER+/HER2+ tumors. This suggests that in a setting where black and white women receive the same initial therapy and follow-up, racial disparities in survival are attenuated or eliminated.

Poster Number 120

Krislyn Boggs, MPH
Emergency Medicine, Clinical Research Coordinator | kboggs@partners.org

Change in Availability of Pediatric Emergency Care Coordinators in US Emergency Departments

INVESTIGATORS: K. M. Boggs, J. A. Espinola, A. F. Sullivan, R. D. Freid, K. Hasegawa, C. A. Camargo Jr.

In 2006, the Institute of Medicine recommended that every emergency department (ED) should appoint both physician and nurse pediatric emergency care coordinators (PECCs), but many EDs still lack even one. PECCs focus on managing pediatric emergency care in the ED. We investigated changes in availability of PECCs in US EDs. We conducted national surveys of US EDs in 2015 and 2016. Using the National Emergency Department Inventory-USA database, we identified 5,237 open in both years. The survey was mailed to each ED director up to three times and then we contacted nonresponding EDs by phone. Overall, 3,819 (73%) EDs responded to both surveys.

Among the 3,769 EDs with PECC data in both 2015 and 2016, at least one PECC was reported by 653 (17%) EDs in 2015 and 714 (19%) in 2016 (P<0.001). Compared with EDs that reported no PECC in both years, EDs that first reported a PECC in 2016 were more likely to have an annual total ED visit volume of ≥10,000, be in hospitals that have a separate pediatric ED, and be located in an urban area (all P<0.05). EDs that first reported a PECC in 2016 were as likely to receive telemedicine services as EDs without PECCs in both years (P=0.55). Availability of at least one PECC increased slightly between 2015 (17%) and 2016 (19%), but ~80% of EDs continue without one. We will continue to survey US EDs to track annual changes in PECC prevalence while also seeking novel ways to increase PECC prevalence nationwide.

Poster Number 121

Kelsy Greenwald, MD
Emergency Medicine, Resident | kgreenwald1@partners.org

Feasibility and Acceptability of Home-based HIV Testing Among Refugees: A Pilot Study in Nakivale Refugee Settlement in Southwestern Uganda

INVESTIGATORS: K. N. O'Laughlin, W. He, K. E. Greenwald, J. Kasozi, Y. Chang, E. Mulogo, Z. M. Faustin, P. Njogu, R. P. Walensky, I. V. Bassett

We conducted a pilot study to determine the feasibility and acceptability of home-based HIV testing in Nakivale Refugee Settlement in Uganda and to compare home-based and clinic-based testing participants. From February-March 2014, we visited homes in 3 villages in Nakivale up to 3 times and offered HIV testing. We evaluated the proportion of individuals encountered (feasibility) and assessed participation in HIV testing among those encountered (acceptability). We compared characteristics of home-based and clinic-based testers (from a prior study). We examined the relationship between time of visit, sex, and number of individuals at home on willingness to test. Of 566 adults living in 319 homes, we encountered 507 (feasibility = 90%). Home-based HIV testers totaled 378 (acceptability = 75%). Compared to clinic-based testers, home-based testers were older (median age 30 [IQR 24-40] vs 28 [IQR 22-37], p<0.001), more likely refugee than Ugandan national (93% vs 79%, <0.001), and more likely to live ≥1 hour from clinic (74% vs 52%, <0.001). HIV prevalence was lower (not significant) in home-based vs. clinic-based testers (1.9 vs 3.4% respectively, p=0.27). Testing was not associated with time of visit (p=0.50) or sex (p=0.66), but for each additional person at home, the odds of accepting HIV testing increased by >50% (OR 1.52, 95%CI 1.12-2.06, p=0.007). Home-based HIV testing in Nakivale Refugee Settlement was feasible, with 90% of eligible individuals encountered within 3 visits, and acceptable with 75% willing to test for HIV, with a yield of nearly 2% individuals tested identified as HIV-positive.

Poster Number 122

A. Sassan Sabouri, MD
Anesthesia, Critical Care and Pain Medicine, Instructor | asabouri@partners.org

Effect of thoracic epidural analgesia and transversus abdominis block (TAP) on hospital length of stay after radial cystectomy

INVESTIGATORS: R. Steinhorn, T. Yamanni, V. Hu, L. Hung, F. McGovern, A. S. Sabouri

This study investigates whether patients with TAP Block may have shorter length of hospital stay compare to patient with thoracic epidural analgesia after radical cystectomy. The primary outcome was length of hospital stay (LOS) in days; secondary outcomes included intraoperative opiate use, intraoperative vasopressor requirement, postoperative PCA days, and postoperative days until mobilization. After IRB approval, data was collected from a chart review of 131 adult patients from 5 different surgeons at Massachusetts General Hospital between 3/2015 and 9/2017, who received a cystectomy under general anesthesia supplemented by an epidural (N=68), TAP catheters (N=56), or TAP single shot block (N=7). Demographic comparison between two groups revealed no significant difference. No significant difference in LOS was found between patients who received epidurals, TAP catheters, or TAP blocks (p=0.2), although the direction of the mean trended towards longer LOS in the epidural group. Patients who received epidurals required fewer intraoperative morphine equivalents per minute than patients who received regional techniques, while intraoperative vasopressor requirements were statistically similar between groups. Patients who received TAP blocks or catheters mobilized more quickly postoperatively than patients with epidurals. Receiving regional versus neuraxial analgesia to supplement general anesthesia during cystectomy did not significantly impact LOS. Although intraoperative opioid requirements were decreased in patients with epidurals, they utilized opiate PCAs for a similar length of time as patients who received TAP blocks or catheters.

Poster Number 123

Jason Wasfy, MD
Medicine, Assistant Professor | jwasfy@mgh.harvard.edu

County Community Health Associations of Net Voting Shift in the 2016 U.S. Presidential Election

INVESTIGATORS: J. H. Wasfy, C. Stewart, V. Bhambhani

In the U.S. presidential election of 2016, substantial shift in voting patterns occurred. The extent to which this shift was related to public health status is unclear. As such, we sought to determine the extent to which county community health was associated with changes in voting between the presidential elections of 2016 and 2012. Using county-level measures of public health, we performed a principal component analysis (PCA) using principal axis method to extract the components to create a generalized measure of unhealthiness. We then used linear regression to test the association of that variable with he percentage of Donald Trump votes in 2016 minus percentage of Mitt Romney votes in 2012 ("net voting shift") after adjustment for county-level demographic variables. The mean county net voting shift was 5.4% (+/- 5.8%). Of 3,009 counties, 2,641 (87.8%) had positive net voting shift (towards Trump) and 368 counties (12.2%) had negative net voting shift (away from Trump). The first principal component ("unhealthy") accounted for 68% of total variance in the data. Higher normalized unhealthy score was associated with with positive net voting shift (22.1% shift per unit unhealthy, $p < 0.0001$). This association was stronger in states that switched Electoral College votes from 2012 to 2016 than in other states (5.9% per unit unhealthy, $p < 0.0001$). Substantial association exists between a shift toward voting for Donald Trump in 2016 relative to Mitt Romney in 2012 and poor public health, suggest a possible role for health status in political choices.

Poster Number 124

Hajera Amatullah, PhD, MPH
Medicine, Research Fellow | hamatullah@mgh.harvard.edu

Crohn's disease-associated epigenetic reader SP140 orchestrates macrophage transcriptional programs through control of DNA unwinding mechanisms

INVESTIGATORS: H. Amatullah, K. L. Jeffrey

SP140 is an epigenetic reader with immune-restricted expression and single nucleotide polymorphisms (SNPs) within SP140 associate with Crohn's Disease. Our lab previously showed SP140 to be a master orchestrator of macrophage identity and function through occupancy and repression of lineage-inappropriate genes such as HOX genes. SP140 depletion in mouse or human macrophages resulted in severely compromised microbe-induced activation. Hematopoietic-specific depletion of Sp140 in mice resulted in exacerbated dextran sulfate sodium (DSS)–induced colitis. The specific mechanism by which SP140 imparts its epigenetic function to modulate transcription is unknown. To determine the mechanism by which the epigenetic reader SP140 controls macrophage transcriptional programs for identity and function at steady state and in response to microbial stimulation, we overexpressed FLAG-SP140 and FLAG-Empty Vector constructs in HEK293 cells, immunoprecipitated SP140, and performed Mass Spectrometry (MS) to reveal SP140 binding partners. Co-immunoprecipitation (IP) experiments were conducted to validate the top SP140 interacting hits. We deleted SP140 in human THP-1 and mouse immortalized macrophages and assessed expression and function of SP140 interacting proteins. Results: The top SP140 interacting proteins are involved in DNA unwinding (Topoisomerase I (Topo1), II alpha (Top2A) and II beta (Top2B); DNA-PK and SUPT16H (FACT complex 160 subunit) which were all confirmed by co-IP experiments. Deletion of SP140 resulted in increased activity of Top2 and Top1. Conclusions: SP140 is an epigenetic reader protein that links histone modifications to transcriptional regulation through control of DNA unwinding mechanisms. We are exploring if this function of SP140 is altered in Crohn's Disease patients harboring SP140 SNPs.

Poster Number 125

Galit Frydman, DVM
Surgery, Research Fellow | gfrydman@mit.edu

Megakaryocytes are functional innate immune cells and may play a role in the pathophysiology of sepsis

INVESTIGATORS: G. H. Frydman, F. Ellett, J. Jorgensen, L. Zuckerberg, M. Selig, S. Tessier, K. Wong, D. Olaye, S. Kauffman, C. Vanderburg, D. Irimia, J. G. Fox, R. G. Tompkins

Megakaryocytes (MKs) are precursors to platelets, one of the most abundant cell types in the peripheral circulation. While platelets are under study for their role in inflammation, the role of MKs within the innate immune system has not yet been explored. We have performed a set of comprehensive in vitro experiments demonstrating that both cord blood-derived MKs and MKs from a megakaryoblastic lineage have innate immune cell functions, including: phagocytosis, formation of extracellular traps, and chemotaxis towards pathogenic stimuli. Megakaryocytes were also seen to directionally release platelets towards pathogenic stimuli. Evaluation of samples from patients with sepsis revealed that there were increased numbers of CD61+ cells in the kidneys, lungs and peripheral blood; suggesting that these cells may have a role outside of platelet formation during severe inflammation and infection. This new potential role of the megakaryocyte as a functional innate immune cell may have significant implications for their role both inside and outside the bone marrow during different disease states.

Poster Number 126

Nitya Jain, PhD
Pediatrics, Assistant Professor | njain@ccib.mgh.harvard.edu

Microbiota and plasmacytoid dendritic cells influence thymic T cell development in early life

INVESTIGATORS: M. Ennamorati, K. Clerkin, S. Halvorsen, K. Berry, C. Porter, H. Wang, V. Yeliseyev, M. Weglarz, L. Bry, S. Sassi, B. Seed, N. Jain

The immune system faces unique challenges in the period immediately following birth. Thrust from a sterile fetal environment, it is abruptly exposed to an immense array of foreign antigens, the major burden of which is in the form of the microbiota newly colonizing the gastrointestinal tract. Adaptive immunity at the post-natal stage is largely immature, and the newborn's immune system relies initially on innate immune effectors for protection against infections. In the early postnatal period, increasing numbers of innate and adaptive lymphocytes mature in the thymus and migrate to populate peripheral lymphoid organs including intestinal mucosal tissue. Intestinal microbiota regulate the maturation of the immune system at mucosal sites and also influence host physiology and function beyond the mucosal tract such as the brain and the pancreas. However, whether and how intestinal microbial colonization influences immune cell development in the thymus remains unknown.

In this study, intestinal microbiota affected the development of thymic innate-like cell subsets in neonates. Altered development of these cells in germfree mice was restored by colonization with Bacteroides fragilis, but not with a polysaccharide antigen A (PSA) negative isogenic strain. Plasmacytoid dendritic cells (PDCs) were found to be one class of antigen presenting cell that can transit from the intestine to the thymus during the postnatal period. Enterothymic communication appears to be one mechanism by which neonates regulate immune responses to intestinal microbial load.

Poster Number 127

Batul Kaj, MBBS
Pediatrics, Research Fellow | bkaj@partners.org

Distinct Microbiota in Primary Sclerosing Cholangitis with Inflammatory Bowel Disease and Primary Biliary Cholangitis

INVESTIGATORS: B. Kaj, G. Birzu, E. X. Li, J. L. Kaplan, C. J. Moran, U. Shah, K. Korolev, D. S. Pratt, H. S. Winter

The intestinal microbiome is thought to play a role in metabolic and immune responses in autoimmune cholestatic liver disease, including primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC). The association of PSC with inflammatory bowel disease (IBD), as well as successes of antibiotic treatment in both IBD and PSC, may implicate the gut-liver axis in the pathogenesis of PSC. We sought to identify differences in the intestinal microbiome between patients with IBD alone, PSC-IBD, PBC and healthy controls. Methods: Stool samples and metadata were collected from 60 participants, including 16 PBC patients, 20 PSC-IBD patients with ulcerative colitis (UC, n = 13) or Crohn's (n = 7), 12 IBD only patients with UC (n=6) or Crohn's (n=6), and 12 controls. V4 region amplicons of 16s-rRNA were sequenced on an Illumina MiSeq platform. Results: As expected, the intestinal microbiota differed in patients with both PSC-IBD and PBC compared to controls. Intestinal microbiota also differed in composition between PBC and PSC. Combining analyses from all subjects demonstrated that the microbiome of PSC-IBD patients was most similar to patients with UC. Conclusions: This study demonstrates that the intestinal microbiota in patients with PSC-IBD and PBC differ from each other and from controls. Microbiota in patients with PSC-IBD are similar to those with UC alone. Identification of specific taxa responsible for these distinctions might provide evidence for perturbations in the microbiome of patients with IBD that trigger hepatic inflammation. Further characterization of distinct microbial profiles may identify novel therapeutic targets or biomarkers.

Poster Number 128

Ethan Lerner, MD
Dermatology, Associate Professor | elerner@mgh.harvard.edu

Direct antigen-induced neural activation and recruitment are required for allergic eczema development

INVESTIGATORS: S. B. Elmariah, T. Luo, E. Azimi, J. Ordovas-Montanes, V. B. Reddy, U. von Andrian, D. Kang, E. A. Lerner

Neuro-immune interactions are critical for amplifying inflammatory signals within epithelia including skin, lung and gut and are implicated in the pathogenesis of atopic dermatitis, a common allergic skin disease. An underlying assumption is that nerve contributions are recruited to potentiate inflammation in response to immune activation. However, the specific upstream signals that engage peripheral nerves remain unclear. In vivo imaging of fluorescently-labeled peripheral nerves in mice during allergic eczema induction demonstrates that cutaneous nerves are pioneers of the allergic process. Hours after antigen exposure, neuropeptidergic fibers undergo pathfinding and arbor expansion, while vaso-immune changes follow weeks later. Using pharmacologic and genetic manipulation, we show that neuronal MrgprA1 mediates calcium activation and neurite outgrowth in direct response to allergic stimulation, and that maintenance of nascent fibers and subsequent inflammation requires neural activity. Our data highlight key cellular and molecular events in disease pathogenesis and shift the therapeutic paradigm for allergic eczema.

Poster Number 129

Nathan Louras, MD
Surgery, Research Fellow | nlouras@partners.org

Effects of Preformed Antibody on Survival Following Pig-to-Primate Liver Xenotransplantation

INVESTIGATORS: N. J. Louras, M. S. Patel, J. A. Shah, A. R. Andrews, Z. Wang, D. H. Sachs, P. A. Vagefi

Xenotransplantation represents the best near-term solution to address the current organ shortage. We have developed a pre-clinical model of pig-to-primate liver xenotransplantation (LXT), and now assess the effect of preformed antibodies (Ab) on post-transplant survival. GalT-KO pig-to-primate LXT was conducted in five animals using immunosuppression consisting of thymoglobulin, tacrolimus, steroids, and co-stimulation blockade (belatacept or anti CD-40). Serum samples taken both pre- and post-transplant were analyzed by a complement-mediated cytotoxicity assay on target cells from GalT-KO miniature swine to determine the presence of Abs, using FACS analysis to evaluate cell death.

All baboon LXT recipients had pre-transplant cytotoxic IgM Ab. Of note, the longest LXT survivors (25- and 29-day) were found to have lower concentration of cytotoxic Ab (Figure). Post-transplant, these two recipients showed an initial increase in cytotoxic Ab formation, reaching the highest concentration on post-operative day 7, followed by a decrease at each time point thereafter. Final pathology for both animals was C4d negative. The other three recipients showed a continuous post-operative increase in concentration of cytotoxic Ab until their death (Days 8, 5, and 7), with two of the three (B1 and B3) demonstrating C4d-positive staining on final pathology. Low concentrations of pre-formed cytotoxic IgM xeno-Abs do not appear to limit post-LXT survival. The fact that recipients surviving nearly one month following LXT showed a reduction in cytotoxic antibodies throughout their post-operative course, suggests that the xeno-Ab barrier may be surmountable with conventional immunosuppression.

Poster Number 130

Crystal North, MD
Medicine, Instructor | cnorth@mgh.harvard.edu

Systemic Inflammation, Immune Activation and Impaired Lung Function among People Living with HIV in Rural Uganda

INVESTIGATORS: C. M. North, D. Muyanja, B. Kakuhikire, A. C. Tsai, R. Tracy, D. S. Kwon, D. C. Christiani, M. J. Siedner

Although both obstructive pulmonary disease and HIV are inflammatory diseases common in sub-Saharan Africa, the relationship between systemic inflammation and lung function among people living with HIV (PLWH) in sub-Saharan Africa is not well described. We measured lung function, serum high sensitivity C-reactive protein, IL-6, sCD14 and sCD163 in 125 PLWH at least 40 years of age who were on antiretroviral therapy for a minimum of three years, and 109 age and sex-similar, community-based HIV uninfected controls in rural Uganda. We modeled the relationship between lung function and systemic inflammation using linear regression, stratified by HIV serostatus and controlling for age, sex, height, tobacco exposure and self-reported biomass exposure.

Approximately half of subjects (46%, [107/234]) were women and the median age was 52 years (IQR 48-55), with no differences by HIV serostatus. Most PLWH (92%, [115/125]) were virologically suppressed on first-line antiretroviral therapy, and median CD4 count was 472 cells/mm3. In multivariable linear regression models adjusted for predicted confounders, higher IL-6 and sCD163 were associated with lower lung function among PLWH, and higher hsCRP was associated with lower lung function among both PLWH and HIV-uninfected controls. sCD14 was not associated with lung function. Macrophage activation and systemic inflammation are associated with lower lung function among PLWH on stable antiretroviral therapy in rural Uganda. Future work should focus on the mechanisms of this relationship and its public health implications.

Poster Number 131

Cory Perugino, DO
Ragon Institute, Instructor | cperugino@partners.org

Identification of a Dominant Auto-Antigen in IgG4-Related Disease Using Monoclonal Antibodies from Patient-Derived Plasmablasts

INVESTIGATORS: C. A. Perugino, S. B. AlSalem, H. Mattoo, V. Mahajan, G. Ganesh, H. Allard-Chamard, E. Della-Torre, J. Kreuzer, W. Haas, J. H. Stone, S. Pillai

IgG4-related disease (IgG4-RD) is characterized by tumor-like, fibroinflammatory masses containing activated B cells, IgG4-expressing plasma cells, and CD4+ cytotoxic T cells (CD4+ CTLs). The oligoclonal expansion of plasmablasts and CD4+ CTLs observed in IgG4-RD suggests an antigen-driven immune response. However, a systematic approach has not been used to identify the auto-antigens targeted by dominantly expanded B and or T lineage cells. Here, we describe a combination approach of single cell cloning and next generation sequencing to identify a dominant auto-antigen in IgG4-RD.

Sequencing Ig genes from single cells revealed that 64% of sorted plasmablasts were derived from a single cell. The two most frequent clones were expressed as human monoclonal antibodies. Both mAbs stained human pancreatic tissue sections and permeabilized pancreatic cancer cell lines. Galectin-3 was identified as the antigen specifically recognized by both antibodies. Sixteen (13.5%) of 121 IgG4-RD patients had elevated plasma levels of galectin-3. The index patient along with 5 other IgG4-RD subjects had anti-galectin-3 antibodies. IgG4 was the dominant IgG subclass of anti-galectin-3 antibodies in 5 of these 6 subjects. After segregating subjects by plasma galectin-3 levels, anti-galectin-3 antibodies were seen in 14.3% of those subjects with elevated levels compared to 4.7% of the subjects with normal levels of this lectin. Galectin-3 was identified as a dominant auto-antigen in IgG4-RD. The presence of anti-galectin-3 autoantibodies correlated inversely with pulmonary involvement. Among individuals with elevated circulating galectin-3 levels, overexpression of this protein may result in a breach of tolerance and the development of autoantibodies.

Poster Number 132

Amar Vedamurthy, MBBS, MS
Medicine, Instructor | amarv1986@gmail.com

Long-term Outcomes of Immunosuppression-Naïve Steroid Responders Following Hospitalization for Acute Severe Ulcerative Colitis: The When to Step Up Study

INVESTIGATORS: A. Vedamurthy, A. N. Ananthakrishnan, L. Xu, J. Luther, F. Colizzo, J. Garber, H. Khalili

Acute severe ulcerative colitis (ASUC) is a severe complication of ulcerative colitis (UC) that is associated with significant morbidity, treatment refractoriness and need for colectomy. Patients who do not adequately respond to the initial intravenous steroid therapy receive medical rescue therapy with infliximab or cyclosporine or undergo surgery for their refractory disease. However, there is limited guidance on management of steroid responders in this setting. While it is well established that Crohn's disease (CD) is progressive and benefits from early institution of immunosuppressive therapy, such a paradigm is less well established in UC and thresholds for therapy escalation remain poorly defined. In immunosuppression-naïve patients, whether a single hospitalization for ASUC is a sufficient threshold to escalate to immunomodulator or biologic therapy is unknown. Our study included a total of 133 immunosuppressive-naïve ASUC patients among whom 56 (42%) escalated therapy to thiopurine (93%) or biologic (7%) post-hospitalization. Here we show that immunosuppression-naïve ASUC patients who respond to intravenous steroids remain at high risk for colectomy with 8% receiving such surgery within 1 year. Therapy escalation was not associated with a reduction in this risk.

Poster Number 133

Marianna Almpani, MD
Surgery, Research Fellow | malmpani@partners.org

Targeting Bacterial Quorum Sensing to Improve Intestinal Barrier Function Following Burn - Site Infection

INVESTIGATORS: M. Almpani, F. Adiliaghdam, M. H. Gharedaghi, R. Hodin, L. G. Rahme

Burn-site infections have been associated with deranged intestinal integrity. Pseudomonas aeruginosa is one of the most common causative microorganisms. We have developed several potent anti-virulence agents targeting the P. aeruginosa MvfR quorum-sensing (QS) system. We assessed whether MvfR inhibition could ameliorate the intestinal barrier dysfunction following burn-site infection. Following induction of a 30% dorsal burn in C57BL/6 mice, a clinical P. aeruginosa isolate (PA14) was intradermally inoculated at the burn eschar. MvfR inhibitor was intravenously administered at 2, 4, 8 and 12 hours following burn and infection. Mice were gavaged with FITC-dextran and permeability was determined by serum FITC-dextran concentration. Mesenteric lymph nodes (MLNs), intestine, and feces were collected for qPCR and ELISA experiments, colony-forming units (CFUs) assessment and confocal microscopy imaging.

MvfR function exacerbates the post-burn intestinal hyperpermeability, increasing FITC-dextran flow from the intestine to the systemic circulation. Our novel anti-virulence MvfR inhibitors significantly decrease the flux out of the gut, diminish bacterial translocation from the intestine to MLNs, and improve tight junction integrity. Inhibitor administration alleviates the intestinal inflammation, as shown by the decreased ileum TNF-α, colon IL-6, and fecal lipocalin-2 levels, while it reduces systemic PA14 dissemination and endotoxin circulation. Collectively, our results suggest that MvfR function is crucial in the intestinal integrity deterioration following PA14 burn-site infection, while inhibition of this QS system mitigates gut hyperpermeability, by attenuating the derangement of morphological and immune aspects of the intestinal barrier.

Poster Number 134

Kimberly Blumenthal, MD, MS
Medicine, Assistant Professor | kblumenthal1@partners.org

The Impact of a Reported Penicillin Allergy on Surgical Site Infection Risk

INVESTIGATORS: K. G. Blumenthal, E. E. Ryan, Y. Li, H. Lee, J. L. Kuhlen, E. S. Shenoy

A reported penicillin allergy may compromise receipt of recommended antibiotic prophylaxis intended to prevent surgical site infections (SSI). Most patients with a reported penicillin allergy are not allergic. We determined the impact of a reported penicillin allergy on the development of SSIs. In this retrospective cohort study of Massachusetts General Hospital hip arthroplasty, knee arthroplasty, hysterectomy, colon surgery, and coronary artery bypass grafting (CABG) patients from 2010-2014, we compared patients with and without a reported penicillin allergy. The primary outcome was a SSI, as defined by the Centers for Disease Control and Prevention's National Healthcare Safety Network. The secondary outcome was perioperative antibiotic use. Of 8,385 patients who underwent 9,004 procedures, 922 (11%) reported a penicillin allergy and 241 (2.7%) had a SSI. In multivariable logistic regression, patients reporting a penicillin allergy had a 1.51 [1.02, 2.22] increased odds of SSI. Penicillin allergy reporters were administered less cefazolin (12% vs 92%, $p<0.001$) and more clindamycin (49% vs 3%, $p<0.001$), vancomycin (35% vs 3%, $p<0.001$), and gentamicin (24% vs 3%, $p<0.001$) compared to those without a reported penicillin allergy. The increased SSI risk was entirely mediated by the patients' receipt of an alternative perioperative antibiotic; from 112 to 124 patients with reported penicillin allergy would need allergy evaluation to prevent one SSI. Patients with a reported penicillin allergy had a 50% increased odds of SSI, attributable to the receipt of second-line perioperative antibiotics. Clarification of penicillin allergies as part of routine preoperative care may decrease SSI risk.

Poster Number 135

Bjorn Corleis, PhD
Ragon Institute, Research Fellow | bcorleis@mgh.harvard.edu

Smoking induces recruitment of monocytes into the alveolar space and contributes to accelerated growth of Mycobacterium tuberculosis

INVESTIGATORS: B. Corleis, J. L. Cho, A. H. Linder, A. Yan, A. Dickey, A. E. Schiff, B. D. Medoff, D. S. Kwon

20-30% of active TB cases worldwide are related to tobacco smoking. In humans and mice, smoking leads to an increase of total macrophage numbers in the alveolar space, and it has been suggested that monocyte-derived macrophages may be more permissive to Mycobacterium tuberculosis (M tb) growth relative to resident macrophages in the lung. We recruited healthy current- and never-smokers for collection of bronchoalveolar lavage (BAL). BAL macrophages and blood monocytes were analyzed using flow cytometry. Monocytes, BAL macrophages or monocyte-derived macrophages (MDMs) were infected with M tb in vitro. We found a significant increase in a population of small macrophages in BAL from smokers compared to non-smokers. Half of the small macrophages expressed surface CD93+, a marker which distinguished circulating monocytes from large alveolar macrophages and suggests that these small macrophages are derived from newly recruited monocytes. BAL fluid from smokers recruited blood monocytes in vitro and significantly higher concentrations of the chemokine CCL11 in BAL fluid correlated with the number of CD93+ small macrophages in BAL. Virulent M tb induced a hyper-inflammatory response in human monocytes with significantly higher intracellular growth compared to MDMs or large alveolar macrophages in vitro. In conclusion, our data indicate that smoking leads to higher numbers of total BAL macrophages by the CCL11 mediated recruitment of circulating CD93+ monocytes into the alveolar space. Importantly, monocytes were highly susceptible to M tb intracellular growth, suggesting that extensive monocyte infiltration plays a significant role in smoking associated risk for active tuberculosis.

Poster Number 136

Jeffrey Duncan, BS
Medicine, Research Technician | jeffrey.duncan@mgh.harvard.edu

Host cytoskeleton activates a bacterial protein delivery system

INVESTIGATORS: J. K. Duncan, B. C. Russo, A. L. Wiscovitch, A. Hachey, M. B. Goldberg

Shigella commonly cause diarrhea in humans. Shigella require a specialized secretion system (T3SS) to invade colonic enterocytes, which is essential for disease. The T3SS, which is conserved among ~30 pathogens, injects effector proteins into the cytosol of eukaryotic cells. T3SS effectors remodel cellular processes in a way that promotes disease. The injection of effectors depends on docking of the T3SS on the plasma membrane. The T3SS delivers bacterial proteins (IpaC, IpaB) that form a pore in the host plasma membrane, onto which the T3SS then docks. Docking activates secretion of effectors through a gated channel in the T3SS that connects the bacterial cytoplasm with the host cell cytoplasm. Thus, docking is a critical step in pathogenesis.

Docking requires the interaction of the plasma membrane pore protein IpaC with intermediate filaments, cytoskeletal proteins. In the absence of intermediate filaments, the pore assembles in the plasma membrane, but the T3SS is not activated. We hypothesized that intermediate filaments cause the conformation of IpaC to change in a way that enables docking. We show that interaction of IpaC with intermediate filaments enabled residues of IpaC to become accessible on the cell surface. This conformational change was associated with docking. Moreover, locking IpaC in a pre-docking conformation by induced crosslinked caused a significant defect in docking. These data indicate that the interaction of IpaC with intermediate filaments induces a conformational change in the T3SS pore protein IpaC that is associated with docking and may serve as a critical signal for the activation the T3SS.

Poster Number 137

Brie Falkard, PhD
Medicine, Research Fellow | bfalkard@partners.org

Bivalent Oral Cholera Vaccine Induces Memory B Cell Responses

INVESTIGATORS: B. W. Falkard, R. C. Charles, L. M. Mayo-Smith, W. R. Matias, J. E. Teng, P. Xu, P. Kovac, E. T. Ryan, M. F. Franke, L. C. Ivers, J. B. Harris

Cholera has become endemic in Haiti since its introduction in 2010. A killed whole cell bivalent oral cholera vaccine (BivWC) has now been used in multiple countries; however, a complete understanding of the immune response to this vaccine is still lacking. The presence of Memory B Cells (MBCs) are considered the best immunological indicator of protection from cholera infections. To determine whether this vaccine generates detectable circulating MBC responses, we followed a cohort of 73 Haitian adults who received two doses of BivWC (Shanchol) for 1 -year following vaccination. We assessed immune responses at day 0 (baseline), day 7 (7 days after the first vaccination), day 21 (7 days after the second vaccination), and again on days 44, 90, 180, and 360 following vaccination. We observed a significant increase in circulating IgA MBC responses targeting the O-specific polysaccharide (OSP; Ogawa and Inaba) of Vibrio cholerae 01, starting 21 days following initiation of vaccination. We also observed an increase in the level of circulating IgG memory B cells targeting V. cholerae 01 Ogawa OSP starting at day 21 and remaining significantly elevated for 6 months following initiation of vaccination; the Ogawa serotype has been the predominant circulating strain of V. cholerae in Haiti. These results provide evidence that the bivalent oral cholera vaccine is capable of inducing and boosting systemic memory B cell responses targeting V. cholerae and may be responsible for generating protection.

Poster Number 138

Dahlene Fusco, MD, PhD
Medicine, Instructor | dnfusco@mgh.harvard.edu

A Unique Relatively Immunocompetent Mouse Model for Host Flaviviral Interaction Studies

INVESTIGATORS: K. Bellovoda, M. Menara, S. S. Cheon, F. Gootkind, M. Saint-Geniez, M. Troulis, D. N. Fusco

Relative contributions of virus and host antiviral response to ZIKV teratogenicity remain incompletely understood. Flaviviral infections trigger host interferon(IFN) antiviral response. IFN suppresses ZIKV in vitro. However, ZIKV subverts IFN signaling by inhibiting STAT1/2 in humans, resulting in highly activated upstream IFN pathways with limited antiviral effect. In mice, in contrast, IFN signaling effectively inhibits flaviviridae due to differences in STAT. Flavivirus-permissive mouse models have been generated by complete removal of IFN signaling events (IFNAR1/2 knockouts). These highly immunocompromised models fail to recapitulate inflammatory events that cause flaviviral pathology in man. Lack of relevant small animal models for flaviviral host interactions has hampered development of antiviral countermeasures. We performed RNAi screening to identify host factors required for IFN-mediated suppression of hepatitis C virus and DENV and identified 56 HCV/DENV IFN effector genes. Preliminary data reveals that one of these genes, HELZ2, is required for IFN-mediated suppression of ZIKV in cell lines. We are now comparing ZIKV teratogenicity in HELZ2-/- vs WT mice. We have successfully detected ZIKV RNA in liver, spleen, blood of dams, and fetal tissue, of HELZ2 knockout mice, revealing ZIKV susceptibility of this unique mouse model. Comparisons between WT and HELZ2 infected mice using viral PCR, skeletal imaging, and retinal pathology are presented. A major advantage of HELZ2-/- mice, compared to current standard IFNAR-/- mice, is maintenance of first steps of IFN signal transduction, allowing improved resolution of host innate inflammatory contribution to pathology. We anticipate these models will serve as useful tools for improved countermeasure testing.

Poster Number 139

Tian Lin, MD, PhD
Pediatrics, Instructor | tlin7@mgh.harvard.edu

Different bactericidal and inflammatory activities of human and mouse blood

INVESTIGATORS: T. Lin, S. Moorlag, J. Liu, M. Ahmed, S. Thundivalappil, F. Riley, S. Warren

Mouse models have traditionally served as a selective gate for drugs developed for treating patients with inflammation and sepsis. However, studies have shown that mice are several orders of magnitude more resistant than humans to intravenous challenge with LPS, a cell wall component of Gram-negative bacteria. Detection of colony counts of 10^6 live bacteria per milliliter of blood in mice is common in many mouse models of bacterial challenge. In contrast, human adults with Gram-negative sepsis generally either have no bacteremia or bacteremia with less than 100 bacterial colonies per milliliter of blood. This substantial difference between mice and humans during infections is unexplained, unstudied and is usually simply ignored.

We studied and compared the growth of four encapsulated, clinically relevant bacterial strains of E. coli and one strain of Pseudomonas. aeruginosa in fresh human and mouse whole blood. We also compared the phagocytosis of killed bacteria by leukocytes and cytokine production in human and mouse blood after exposure to live bacteria. We found that all strains of E. coli and Pseudomonas studied grew in mouse blood and plasma but were killed in human blood and plasma. More E. coli and Pseudomonas were phagocytosed by leukocytes in human than mouse blood. Furthermore, pro-inflammatory cytokines were produced in human but not mouse blood after live bacterial inoculation. In conclusion, our results suggest that bacterial killing, phagocytosis, and cytokine induction in the blood compartment during human bacteremia with Gram-negative bacteria are probably not very well mimicked in mouse models of bacterial challenge.

Poster Number 140

Alejandro Llanos-Chea, MD
Pediatrics, Clinical Research Fellow | allanos-chea@mgh.harvard.edu

A Novel Enteropathogen Infection Model in Human-Derived Organoid Monolayers

INVESTIGATORS: A. Llanos-Chea, K. P. Nickerson, L. Ingano, S. Senger, A. Fasano, C. S. Faherty

Enteric bacteria such as Shigella and Salmonella cause severe infections to the gastrointestinal (GI) epithelium. A reproducible infection model is critical. Unfortunately, animal models for human-specific pathogens are difficult to develop and do not adequately replicate human infection. We adapted an organoid model from human tissue to study human-specific responses to Shigella and Salmonella infection. Organoids were derived from biopsies of the terminal ileum (TI) and cecum. Crypt stem cells were expanded and differentiated to give rise to a 2-D polarized GI epithelium monolayer. We conducted infection assays for Shigella flexneri and Salmonella enterica serovar Typhi. Cellular markers by quantitative PCR confirmed GI epithelium differentiation. For infection, wild-type S. flexneri strain 2457T efficiently infected the cecum following apical bacterial administration to mimic the natural infection process; with 15% bacterial recoveries for adherence and 0.02% for invasion. When a non-invasive mutant was used for invasion, 5.8-fold less bacteria were recovered relative to 2457T, indicating most bacteria recovered (83%) were intracellular. Similarly, S. Typhi strain Ty2 efficiently invaded the TI, with ~1% recovery of bacteria invading enterocytes apically. For both pathogens, we identified bacteria in the basolateral space and within the enterocytes through IF and TEM analyses. We have developed an efficient and reproducible model; where the bacterial culture conditions coupled with the human-specific model serve as the most accurate human-like environment to study the natural infection processes of Shigella and Salmonella to date. This model will facilitate an improved understanding of host-pathogen interactions and provide new insights for novel therapeutic development.

Poster Number 141

Kourtney Nickerson, PhD
Pediatrics, Research Fellow | kpnickerson@partners.org

Bile salt-mediated adherence factor expression drives Shigella flexneri mucosal attachment and infection

INVESTIGATORS: K. P. Nickerson, R. B. Chanin, A. L. Chea, J. R. Sistrunk, D. A. Rasko, D. V. Kumar, S. K. Dogiparthi, B. J. Kusber, J. Ding, C. S. Faherty

Globally, diarrhea causes one in ten pediatric fatalities, predominantly due to the human-specific bacterial pathogen Shigella flexneri. Antibiotic resistance has accelerated the need to develop effective vaccines, which has classically been limited by an incomplete understanding of Shigella virulence activation in humans. Shigella is exposed to the bactericidal host factor bile during small intestine transit prior to causing infection in the colon. Our group has pioneered work defining the role of bile salts in the induction of adherence factor expression required for attachment to colonic epithelial cells as well as for biofilm formation essential to survive bile exposure. By integrating bile salts into growth media, we identified adherence factor gene expression by RNA-sequencing and RT-PCR analyses. Transmission electron microscopy and gene deletions confirmed the transcriptomic data. Furthermore, gene deletions resulted in diminished epithelial cell binding and reduced early biofilm formation. We are currently utilizing novel human organoid-derived epithelial monolayers as a host-specific model to identify adherence factor binding specificity. Combined with the mutation analyses, substrate-specific binding assays have identified affinity for extracellular matrix structures only for a few factors, suggesting functional differences between the Shigella adherence structures. For the first time, this work demonstrates that Shigella produces adherence factors when grown under in vivo conditions. The kinetics and specifics of adhesin-host interaction are under active investigation and represent lucrative targets for vaccine development. This work expands the infection paradigm for Shigella and underscores the importance of context in microbiological research, particularly for innovative vaccine development strategies.

Poster Number 142

Kathleen Powis, MD, MPH
Medicine, Assistant Professor | kpowis@mgh.harvard.edu

Lower estradiol in HIV-infected pregnant women on dolutegravir-based ART in Botswana

INVESTIGATORS: K. M. Powis, J. Jao, J. Legbedze, N. Mmasa, S. W. Kgole, S. Kala, A. Bhattacharya, T. Mohamed, E. Van Widenfelt, J. Makhema, S. Moyo, C. Petlo, M. Geffner, E. J. Abrams, L. Serghides

Estradiol (E2) is an important pregnancy hormone. Protease inhibitors and efavirenz alter E2 in pregnancy. Limited data exists on dolutegravir (DTG). We evaluated associations between DTG use in pregnancy and maternal E2 levels. The Tshilo Dikotla study is prospectively enrolling HIV+ and HIV- pregnant women ≥18years old in Botswana. All HIV+ women are taking DTG/tenofovir/emtricitabine. Levels of E2 and sex-hormone binding globulin (SHBG) were measured by ELISA in plasma collected between 24-29 weeks gestation and used to calculate bioavailable E2 (bE2). To normalize distribution, bE2 was log-transformed. Linear regression models were fit to assess the association between maternal HIV status/DTG use and bE2.

Plasma from the first 118 pregnant women [47 HIV+] enrolled in the study were analyzed. HIV+ women were older (27 vs 25 years; p= 0.02) and of higher gravidity (3 vs 1; p<0.01). There was no difference in median body mass index between groups (p=0.38). Median time on antiretroviral treatment (ART) at time of plasma collection was 11.7 weeks. Median bE2 was lower in HIV+ vs HIV- [461 pg/mL, Interquartile Range (IQR) 350-778 vs 588 pg/mL, IQR 451-898; p<0.01] (Figure 1). Log bE2 remained lower in HIV+/DTG women after adjusting for maternal age and gravidity (β:-0.10; p=0.03). HIV+ women receiving DTG-based ART in pregnancy had lower bE2 compared with HIV- women. With the potential for expanded global use of DTG in pregnancy, further research and national surveillance data are needed to inform the safety of DTG for pregnant women and their infants.

Poster Number 143

Erica Shenoy, MD, PhD
Medicine, Assistant Professor | eshenoy@partners.org

A generalizable, data-driven approach to predict daily risk of Clostridium difficile infection at two large academic health centers

INVESTIGATORS: J. Oh, M. Makar, C. Fusco, R. McCaffrey, K. Rao, E. E. Ryan, L. Washer, L. R. West, V. B. Young, J. Guttag, D. C. Hooper, E. S. Shenoy, J. Wiens

An estimated 293,300 healthcare-associated cases of Clostridium difficile infection (CDI) occur annually in the US. To date, research has focused on developing risk prediction models for CDI that work well across institutions. However, this one-size-fits-all approach ignores important hospital-specific factors. We focus on a generalizable method for building facility-specific models. We demonstrate the applicability of the approach using electronic health records (EHR) from the University of Michigan Hospitals (UM) and the Massachusetts General Hospital (MGH). We utilized EHR data from 191,014 (UM) and 65,718 (MGH) adult admissions. We extracted patient demographics, admission details, patient history, and daily hospitalization details, resulting in 4,836 (UM) and 1,837 (MGH) features. We used L2-regularized logistic regression to learn the models, and measured the discriminative performance of the models on held-out data from each hospital.

On the UM and MGH test data, the models achieved AUROCs of 0.82 (CI:0.80-0.84) and 0.75 (CI:0.73-0.78), respectively. Some predictive factors were shared between the two models, but many of the top predictive factors differed across facilities. We presented a data-driven approach to building models for estimating daily patient risk for CDI, and used it to build institution-specific models at two large hospitals with different patient populations and EHR systems. In contrast to traditional approaches that focus on developing models that apply across hospitals, our generalizable approach yields risk-stratification models tailored to an institution. Use of these hospital-specific models allows for earlier and more accurate identification of high-risk patients and better targeting of infection prevention strategies.

Poster Number 144

Shariq Usmani, PhD
Medicine, Research Fellow | smusmani@mgh.harvard.edu

HIV-1 Balances the Fitness Costs and Benefits of Disrupting the Host Cell Actin Cytoskeleton Early After Mucosal Transmission

INVESTIGATORS: S. M. Usmani, T. T. Murooka, M. Deruaz, W. H. Koh, R. R. Sharaf, K. A. Power, P. Lopez, R. Hnatiuk, V. D. Vrbanac, A. M. Tager, T. M. Allen, A. D. Luster, T. R. Mempel

Defining distinct host-virus interactions and the processes that facilitate de novo infections during HIV-1 transmission are of paramount importance for vaccine design and preventive strategies. Here, using multiphoton intravital microscopy of humanized mice, we show that HIV-1 impairs migratory behavior of infected cells in vivo. Systematic analysis revealed that HIV specifically employs multifunctional Nef protein for this function and not Vpu or Env. Deleting Nef restored the migratory properties of the infected cells. Furthermore, we found that Nef's effect on cell migration was dependent on a single residue at the C-terminal of Nef-F191. F191 is responsible for Nef-mediated PAK2 activation, which in turn causes dysregulation of actin cytoskeleton in infected cells. Interestingly, Nef-F191 motif is also highly conserved among various transmitted/founder HIV strains suggesting a potentially important role during the early stages of transmission. In order to test if this interaction has any relevance during transmission, we devised an in vivo competitive fitness assay after low-dose intravaginal inoculation to compare the replication of individual viruses by next generation illumine sequencing. We found that while this function significantly slows viral dissemination during initial stages, it subsequently enhances virus's ability to produce high viral loads in tissues and plasma. Thus, our study reveals an important role for Nef protein at early stages of HIV infection and demonstrates how a specific molecular function of this HIV protein, which initially appears to be counterproductive for the virus, in fact is beneficial for its systemic spread and persistence at the later stages of the infection.

Poster Number 145

Shuai An, MD, PhD
Orthopedics, Research Fellow | san2@mgh.harvard.edu

Early Outcomes of Revision Surgery for Head-Neck Taper Corrosion of Metal-on-Polyethylene THA with Pseudotumors

INVESTIGATORS: S. An, P. Arauz, Y. Peng, Y. Kwon

Modularity in total hip arthroplasty (THA) allows surgeons to optimize implant reconstruction to patient anatomy intraoperatively. Recently, adverse local tissue reactions (pseudotumors) due to tribocorrosion of modular head-neck taper junctions in contemporary THA are emerging as an important reason for failure requiring revision surgery. However, the clinical outcomes of revision surgery remain largely unknown. We aim to report early complication rates and outcome of revision surgery for this particular type of patient. Forty-four revision surgeries with metal-on-polyethylene THA were evaluated (averaged time from index surgery to revision: 77 months; minimum follow-up of 12 months). The indication for revision surgery was the presence of symptomatic pseudotumours on MRI with elevated metal ion levels. A high rate of early complications (14%) and re-revisions (7%) was observed after revision surgery. One patient experienced recurrent dislocations, which required a re-revision. A second patient experienced dislocations followed by acetabular component aseptic loosening, both of which required re-revisions. Another patient had a single dislocation. Three cases of superficial infections occurred, all of which were treated using antibiotics. From pre-revision to post-revision, the mean serum levels of cobalt decreased from 7.7µg/L to 3.0µg/L, the mean cobalt/chromium ratio decreased from 5.5 to 1.7, and the mean HHS improved from 54.7 to 71.1. This is currently the largest cohort follow-up on the clinical outcomes of revision due to head-neck taper corrosion in metal-on-polyethylene THA with associated pseudotumours. This information provides clinically useful information for pre-operative counseling of THA patients undergoing revision surgery for head-neck taper corrosion.

Poster Number 146

Hubert Aviolat, PhD
Neurology, Research Fellow | haviolat@mgh.harvard.edu

Exploiting bias in mutant Huntingtin quantification to improve immunoassays and develop the first assay for the quantification of CAG instability at the protein level

INVESTIGATORS: H. Aviolat, H. E. Richey, E. Sapp, M. Iuliano, K. B. Kegel-Gleason, M. DiFiglia

Huntington's disease (HD) is due to a CAG repeat expansion resulting in an extended polyglutamine (polyQ) tract within huntingtin (HTT) protein. Accurate quantification of HTT is an important issue for clinical trials that aim to lower mutant HTT (mHTT) and to track disease progression as mHTT detected in cerebrospinal fluid could be a biomarker. To specifically quantify mHTT, researchers use an antibody (Ab) that targets the expanded polyQ region. Since, the Ab has increasing avidity with increasing polyQ length, there could be bias when quantifying mHTT from a batch of patient samples with different lengths of polyQ tracts or from the same sample that has somatic instability of CAG repeat (progressive length increases over time). In preliminary results using purified truncated recombinant HTT proteins with variable polyQ lengths (Q19/Q25/Q32/Q38/Q44), we showed by Meso Scale immuno-based Detection assay that there is an exponential polyQ length-dependent bias (R2>0.99; HTT signal doubles with only 6 additional glutamine residues). Similar exponential correlation was found when quantifying mHTT from brains of HD KI mice with different CAG repeats lengths in HTT (Q50/Q80/Q111/Q140/Q175; R2>0.98). All together, these data raise the question of what the results of currently used immuno-based methods for measuring mHTT represent? The aim of this project will be to accurately define a polyQ length-dependent correction factor in order to increase accuracy of immuno-based detection assays. Moreover, by exploiting the presence of quantification bias, we plan to develop the first assay to quantify the average size of polyQ tracts in a patient sample.

Poster Number 147

Jun-Seok Bae, PhD
Neurology, Research Fellow | jbae5@mgh.harvard.edu

Alzheimer's mutation within beta-amyloid region [K16N] inhibits alpha-secretase cleavage of APP in CRISPR/Cas9-edited cells

INVESTIGATORS: J. Bae, D. M. Romano, R. E. Tanzi, J. Suh

CRISPR/Cas9-mediated gene editing technology has been proved very powerful in studying pathogenic mechanism and developing novel therapies for many diseases. To investigate the molecular mechanism of a novel mutation found in an early-onset Alzheimer's disease (AD) family, in this study, we introduced the mutation [K16N] in the amyloid precursor protein (APP) into the genome of a cell line by CRISPR/Cas9. To validate the feasibility of this gene editing technology, we also generated cell clones harboring a well-characterized early-onset AD mutation in APP (Swedish). Biochemical analysis of several clones for each mutation revealed APP Swedish mutation at beta-secretase cleavage site significantly increases beta-cleavage of APP and Abeta generation. Instead, homozygous APP K16N mutation, located at exact alpha-secretase cleavage site, decreased both sAPPalpha (>90%) and APP-CTFalpha (~50%), with minimal changes in sAPPbeta level. Variations among different cell clones were rarely notable for the changes in APP processing. Together, these findings suggest that APP K16N mutation inhibits alpha-secretase cleavage of APP, and that CRISPR/Cas9-mediated gene editing may be utilized to uncover the pathogenic mechanisms of both previously known and novel AD mutations in cell-based system.

Poster Number 148

Trevor Balena, PhD
Neurology, Research Fellow | tbalena@mgh.harvard.edu

Protracted post-traumatic neuronal death in the developing hippocampus

INVESTIGATORS: T. Balena, N. Rahmati, Y. Saponjian, K. J. Staley

Delayed neuronal death (DND) is of interest as a means to explain clinical deterioration after acute brain injury. However, mechanisms underlying DND and its relationship to apoptosis remain poorly understood. We evaluated the death of neurons in a chronically epileptic in vitro preparation in which multiphoton microscopy could be performed over a period of several days. Organotypic hippocampal slice cultures were made from wild-type C57BL/6J mice, and imaged with transgenic fluorophores as well as the Na+ dye SBFI-AM. The earliest detectable events in the neurons post-trauma were an increase in caspase activity (as indicated by FLICA positivity), a reduction in the emission of virus-induced and transgenically-expressed fluorescent proteins (TurboRFP and Clomeleon, respectively), and an apparent retraction of all dendrites and axons. Next, neuronal membrane permeability progressively increased over several days and esterifed dyes such as SBFI-AM and Fura-AM permeated the cytosol. Cell membrane permeability to Na+ increased, which was associated with decreased membrane potential and increased cytoplasmic Na+ concentration with minimal change in neuronal volume. Cytoplasmic Ca2+ also increased a few hours before cells became unrecognizable. The terminal event was a sudden reduction in neuronal volume that appeared to be associated with engulfment by microglia. Mitochondrial potentials and Na+/K+ ATPase activity were sustained throughout the process. Membrane permeability was reduced by cyclooxygenase (COX-2) inhibitors and Bax antagonists, but no intervention completely reversed the process. Overall, we describe here a new in vitro model of delayed neuronal cell death in the developing hippocampus.

Poster Number 149

Joseph Biederman, MD
Psychiatry, Professor | jbiedrman@partners.org

Evidence of Poor Patient Engagement in Treatment for ADHD: A 17-year Electronic Medical Records Data Mining Study from a Large Health Care Organization

INVESTIGATORS: J. Biederman, R. Fried, M. Fitzgerald, B. Storch, A. Pulli, K. Y. Woodworth, I. Biederman, T. Spencer, S. Faraone, R. Perlis

ADHD is a prevalent and morbid neurobiological disorder that has been associated with a wide range of adverse outcomes. Data from large datasets documents that stimulants decrease the risks for many adverse outcomes, yet compliance with stimulants remains very poor. The main aim of the present study was to evaluate objective contemporaneous rates and correlates of patient engagement in ADHD treatment. Prescription and demographic data were extracted from the Partners HealthCare Research Patient Data Registry (RPDR) for patients younger than 17 years of age who were prescribed a CNS stimulant between 2006 and 2016. The sample consisted of 2,685 patients, with an average age of 12.4 ± 3.0 years. Seventy-three percent were male, 72% were Caucasian, and 92% spoke English as their primary language. Here we show that patient engagement in stimulant treatment for ADHD is poor. A decade of electronic medical record (EMR) data from the large health care organization shows that only 57% of close to 3,000 patients engaged in treatment. These findings provide compelling evidence for poor rates of patient engagement in stimulant treatments for ADHD and are consistent with findings reported in a recent literature review.

Poster Number 150

Rachel Buckley, PhD
Neurology, Research Fellow | rfbuckley@mgh.harvard.edu

Sex-specific effects on cognitive decline in preclinical Alzheimer's disease: findings from ADNI, AIBL and HABS

INVESTIGATORS: R. F. Buckley, E. C. Mormino, M. J. Properzi, D. M. Rentz, A. P. Schultz, K. A. Johnson, R. E. Amariglio, R. A. Sperling

Population studies of Alzheimer's disease (AD) dementia risk suggest that females exhibit incidence rates than males, even after adjusting for longevity and competing mortality. In addition, meta-analyses report increased risk for progression to AD dementia in females carrying the genetic risk for sporadic AD (from the apolipoprotein e4 (APOE4) allele) relative to male carriers. APOE4 increases risk for cognitive decline and abnormal levels of neocortical amyloid burden. It is unclear, however, where along the Alzheimer's disease biological cascade sex may modify the effect of APOE4 and amyloid to influence cognitive decline. In this study, we assessed cognitive performance in 755 cognitively-normal individuals over 7 years (Age=73 years, 55% female). Information about neocortical amyloid burden was attained via positron emission tomography. We fit mixed-effects models of cognitive change by sex controlling for age, education and baseline cognitive performance with quadratic time effects. We then modeled separately the contributions of sex*APOE and sex*amyloid, and finally, a three-way interaction between sex*APOE4*amyloid. We found that sex alone did not influence cognitive decline, however, females with high amyloid exhibited significantly faster cognitive decline than males. No sex*APOE4 interaction was found. The sex*APOE4*amyloid interaction was significant, suggesting that female APOE4 carriers with high amyloid exhibited the steepest decline in cognition compared with their male counterparts, however, this effect was weak. Our results suggest that females demonstrate greater cognitive decline than males in the setting of high amyloid burden. These results support epidemiological findings, and elucidate a potential underlying mechanism behind female vulnerability to AD dementia.

Poster Number 151

Marc Aurel Busche, MD, PhD
Neurology, Research Fellow | mabusche@mgh.harvard.edu

Amyloid-β:tau interaction silences neural circuits in vivo

INVESTIGATORS: M. A. Busche, S. Wegmann, J. Schiantarelli, S. Dujardin, B. T. Hyman

The presence of amyloid-β (Aβ) plaques and tau neurofibrillary tangles (NFTs) in the neocortex is linked to neural circuit failure and cognitive decline in Alzheimer´s Disease (AD). However, the underlying neuronal mechanisms are unknown. By employing in vivo two-photon calcium imaging in AD mouse models that develop both plaques and NFTs, we reveal that cortical circuit impairment is determined by tau rather than Aβ, and is characterized by a massive silencing of neurons. We show that the presence of soluble, nonfibrillar tau is sufficient for neuronal silencing, and that NFTs are not required. Surprisingly, in the presence of Aβ, suppression of tau was ineffective in restoring normal neuronal functions. Our results provide a cellular explanation for why brain circuits become silent in AD, and cognitive decline is more closely related to tau than to Aβ. We provide experimental evidence that the synergy between tau and Aβcan lead to an unexpected resistance of neuronal circuit impairment to treatment.

Poster Number 152

Alison Cloutier, MS
Neurology, Clinical Research Coordinator | acloutier1@mgh.harvard.edu
Visualization of Stroke Recovery Trajectories After Ischemic Stroke that Caused Upper Extremity Weakness

INVESTIGATORS: A. M. Cloutier, D. J. Lin, L. R. Hochberg

Stroke affects approximately 800,000 people per year in the United States alone and is the leading cause of acquired adult disability. Upper extremity impairment after stroke is the most common cause of this disability. Currently, therapy options for upper extremity motor rehabilitation after stroke are limited. Critical prerequisites to clinical translation of novel therapies and technologies for upper extremity rehabilitation are quantifying the trajectory of recovery after stroke and understanding the impact of arm impairment on function and disability. The aims of this study are to: 1) quantify and understand the trajectory of stroke recovery and 2) understand the impact of arm impairment on function and disability. We have developed a method to quantify and visualize upper extremity motor recovery following ischemic stroke. This method incorporates the different axes of the World Health Organization International Classification of Functioning, Disability, and Health (ICF), specifically structure-function, activity, and participation, which provides a general framework for understanding disability after stroke. Forty-five MGH consecutively enrolled patients with upper extremity weakness after stroke are being assessed using this method starting during the acute inpatient stay, at 6 weeks, 3 months, and 6 months post-stroke. Novel visualizations of stroke recovery using radar plots allow us to provide real-time feedback to patients and potentially to aid therapists in developing personalized neurorehabilitation strategies. These preliminary results provide a framework to understand recovery after stroke and to study how quantification of recovery affects (1) patient-provider engagement and (2) targeted therapies for stroke rehabilitation.

Poster Number 153

Weihua Ding, MD
Anesthesia, Critical Care and Pain Medicine, Research Fellow | wding@mgh.harvard.edu
An improved approach for trigeminal neuropathic pain in rat

INVESTIGATORS: W. Ding, L. Yang, Z. You, S. Shen, J. Doheny, S. Zhu, J. Mao

The number of studies on trigeminal nerve injury using animal models is limited. There are many difficulties in generating animal models of trigeminal nerve injury. Chronic constriction injury of trigeminal nerve in rodent was developed in the 1990s by Vos et al and has been used as the major animal model to study trigeminal neuropathic pain. The surgical procedure for chronic constriction injury of trigeminal nerve (CCI-TN) is more complicated than that for other peripheral nerves (e.g. sciatic nerve). A simplified and improved CCI-TN procedure will facilitate the research on orofacial pain including trigeminal neuralgia. The aim of the present research was to improve on the current surgical procedure to produce chronic constriction injury of trigeminal nerve model in rodents. Study Design: An experimental study. This study took place in the Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Harvard Medical School. Experiments were carried out on male Sprague–Dawley rats. Chronic constriction injury was produced by loose ligation of the distal infraorbitol nerve (dCCI-IoN). The changes in spontaneous and evoked behavior were measured after surgery. We have demonstrated a simple, minimally invasive, and time saving procedure to consistently induce nociceptive behavior in rats after dCCI-IoN.

Poster Number 154

Amanda Dios, PhD
Neurology, Research Fellow | amanda.dios@gmail.com

HDAC levels are not altered in amyotrophic lateral sclerosis

INVESTIGATORS: A. M. Dios, S. Babu, E. J. Granucci, K. A. Mueller, J. D. Berry, N. Atassi, J. Hooker, G. Sadri-Vakili

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons. Currently there are no therapies that can extend lifespan beyond a few months in ALS, demonstrating a clear and urgent need for the development of novel treatments. HDACs are epigenetic erasers that play a critical role in regulating gene expression by catalyzing the removal of acetyl groups from lysine residues on histones, thereby promoting condensation of chromatin and inactivation of transcription. HDACs have been implicated in several neurodegenerative diseases, including ALS. In ALS, histone acetylation is reduced and there is an increase in HDAC2 levels in the ventral horn of the spinal cord in ALS patients. More importantly, a phase II human ALS trial demonstrated that sodium phenylbutyrate, an HDAC inhibitor, significantly increased histone acetyaltion in blood and that the drug was safe and well tolerated. Thus, HDAC inhibitors are considered as a potential neuroprotective treatment for ALS. Our study aimed to further characterize alterations in histone acetylation and HDACs in ALS. Our results indicate that there is no change in HDAC 1, 2, 3, 4, 6 or 8 levels in the motor cortex or the ventral horn of the lumbar spinal cord of patients with ALS compared to controls. In addition, there is no changes in acetylation of histone H3 or H4 in ALS compared to control. Furthermore, these findings are confirmed in vivo using PET imaging with a novel tracer, Martinostat, which selectively binds to HDAC 1-3 and HDAC6.

Poster Number 155

Francisco Flores, PhD
Anesthesia, Critical Care and Pain Medicine, Instructor | fflores@mgh.harvard.edu

Effects of Dexmedetomidine on Local Cortical Networks

INVESTIGATORS: F. J. Flores, S. B. Kodandaramaiah, J. An, E. S. Boyden, C. Forest, E. N. Brown

Understanding the neural correlates of consciousness remains a fundamental task in neuroscience for both clinical and scientific reasons. In particular, changes in the membrane potential of cortical neurons has been associated with different states of consciousness, such as wakefulness, sleep, and general anesthesia. We used automated, in vivo whole-cell recordings to observe the membrane potential of mice's somatosensory neurons, before and after the administration of sedative doses of dexmedetomidine (DEX), a commonly used sedative drug. DEX molecular actions result in blockade of noradrenergic input to cortical neurons, and therefore, we hypothesized that it should produce sustained hyperpolarization, consistent with the role of noradrenaline in the depolarization of the membrane potential. However, we observed that after DEX-induced sedation, the membrane potential exhibited slow fluctuations (one to two per second) between hyperpolarized and depolarized states, very similar to those we observed during quiet wakefulness. Simultaneous in vivo recordings in pairs of neurons revealed that the slow fluctuations were synchronized. These results suggest that DEX effects are more consistent with a decrease in excitation rather than an increase in inhibition, which might explain why DEX effects are similar to natural sleep. Moreover, sustained synchronization of neural activity it is not consistent with conscious states, further adding to DEX unconsciousness-inducing mechanisms.

Poster Number 156

Yi Gong, PhD
Center for Genomic Medicine, Research Fellow | ygong4@mgh.harvard.edu

Optimizing Intrathecal Adeno-Associated Viral Vector-mediated Gene Delivery for Adrenomyeloneuropathy

INVESTIGATORS: Y. Gong, F. Laheji, A. Berenson, G. Gao, R. Kok, S. Kemp, C. Maguire, F. Eichler

Mutations in the gene encoding the peroxisomal ATP binding cassette transporter (ABCD1) cause elevations in very long chain fatty acids (VLCFA) and the neurodegenerative disease adrenoleukodystrophy. In most adults this manifests as the spinal cord axonopathy, adrenomyeloneuropathy (AMN). A challenge in AMN is how to achieve functional gene correction to the entire spinal cord while minimizing leakage into the systemic circulation. Here we show that we can use an osmotic pump to deliver adeno-associated virus (AAV) vector into the lumbar CSF space in mice. We report that recombinant AAV serotype 9 (rAAV9) achieves efficient gene transfer across the spinal cord and dorsal root ganglia. Slow continuous delivery of rAAV9- encoding green fluorescent protein (GFP) over 24 hours via intrathecal osmotic pump delivery led to a 10-fold higher expression of GFP across all segments of the cord compared to other routes of injection including intavenous, intracerebroventricular and intratehcal bolus injection while reducing systematic leakage into peripheral organs. In the Abcd1-/- mouse, gene correction after AAV9-CBA-hABCD1 delivery led to a 30% decrease of VLCFA levels in spinal cord compared to controls. Astrocytes, vascular endothelial cells and neurons were the major cell types transduced in the spinal cord. Importantly, rAAV9 delivered intrathecally by osmotic pump greatly reduced systemic leakage into peripheral organs, particularly liver and heart tissue.

Poster Number 157

Jessica Gracias, MS
Center for Genomic Medicine, Research Technician | jgracias@mgh.harvard.edu

Increased microglial synapse elimination in patient-specific models of Schizophrenia

INVESTIGATORS: J. F. Gracias, C. M. Sellgren, B. Watmuff, C. P. Goold, J. M. Thanos, T. Fu, J. Wang, R. Karmacharya, H. E. Brown, S. D. Sheridan, R. H. Perlis

The primary etiology of schizophrenia remains largely elusive. Studies have shown reductions in synaptic density in postmortem brains from individuals with schizophrenia. One putative mechanism of decreased synaptic density suggested by rodent models is aberrant synapse elimination by microglia during neurodevelopment. We have developed a novel method of deriving microglia-like cells from peripheral blood mononuclear cells. We validated this technique by microglial-specific gene and marker expression using a customized gene expression panel (Nanostring) of known microglial markers as well as global gene array (Affymetrix) and immunocytochemistry. Functional synaptic pruning was also confirmed by co-culture with neural cultures. Using synaptosomes from patient-iPSC derived neural cultures with patient derived microglia-like cells, we developed high throughput methods that enable us to model synaptic pruning in vitro. Using multiple image-based assays we demonstrate that synapse elimination is increased in schizophrenia-derived cellular models. We show that excessive synaptic pruning in schizophrenia reflects independent abnormalities in microglia-like cells as well as in synaptic structures. We also show that schizophrenia risk-associated common variants within complement factor 4 genes partially contribute to this observed increased uptake. Finally, we demonstrate that the antibiotic minocycline reduces synapse uptake in vitro. Pharmacologic interventions targeting synaptic pruning merit investigation for their potential to delay or prevent the onset of schizophrenia in high-risk individuals.

Poster Number 158

Eric Granucci, MS
Neurology, Research Technician | eric.granucci@gmail.com

Increasing Urate Levels Delays Disease Onset in the SOD1G93A Mouse Model of Amyotrophic Lateral Sclerosis

INVESTIGATORS: E. J. Granucci, K. E. Glajch, K. Tsioras, K. A. Mueller, A. M. Dios, Y. Xu, R. Bakshi, X. Chen, J. Pereira, S. Paganoni, M. A. Schwarzchild, E. Kiskinis, G. Sadri-Vakili

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive loss of upper and lower motor neurons resulting in impaired motor function, paralysis, and death by respiratory failure. Although the exact mechanisms involved in the onset and progression of the disease remain unknown, an increase in oxidative stress has been implicated. One of our major endogenous defenses against oxidative stress is urate, an antioxidant and byproduct of purine metabolism. Recent findings have highlighted neuroprotective potential of urate in neurodegenerative diseases such as Parkinson's disease (PD), Huntington's disease, Alzheimer's disease (AD) and ALS. However, little is known about neuroprotective effects of urate in ALS. Here we assessed the neuroprotective effects of increasing urate levels in the SOD1 G93A mouse model of ALS. To increase CNS urate levels, mice with a mutation in the gene (UOx) encoding urate oxidase, enzyme responsible for urate metabolism, were crossed with transgenic (Tg) SOD1 G93A mice to generate Tg SOD1/UOx-/- mice and littermate controls. We assessed body weight, neurological score, motor function, disease onset and progression. Our results demonstrate that elevated urate levels are associated with a twenty-day delay in the onset of hind limb paresis in the Tg SOD1/UOx-/- mutant mice compared to Tg SOD1/UOx+/+ or wild-type littermates. Ongoing studies are assessing the effects of increased urate on motor neuron counts and neuromuscular junction integrity. Together these findings demonstrate that, similar to PD, increasing urate levels may provide therapeutic benefits in ALS and support ongoing translational studies of urate elevation in patients with ALS.

Poster Number 159

Elizabeth Halpin, BA
Anesthesia, Critical Care and Pain Medicine, Research Technician | eshalpin@mgh.harvard.edu

Variable Length Modifiers in Cysteine Substituted GABAA Receptors as Molecular Rulers for Mapping Anesthetic Binding Sites

INVESTIGATORS: E. S. Halpin, A. Nourmahnad, S. A. Forman

Etomidate is a potent general anesthetic that acts by modulating GABAA receptors via its binding sites in $\beta+/\alpha-$ transmembrane inter-subunit clefts. Residues near bound etomidate in $\alpha1\beta3\gamma2L$ GABAA receptors, including β3M286, have been identified using photolabel derivatives of etomidate and substituted cysteine modification-protection (SCAMP) using a large sulfhydryl modifying reagent, p-chloromercuribenzene sulfonate (pCMBS). However, no detailed structural data on etomidate binding to its site is available. We hypothesized that covalently modifying β3M286C with variable-length adducts could more precisely locate etomidate binding sites in structural homology models.

Here we show that the distance between the modifiable sulfhydryl group of β3M286C and bound etomidate is between the length of –S-propyl and –S-butyl adducts. This variable-length SCAMP approach may be applicable at other cysteine-substituted residues near the etomidate site, enabling a more precise localization of the ligand binding site in structural models of GABAA receptors.

Poster Number 160

Reine Ibala, BS
Anesthesia, Critical Care and Pain Medicine, Clinical Research Coordinator | ribala@mgh.harvard.edu

Decreased EEG Alpha/Beta Power During Sevoflurane General Anesthesia is Associated with Preexisting Cognitive Impairment

INVESTIGATORS: R. W. Ibala, E. Y. Hahm, J. A. Gitlin, A. Nozari, E. F. Garcia, M. A. Nebreda, S. Maher, O. Johnson-Akeju

Intraoperative EEG biomarkers of post-operative delirium (POD) are actively being sought. The prominent GABAergic anesthetic EEG (alpha [8-12 Hz] and beta [13-33 Hz]) oscillatory dynamics, which are generated by cortical pyramidal and low threshold spiking interneurons, may reflect the robustness of cortical circuits underlying cognitive processes. We hypothesized that patients with clinically diagnosed cognitive impairment (dementia), as well as "cognitively normal" patients who developed POD, would demonstrate decreased alpha/beta power during general anesthesia. We prospectively collected intraoperative EEG data of orthopedic trauma patients. POD and/or pre-existing cognitive impairment were assessed by a board-certified geriatrician and medical record review. We estimated and compared EEG spectra from 2-min periods of stable sevoflurane maintenance data from 24 patients (n = 5 dementia [n = 4, POD on dementia]; n = 21 cognitively normal [n = 2, POD]). We constructed a binomial regression model with adaptive elastic net penalty to assess associations between dementia and patient characteristics. Dementia was associated with decreased alpha/beta (10-20 Hz) power. POD in cognitively normal patients was also associated with decreased alpha/beta power. Both findings passed our threshold for statistical significance. Our regression analysis demonstrated an association between point estimates for alpha/beta power and dementia [odds ratio, 1.43; 95% confidence interval, 1.14 to 1.79]. Cognitively normal patients who developed POD similarly demonstrate decreased alpha/beta power – but to a lesser extent – compared to patients with dementia. This suggests that POD may be more closely associated with sub-clinical cognitive impairment than previously appreciated.

Poster Number 161

Mohammad Rashedul Islam, PhD
Medicine, Research Fellow | mrislam@mgh.harvard.edu

The exercise hormone FNDC5/ irisin is required for the exercise-induced improvements of spatial learning and memory

INVESTIGATORS: M. R. Islam, M. F. Young, M. P. Jedrychowski, K. K. Gerber, B. J. Caldarone, H. V. Praag, B. M. Spiegelman, L. E. Bettio, B. R. Christie, C. D. Wrann

Exercise can improve cognitive function by enhancing synaptic plasticity and adult hippocampal neurogenesis, yet the underlying molecular mechanisms remain largely unknown. We have previously demonstrated that FNDC5 and its secreted form 'irisin' are induced by exercise and can regulate hippocampal brain derived neurotropic factor (Bdnf) gene expression. However, the physiological role and importance of FNDC5 in the beneficial effects of exercise on the brain have not been examined. Here, we generated mice lacking FNDC5 (global F5KO mice). The F5KO mice failed to show the typical exercise-induced improvements in spatial learning and memory in the Morris water maze. In addition, the development of newborn neurons generated by exercise in the F5KO mice was abnormal. Measurements of long-term potentiation (LTP) in the dentate gyrus and transcriptional profiling suggest a deficit in synaptic plasticity. These results provide strong in vivo genetic support for a role of FNDC5/irisin in the beneficial effects of exercise in the brain and adult hippocampal neurogenesis. Future work will evaluate the therapeutic potential of FNDC5/irisin in neurological disorders.

Poster Number 162

Smita Jagtap, PhD
Psychiatry, Research Fellow | sjagtap@mgh.harvard.edu

Cellular Modeling of CDKL5-Associated Neurodevelopmental Disorders: Isogenic Wild Type & Mutant CDKL5 Allele Expressing Patient-Derived iPSC & Neural Progenitor Lines

INVESTIGATORS: S. Jagtap, J. Lalonde, J. M. Thanos, J. Wang, J. Ruliera, S. D. Sheridan, S. J. Haggarty, R. H. Perlis

Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5/STK9) gene have been associated with atypical X-linked Rett- and West-like Syndromes resulting in severe neurological disorders characterized by intellectual disability, infantile spasms and seizures occurring predominantly in females. Although mutations in other genes (MECP2, FOXG1, ARX) have been identified in some of these syndromes, less is understood about the role of CDKL5 itself. Modeling CDKL5-related disorders in animals has given insights into the function of CDKL5 in neurodevelopment, but such models may have limitations in investigating human-specific phenotypes and drug responsiveness. We have therefore generated induced pluripotent stem (iPS) cells from a female CDLK5 patient fibroblast line and took advantage of stable X-chromosome inactivation to generate clonal isogenic pairs expressing either the wild type or mutant CDKL5. With these isogenic iPSC clones we have derived stable, expandable neural progenitor cells (NPCs) and differentiated them toward defined neuronal subtypes. Comparisons within the isogenic pairs will be useful to reduce the phenotypic variation due to diverse genetic backgrounds in across-individual comparisons. The NPCs have allowed development of a phenotypic cell-based metabolic potential assay examining mitochondrial abnormalities, amenable to identification of compounds rescuing these phenotypes. We are seeking to characterize additional neurobiological, transcriptional and morphological phenotypes specific to CDKL5 mutations in order to facilitate identification of therapeutic targets for high-throughput functional genomic and pharmacological screens.

Poster Number 163

Marina Kovalenko
Center for Genomic Medicine, Research Technician | mkovalenko@mgh.harvard.edu

Mlh1 modifies Huntington's disease pathogenesis via its impact on somatic CAG repeat expansion

INVESTIGATORS: M. Kovalenko, R. Mouro Pinto, M. Andrew, J. Giordano, V. Wheeler

Recent genome-wide association studies have identified mismatch repair gene, MLH1, as a modifier of onset of Huntington's disease (HD). Understanding the mechanism(s) by which MLH1 modifies disease is critical for directing therapeutic strategies. In HD knock-in mice we previously identified Mlh1 as an enhancer of both somatic CAG repeat expansion and the disease process marked by the nuclear accumulation of mutant huntingtin. These observations lead to the hypothesis that Mlh1 modifies disease via its impact on somatic repeat expansion, but do not rule out the possibility that Mlh1 modifies disease via a separate mechanism, independent of its role in promoting CAG repeat expansion. To rigorously distinguish these possibilities, we crossed Mlh1 knockout mice with HD knock-in mice harboring either pure, somatically unstable CAG repeats or mice harboring CAA-interrupted CAG repeats that are somatically stable. On a Mlh1+/+ background, nuclear huntingtin accumulation was significantly reduced in the interrupted repeat mice relative to the pure repeat mice, consistent with a role of somatic expansion in accelerating this phenotype. In pure repeat mice, Mlh1 knockout also significantly reduced nuclear huntingtin, with an impact similar to that achieved by stabilizing the repeat. In strong contrast, Mlh1 knockout had no impact on nuclear huntingtin accumulation in the interrupted repeat mice. These results indicate that MLH1 likely modifies HD pathogenesis in humans by altering the rate of somatic CAG expansion, supporting targeting somatic expansion as a viable therapeutic strategy. Further, our mouse models provide a novel platform for dissecting disease mechanisms of other human modifier genes.

Poster Number 164

Baoqiang Li, PhD
Radiology, Research Fellow | baoqiang.li@mgh.harvard.edu

Cortical depth specific capillary blood flow homogenization facilitates resting state brain oxygen delivery

INVESTIGATORS: B. Li, I. Sencan, T. Esipova, K. Kilic, J. Porter, M. Moeini, M. Yaseen, B. Fu, S. Kura, F. Lesage, S. Vinogradov, A. Devor, D. Boas, S. Sakadzic

Cortical capillary blood flow and oxygenation are highly heterogeneous. Mapping the absolute capillary blood flow and oxygenation along the capillary path is a key step towards understanding how oxygen is transported and delivered in a complex microvascular network to enable adequate tissue oxygenation. In this work, we applied two-photon microscopic imaging of intravascular oxygen partial pressure (PO2) to measure both oxygen concentration and red blood cell (RBC) flux in cortical arterioles, capillaries, and venules. The PO2 measurements with high signal-to-noise ratio were enabled by a novel oxygen-sensitive phosphorescence probe, PtG-2P. Imaging was performed in awake, head-restrained C57BL/6 mice (n=15), through a chronic sealed cranial window centered over the E1 whisker barrel.

We obtained a detailed mapping of the resting state cortical microvascular PO2 in all arterioles and venules, and both PO2 and RBC flux in most capillaries down to 600 μm depth from the cortical surface (n=6,544 capillaries across all mice). Capillary RBC speed and density were also extracted and all measurements were co-registered with the microvascular angiograms. We characterized the distributions of capillary PO2 and flow as a function of branching order and cortical depth. The results show strong positive correlation between oxygenation and flow in the capillary segments, with an increased correlation in downstream capillaries. We have also observed homogenization of both oxygenation and flow in deeper cortical layers, which may imply a mechanism to improve oxygen delivery without increasing global blood flow in the area with increased metabolism.

Poster Number 165

Bangyan Liu, MS
Center for Genomic Medicine, Research Technician | bliu18@mgh.harvard.edu

Identifying Therapeutic Leads for Parkinson's Disease Using a Gene-Expression Based Screen

INVESTIGATORS: B. Liu, N. Nivanthika, R. Karmacharya

There is significant variability in efficacy of current treatments for Parkinson's disease (PD) and many patients do not have a robust response to current medications. Here we report the establishment of a novel gene-expression-based in silico screen to identify small molecules with therapeutic potentials for PD. We curated a gene-expression profile for PD and sought to pinpoint small molecules that have gene-expression profiles anti-correlated with the profile for PD. We identified GW8510, a cyclin-dependent kinase 2/5 (CDK 2/5) inhibitor, as our top hit in the in silico screen. We validated the in silico result by demonstrating a neuroprotective effect of GW8510 against 1-methyl-4-phenylpyridinium (MPP+), a commonly used neurotoxin for PD modeling, in rodent MN9D cells and in human neural progenitor cells (NPCs). Considering the selective vulnerability of dopaminergic neurons in PD, we are currently testing the effectiveness of GW8510 in human dopaminergic neurons differentiated from induced pluripotent stem cell (iPSC). At the same time, we are investigating the hypothesis that the neuroprotective effect of GW8510 is mediated through attenuating the over-phosphorylation of DRP1 by CDK5. Overall, we illustrated the gene-expression based small-molecule screening as a comprehensive approach to identify compounds with therapeutic potential in neurodegenerative diseases.

Poster Number 166

Ricardo Mouro Pinto, PhD, MSc
Center for Genomic Medicine, Instructor | rmouropinto@mgh.harvard.edu

Identification of genetic modifiers of somatic CAG instability in Huntington's disease by in vivo CRISPR/Cas9 genome editing

INVESTIGATORS: A. Azevedo, R. Murtha, M. Kovalenko, M. Andrew, F. Zhang, V. Wheeler, R. Mouro Pinto

Huntington's disease (HD) is a dominant neurodegenerative disorder caused by a CAG repeat expansion within the huntingtin gene (HTT). Somatic CAG expansions are inversely correlated with patient age of onset and require components of the DNA mismatch repair (MMR) machinery. Notably, DNA repair genes were identified as modifiers of HD age at motor onset in a genome-wide association study (GWAS). Together, these data indicate that somatic CAG expansion is a critical disease driver and that therapeutic targeting of this process will slow the disease course.

Here, with the goal of dissecting underlying mechanisms we report the development of a CRISPR/Cas9-based platform to uncover novel somatic CAG instability modifiers in a HD mouse model. To this end, we generated HD knock-in mice that endogenously express Cas9, and validated sgRNAs targeting known modifier genes that either enhance or suppress somatic CAG expansion based on genetic knockout studies. Following a single tail vein injection of AAV8 or PHP.B-based viruses carrying sgRNAs, we confirmed strong brain and liver transduction using an mCherry reporter, detected a high frequency of frameshift mutations (>70%) at target sites, and successfully suppressed or hastened somatic CAG expansions. In summary, we have successfully developed an in vivo CRISPR/Cas9-based platform that allows for efficient knockout of genes of interest in the brain and liver of adult HD mice. We will next use this tool to test GWAS candidates as potential modifiers of CAG instability, as well as to probe more broadly the role of other candidate genes in this disease-relevant process.

Poster Number 167

Kaly Mueller, MS
Neurology, Research Technician | kmueller3@mgh.harvard.edu

Alterations in Hippo/YAP signaling as a pathogenic mechanism in Amyotrophic Lateral Sclerosis

INVESTIGATORS: K. A. Mueller, E. J. Granucci, J. D. Pereira, A. M. Dios, B. J. Wainger, J. D. Berry, K. Vakili, G. Sadri-Vakili

Understanding the underlying pathogenic mechanisms of Amyotrophic Lateral Sclerosis (ALS) is critical for the development of new therapies. One such candidate is the Hippo signaling pathway, which exerts its activity through the transcriptional activator Yes-Associated Protein (YAP), and is involved in neuronal survival, cellular proliferation, and organ size regulation. Increases in the activity of an upstream regulator of YAP, MST1/2, leads to neuronal death and has been linked to neurodegenerative disorders. In contrast YAP expression and nuclear activity is required for neuronal survival. Previous studies demonstrated that MST1 activity was increased in pre-symptomatic transgenic (Tg) SOD1G93A mouse model; and knockdown of MST1 in the Tg mice increased motor neuron counts, delayed disease onset, and extended survival. Here, we sought to determine whether alterations in Hippo signaling contribute to motor neuron death in ALS. Immunofluorescence and western blots were used to evaluate protein expression and localization in the SOD1G93A mice and post-mortem motor cortex from ALS patients. Our results demonstrated that there is a significant decrease in neuronal nuclear YAP in the motor cortex of ALS patients. In addition, there was a concomitant decrease in YAP target gene expression in the motor cortex of ALS patients compared to healthy controls. Lastly, there was a significant increase in phosphorylated MST1/2 (active form) in the lumbar spinal cord of Tg SOD1G93A mice. Taken together, these findings demonstrate that Hippo signaling is altered in ALS and could provide mechanistic insight into the causes of motor neuron death in ALS.

Poster Number 168

Sandrine Muller, PhD
Surgery, Research Fellow | smuller1@mgh.harvard.edu
Cerebral cortex transcriptome-based network guide discovery of neuronal protein interactions

INVESTIGATORS: S. Muller, A. Kim, T. Li, C. L. Hartl, A. Battle, D. H. Geschwind, K. Lage

To understand the brain, neuroscientists can investigate the molecular basis of cellular signaling using protein-protein interactions (PPIs). However, known PPIs are generally not specific to a tissue or cell type. In recent years, large amounts of tissue-specific gene expression have been acquired, giving more insights into the tissue-specific transcriptomic machinery. Therefore, it should be possible to identify brain-specific protein interactions from gene expression. We hypothesize that tissue-specific transcriptome networks can guide biologists on PPIs specific to a tissue or cell type. Here, we propose a Bayesian algorithm suited for small sample size datasets, modelling the direct biological and biochemical gene relationships as a transcriptome network. As a validation of our method, we compare the inferred interactors from the cerebral cortex transcriptome to the interactive partners derived from CACNA1C pull-down experiments in NGN2 induced neurons. All CACNA1C inferred interactors expressed in our neuronal cell model are genes coding for proteins significantly interacting with CACNA1C in at least one time dependent pull down experiments ($p<0.1$ corr.). Globally, the cerebral cortex-specific transcriptome is significantly enriched for protein expression in cerebral cortex (AUC = 0.69; Protein Atlas) and neuronal cells specificity ($p<0.05$ corr.; GO enrichment for cell-specific compartments). Our cerebral cortex-specific network underlies neuronal specific biological mechanisms and will guide follow-up experiments in our neuronal cell models to better understand neuronal pathways involved in schizophrenia.

Poster Number 169

Eva Rocha, MD
Neurology, Research Fellow | eandraderocha@mgh.harvard.edu
Reduced Infarct Growth with IV Heparin in Acute Ischemic Stroke

INVESTIGATORS: E. A. Rocha, R. Ji, Z. Li, H. Ay, G. S. Silva, G. Sorensen, O. Wu, A. B. Singhal

Background: The role of IV heparin in acute ischemic stroke (AIS) is controversial. In this study, we investigated the effect of IV heparin on ischemic lesion growth.

Methods: We analyzed data on 274 consecutive AIS patients with non-lacunar stroke prospectively enrolled in a study where diffusion/perfusion MRI (DWI-PWI) was completed <12 hrs after last seen well and a follow-up MRI/CT completed after day 4. We excluded patients treated with tPA, and those with MTT-DWI mismatch <20% of the DWI volume or absolute mismatch volume <10 mL. Lesion growth was assessed by (a) Absolute Lesion Growth, i.e. final infarct volume – admission DWI lesion volume, and (b) Percentage mismatch lost (PML), i.e. (final infarct volume – admission DWI volume)/(mismatch volume) x100%. Image analysis was blinded to clinical data. Univariable and multivariable analysis were performed to determine the effects of IV heparin on infarct growth.

Results: N=113 met I/E criteria; 52 received IV heparin. Heparin use was associated with smaller PML ($p<0.05$); there was approximately 5-fold difference in PML between heparin users and non-users. Absolute lesion growth was significantly associated with admission glucose, blood pressure, NIHSS score, DWI volume and stroke etiological subtypes; and there was a trend for association with age and heparin use. Intravenous heparin use was an independent predictor of both PML and absolute lesion growth, and was associated with better 3-month outcomes (modified Rankin scale score 0-2, 80% vs. 57%, $p=0.04$). Conclusion: These data suggest that IV heparin may attenuate the progression of acute brain infarcts.

Poster Number 170

Aneesh Singhal, MD
Neurology, Associate Professor | asinghal@partners.org

Ischemic Stroke with Isolated Upper Limb Weakness: Mechanisms and Outcome

INVESTIGATORS: M. A. Topcuoglu, E. A. Rocha, A. K. Siddiqui, B. B. Mills, G. S. Silva, L. H. Schwamm, G. M. Lamuraglia, A. B. Singhal

Isolated Upper Extremity (iUE) weakness is an uncommon stroke syndrome. Its mechanism and outcome have not been adequately studied. In our Get with the Guidelines-Stroke dataset (n=7643), 87 (8%) patients had isolated UE symptoms and signs, received acute diffusion-weighted MRI, and underwent an adequate stroke work-up with satisfactory arterial imaging. We analyzed their clinical-imaging features, management, and outcome. The mean age was 66 years; 58 men and 29 women; 72% had hypertension, 22% diabetes, 53% hyperlipidemia, and 16% were smokers. 71 patients (82%; Group A) had small infarcts involving the motor cortex (21% single and 61% multiple small infarcts in or around the hand-knob area) and 16 patients (18%, Group B) had small infarcts outside the motor strip including 9 with bi-hemispheric infarcts. The ICA was abnormal (luminal stenosis, or plaque ulceration/thrombus) in 60 (84.5%) patients in Group A, vs. 8 (50%) patients in Group B ($p=0.006$). Stroke etiology (TOAST) was significantly different ($p<0.001$) with Group A having over twice as many patients with large-artery and cryptogenic (including patients with complex plaque or <50% ICA stenosis), and Group B had more cardio-embolic etiology. Recurrence rate was 11.5% (9.3% in same territory) over 1515 patient-days follow up. The syndrome of acute iUE weakness and contralateral hand-knob area infarcts is predominantly associated with mild carotid stenosis and plaque ulceration; this clinical-imaging syndrome may be a major manifestation of vulnerable carotid plaque. iUE weakness can also result from infarcts located outside the motor strip, with more variable mechanisms.

Poster Number 171

Caroline Smith, PhD
Pediatrics, Research Fellow | csmith97@mgh.harvard.edu

Combined exposure to air pollution and maternal stress induces sex-specific, autism-like social behavior deficits in mice

INVESTIGATORS: C. J. Smith, P. K. Tran, K. E. Malacon, S. D. Bilbo

Autism spectrum disorder (ASD) is characterized by impaired social interaction and communication, engagement in repetitive behaviors, and a sex bias in prevalence (higher in males). Air pollution poses a significant threat to public health world-wide. Importantly, recent epidemiological studies suggest that maternal exposure to air pollution during pregnancy/early postnatal development may increase ASD risk. Furthermore, studies have shown that maternal stress during pregnancy increases the severity of ASD symptoms. Our lab has developed a novel mouse model of combined diesel exhaust particle (DEP) and maternal stress (MS) exposure. We hypothesized that this DEP/MS exposure would induce ASD-like behavioral deficits in male offspring only. To test this hypothesis, we assessed the behavior of DEP/MS-exposed or control-exposed mice on a variety of behavioral assays during the adolescent period. In males only, we observed a significantly lower preference for social vs. non-social stimuli in DEP/MS-exposed mice as compared to controls. Furthermore, while control males preferred to interact with a novel peer over their cage mate, DEP/MS-exposed males showed the opposite preference (cage mate over a novel peer). DEP/MS exposure had no effect on marble burying behavior (a measure of repetitive behavior) or on anxiety-like behavior (as measured in an open field) in either sex. We are currently using this model to investigate the neuro-immune mechanisms by which DEP/MS exposure induces sex-specific deficits in social behavior.

Poster Number 172

Yamuna Devi Subburaj, PhD
Neurology, Research Fellow | ysubburaj@mgh.harvard.edu

Gene Expression Analysis Reveals How ABCD1 Alters Tight Junction, Cell Cycle and Extracellular Matrix Function in Human Brain Endothelium

INVESTIGATORS: Y. D. Subburaj, N. Sasidharan, A. C. Berenson, Y. Gong, F. S. Eichler, P. L. Musolino

X-linked adrenoleukodystrophy (ALD) is a debilitating neurological disorder caused by mutations in the peroxisomal half transporter, ABCD1. Blood brain barrier (BBB) disruption with migration of leukocytes to the brain, as indicated by histopathology has for a long time been implicated in the cerebral inflammatory form of the disease. To assess how ABCD1 alters brain endothelial barrier function we performed a gene expression analysis by direct RNA sequencing in human brain microvascular endothelial cells(HBMECs). Bioinformatic analyses identified upregulation of 1527 and downregulation of 1706 genes upon ABCD1 silencing. Preliminary pathway analysis on these data showed that TGF-β pathway is upregualted while NF-κβ, cell cycle and extracellular matrix pathways are significantly downregulated in HBMECs lacking ABCD1. These molecular changes also correspond with our experimental findings demonstrating that lack of ABCD1 in brain microvascular endothelium increases their permeability to monocytes and alters angiogenesis by upregulation of the TGFBeta1 pathway. Our analysis provides novel insights on molecular mechanisms and potential therapeutic targets for cerebral ALD.

Poster Number 173

Ricardos Tabet, PhD
Neurology, Research Fellow | rtabet@mgh.harvard.edu

ALS/FTD C9ORF72 transcripts initiate translation at CUG codon

INVESTIGATORS: R. Tabet, L. Schaeffer, F. Freyermuth, M. Jambeau, C. Lee, M. Workman, C. Lin, K. Jansen-West, J. Jiang, H. Abou-Hamdan, L. Desaubry, T. Gendron, L. Petrucelli, F. Martin, C. Lagier-Tourenne

Expansion of G4C2 repeats in the C9ORF72 gene is the most prevalent inherited form of amyotrophic lateral sclerosis and frontotemporal dementia. Bidirectional transcription of the C9ORF72 locus results in the production of transcripts containing either G4C2 or C4G2 repeats, that undergo repeat-associated non-AUG (RAN) translation to produce toxic dipeptide repeat (DPR) proteins from all reading frames. RAN translation occurs in absence of an AUG start codon, in multiple reading frames of the same repeat-containing transcript, and within coding as well as non-coding regions. To determine cis and trans-factors influencing RAN translation of the human C9ORF72 expansion transcripts, G4C2 RAN translation was assessed in rabbit reticulocyte system and mammalian cell lines, from reporters harboring 66 repeats and different 5' flanking sequences. DPR products were probed with specific antibodies, radiolabeled with [35S]-methionine and visualized by immunofluorescence. Similar to a canonical mechanism of translation, the production of DPR proteins from expanded transcripts requires a 5'cap insertion, involves the initiator methionine and strongly relies on sequences upstream of the repeat. G4C2 RAN translation proceeds by a 5'-3' canonical scanning mechanism to start translation at a near-cognate CUG codon and produces DPR proteins by frameshifting. Consistent with this mechanism, we also demonstrate that G4C2 RAN translation is downregulated by an upstream open reading frame present in abnormally spliced C9ORF72 transcripts. Inhibitors of the pre-initiation ribosomal complex and RNA antisense oligonucleotides targeting the sequence upstream of the repeats inhibit G4C2 RAN translation, confirming a scanning dependent mechanism that may be targeted for therapeutic intervention.

Poster Number 174

Sean Tobyne, MA
Neurology, Clinical Research Coordinator | stobyne@partners.org

Focal thalamic atrophy and clinical disability in progressive multiple sclerosis subtypes

INVESTIGATORS: S. M. Tobyne, A. W. Russo, R. J. Fox, E. C. Klawiter

The thalamus is a connection-dense deep gray matter structure with predilection for atrophy in multiple sclerosis (MS). NeuroNEXT 102/SPRINT-MS is a phase II trial of ibudilast in primary and secondary progressive MS (PPMS & SPMS). Baseline anatomical and diffusion magnetic resonance images from 244 participants (129 PPMS, 115 SPMS, mean age 55.5, mean disease duration 16.4 years, median EDSS 6.0) from the SPRINT-MS clinical trial were processed and analyzed to produce automated segmentations of the thalami. Thalamic volumes were compared between disease subgroups while controlling for age, gender and intracranial volume. 3D thalamic reconstructions were used to perform vertex-wise group comparisons to identify differentially affected regions of the thalamus. The relationships between thalamic atrophy and aggregate clinical and neuropsychological measures were separately analyzed. We show that thalamic volume is decreased in SPMS compared to PPMS (normalized volumes: 0.0069 vs 0.0066, un-normalized volumes: 11269 vs. 10382 mm3, $F = 19.8$, $p < 0.0001$). 3D modelling indicated that atrophy was most pronounced in the posterior poles of the thalami in SPMS compared to PPMS. There was a significant moderate, positive partial correlation between thalamic volume and clinical disability, ($r = 0.166$, $N = 244$, $p = 0.01$) and a weaker, positive partial correlation between thalamic volume and neuropsychological test performance ($r = 0.126$, $p = 0.05$). These results suggest mechanistic differences in atrophy progression between the two disease subgroups, with potential relevance to clinical disability in progressive MS.

Poster Number 175

Susanne van Veluw, PhD
Neurology, Research Fellow | svanveluw@mgh.harvard.edu

Histopathological correlates of diffusion imaging abnormalities in cerebral amyloid angiopathy

INVESTIGATORS: S. J. Van Veluw, Y. D. Reijmer, A. J. Van der Kouwe, A. Charidimou, G. A. Riley, A. Leemans, B. J. Bacskai, M. P. Frosch, A. Viswanathan, S. M. Greenberg

We explored the underlying histopathology of white matter diffusion tensor imaging (DTI) measures with ex vivo MRI in intact brains of patients with cerebral amyloid angiopathy (CAA). Formalin-fixed hemispheres from nine CAA cases (mean age at death 70 years (range: 64-81); seven males two females) and two elderly control cases (a 95-year-old female and a 90-year-old male) were scanned at 3T MRI, including a 1x1x1 mm3 (with 44 directions) DTI sequence. The anterior thalamic radiation (ATR) and inferior longitudinal fasciculus (ILF) were reconstructed using deterministic fiber tractography. Fractional anisotropy (FA) and mean diffusivity (MD) were calculated per tract. Three samples per tract were taken for histopathological analysis. FA was reduced and MD was increased in CAA cases compared to controls, in both the ATR and ILF. FA and MD were significantly correlated with tissue rarefaction, myelin density, and white matter microinfarcts. Additionally, FA was correlated with axonal density. FA and MD did not correlate with oligodendrocyte count, astrocyte count, or gliosis. Finally, cases with moderate-to-severe CAA in the frontal cortex had increased MD in the frontal white matter compared to cases with no-or-mild CAA in the frontal cortex. Taken together, we confirmed previous observations of in vivo DTI measures in CAA patients with ex vivo DTI. Moreover, we found that DTI measures are correlated with markers of white matter tissue integrity, myelin and axonal density, but not with markers of inflammation or oligodendrocytes. Our findings have important implications for the interpretation of DTI in the context of small vessel disease.

Poster Number 176

Alik Widge, MD, PhD
Psychiatry, Assistant Professor | awidge@partners.org

Multi-site, Precisely Timed Electrical Stimulation Re-wires Networks of Mental Illness

INVESTIGATORS: A. S. Widge, M. Lo, E. Blackwood, M. R. Milad

Mental disorders, the single largest cause of disability world-wide, are circuit disorders. They arise from dysfunctional information transfer between brain regions. That transfer depends on two electrical properties of brain circuits: synchrony (coordinated low-frequency electrical rhythms) and synaptic connectivity. The challenge is that there is no way to specifically change either property within a defined brain circuit. Electrical brain stimulation, a known-safe treatment paradigm, might be a path to that circuit change. Existing stimulators, however, have inconsistent effects on circuit properties. Here we show that synchrony and connectivity can be changed through brain stimulation, but only through specific, carefully-designed stimulation sequences. We first developed an "open loop" paradigm, where electrical pulses applied to two brain areas with precise inter-pulse timing bring them into synchrony. That brief synchrony led to long-term connectivity changes, lasting for hours from only 30 minutes of stimulation. We then advanced this to a "closed loop" paradigm, where we stimulated a "downstream" brain area in tune with activity in an "upstream" region. The two regions became strongly entrained (Z-score of 30 over their baseline synchrony), and that entrainment again lasted for hours post-stimulation. We have demonstrated both techniques at the electrophysiologic level in rodents, in a prefrontal-cortex-to-amygdala circuit known to be crucial for emotional self-regulation. We are now gathering evidence that these connectivity changes shape animals' fear behavior. These circuit-oriented stimulation methods hold great promise for treating mood, anxiety, and trauma disorders.

Poster Number 177

Limin Wu, MD, PhD
Pediatrics, Research Fellow | lwu11@mgh.harvard.edu

Evidence for the importance of the RIPK3 scaffold function in the pathogenesis of cell death, inflammation, and functional outcome after cerebral contusion

INVESTIGATORS: L. Wu, S. Lule, J. Chung, W. Edmiston, M. Whalen

Introduction: RIPK1/ RIPK3 mediate necroptosis, apoptosis, and inflammation depending on cell type and injury context. Although the kinase domain of RIPK1/3 is required for programmed necrosis, kinase independent scaffold functions of RIPKs can initiate apoptosis and inflammation. As master regulators of these pathophysiological processes, RIPKs are compelling therapeutic candidates to inhibit secondary injury after acute brain injury.
Methods: WT and RIPK1 and RIPK3 kinase dead mice, as well as RIPK3 KO mice were subjected to CCI. HMGB1 release was used to assess necrosis/inflammation, IL-1β ELISA was used on brain and cerebrospinal fluid samples, immunoprecipitation and Western blot were used to assess RIPK1-RIPK3-MLKL interaction. Microglia and brain macrophages were isolated by FACS, Nanostring technology was used to assess microglial transcription in RIPK3 KO and WT mice, and behavioral tests were used to assess functional outcome.
Results: RIPK3 and MLKL were induced after CCI. RIPK1-RIPK3-MLKL interaction was observed at 3 h but not at later time points. HMGB1 release was significantly inhibited in RIPK3 KO compared to all other groups. RIPK1 kinase dead mice had reduced wire grip deficits, whereas RIPK3 KO mice had reduced Morris water maze deficits, wire grip deficits, and NORT deficits vs. wild type and RIPK1 kinase dead. RIPK3 KO mice had undetectable CSF IL-1β but similar transcription profiles as WT in microglia at 48 h. Conclusions: RIPK3 scaffold function appears to be the predominant mechanism of secondary injury. Further studies are needed to elucidate druggable targets associated with RIPK3 scaffold function to improve outcome after contusion TBI.

Poster Number 178

Andrea Edlow, MD
Obstetrics and Gynecology, Assistant Professor | aedlow@partners.org

Sex-specific effects of maternal obesity on offspring hippocampal learning and memory

INVESTIGATORS: R. M. Glass, A. G. Edlow

1 of 3 reproductive-age women in the United States is obese at conception. Maternal obesity (MATOB) is associated with adverse neurodevelopmental outcomes in children, yet the underlying mechanisms remain unclear. There is evidence that offspring sex may play a role in neurodevelopmental outcome. We sought to explore the effect of MATOB on hippocampal-dependent learning tasks in male and female offspring. Female C57BL/6 mice were fed a 60% high-fat diet (HFD) or a 10% fat control diet (CD) for 12 weeks prior to mating. During pregnancy/lactation, obese dams continued on the HFD, or transitioned to CD. Lean dams remained on CD. Offspring were weaned at 3 weeks and placed on a CD. Hippocampal-dependent learning of male and female pups was evaluated with fear conditioning at 4 weeks (juvenile) and Morris Water Maze at 11 weeks (adult). Microglial (Iba-1) staining of the embryonic day 17.5 (e17.5) hippocampus is in process. Here we show that both male and female offspring exposed to MATOB in utero displayed hippocampal learning deficits in juvenile life (reduced freezing in fear conditioning, $p<0.0001$). These deficits persisted into adult life in male offspring only (increased total latency to platform $p=0.02$, increased total distance traveled $p=.01$, and decreased time spent in target quadrant $p=0.008$ in the Morris Water Maze). Preliminary Iba-1 staining showed increased microglial activation at e17.5 in the hippocampus compared to the cortex in all 3 diet groups; quantification of this activation by sex and MATOB status is underway.

Poster Number 179

Malavika Prabhu, MD
Obstetrics and Gynecology, Clinical Research Fellow | mprabhu@partners.org

Liposomal bupivacaine block at the time of cesarean delivery to decrease post-operative pain: A randomized controlled trial

INVESTIGATORS: M. Prabhu, M. A. Clapp, E. McQuaid-Hanson, S. Ona, T. O'Donnell, K. James, B. T. Bateman, B. J. Wylie, W. H. Barth, Jr.

The objective is to evaluate whether liposomal bupivacaine incisional block decreases postoperative pain and represents an opioid-sparing strategy after scheduled cesarean delivery. In a single-blinded, randomized controlled trial among opioid naïve women undergoing cesarean delivery, liposomal bupivacaine or placebo was infiltrated into the fascia and skin prior to fascial closure. Using an 11-point numerical rating scale, the primary outcome was pain score with movement at 48 hours postoperatively. Pain scores and opioid consumption were not normally distributed and were summarized as medians (interquartile range, IQR) and compared using the Wilcoxon rank sum test. Between March 2017 and September 2017, 249 women were screened, 103 women enrolled, and 80 women presented for scheduled cesarean delivery and were randomized. One woman in the liposomal bupivacaine group was excluded after randomization due to a vertical skin incision, leaving 39 patients in the liposomal bupivacaine group and 40 in the placebo group. Baseline characteristics between groups were similar. The median (IQR) pain score with movement at 48 hours postoperatively was 4 (2-5) in the liposomal bupivacaine group and 3.5 (2-5.5) in the placebo group, $p=0.88$. The median (IQR) opioid use was 37.5 (7.5-60) oral morphine milligram equivalents (MME) in the liposomal bupivacaine group and 37.5 (15-75) MME in the placebo group in the first 48 hours postoperatively, $p=0.44$. Compared with placebo, liposomal bupivacaine incisional block at the time of cesarean delivery resulted in similar postoperative pain scores and opioid consumption in the first 48 hours postoperatively.

Poster Number 180

Ishita Basu, PhD
Psychiatry, Instructor | ibasu@mgh.harvard.edu

Deep brain stimulation in the striatum improves cognitive flexibility by modulating High Gamma power in the dorsal anterior cingulate of human subjects

INVESTIGATORS: I. Basu, A. Yousefi, A. C. Paulk, K. Farnes, B. Crocker, U. T. Eden, D. D. Dougherty, S. S. Cash, E. N. Eskandar, A. S. Widge

Deep brain stimulation (DBS) is used to treat drug resistant pathologies such as movement disorders and epilepsy and is being trialed for managing psychiatric disorders. Impaired cognitive flexibility and top-down control are found across anxiety and mood disorders. A DBS paradigm that improves cognitive flexibility is expected to relieve some of the symptoms associated with such disorders. Cognitive Flexibility is the ability to rapidly shift one's attention and behavioral strategy in response to changes in the environment which can be assessed with a Multi-Source Interference Task (MSIT). We had 6 patients undergoing invasive monitoring for epilepsy surgery, perform the MSIT over several blocks of trials. Short trains of dorsal and ventral striatal stimulation at 130 Hz, 6 mA were delivered during some of these trials. We found a significant effect ($p<0.01$) of dorsal striatal stimulation in improving cognitive flexibility in all patients. We calculated a Spearman's correlation coefficient between the cognitive flexibility state estimated from reaction time and High Gamma Power (HGP, 65-200 Hz) in dorsal anterior cingulate (dACC) during the first 0.5 seconds after image onset. We found in 4/6 patients that HGP in dACC had a positive correlation with the cognitive flexibility state. These were also the same patients who had a significant improvement in flexibility with stimulation. From initial analysis, we conclude that dorsal striatal stimulation improves cognitive flexibility by modulating HGP in the dACC.

Poster Number 181

Karmel Choi, PhD
Psychiatry, Clinical Research Fellow | kwchoi@mgh.harvard.edu

Genetic Risk and the Protective Effect of Unit Cohesion on Post-Deployment Depression in U.S. Army Personnel

INVESTIGATORS: K. W. Choi, C. Y. Chen, R. J. Ursano, R. C. Kessler, M. J. Wang, M. B. Stein, J. W. Smoller, Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium

Genome-wide association studies have begun to demonstrate the extent to which genetic variation contributes to risk for depression. Protective factors may modify this risk, illuminating opportunities for prevention. In a unique prospective sample of 4,900 active duty soldiers from the Army Study of Risk and Resilience in Servicemembers (Army STARRS), this study examined whether a candidate protective factor at pre-deployment—unit cohesion—could modify genetic risk for depression over the course of deployment. To define genetic risk, we constructed polygenic risk scores for depression using summary statistics from a well-powered reference study (N=461,134), summing the number of index alleles at each genetic variant weighted by their effect size. Scores were normally distributed across individuals and divided into three categories of genetic risk: low (quintile 1), intermediate (quintiles 2-4), and high (quintile 5). We tested the effect of unit cohesion within these genetic risk categories, adjusting for sex, age, population stratification, and deployment stress. Here, we show that, across all categories of genetic risk, individuals who reported higher unit cohesion at pre-deployment had lower risk for developing post-deployment depression. Even among those at highest genetic risk, stronger unit cohesion was associated with significantly reduced odds of depression (aOR=.76, [.61-.95]). This genetically informed prospective study of active duty soldiers illustrates the potential of protective factors to buffer psychiatric risk following exposure to traumatic stress. Importantly, intervenable factors such as social cohesion may protect against depression even among those most genetically susceptible, and represent promising targets for promoting resilience in at-risk populations.

Poster Number 182

Alyson Dechert, BA
Psychiatry, Clinical Research Coordinator | adechert@mgh.harvard.edu

Evaluation of a New Scale for Medical Marijuana Effect Expectancy

INVESTIGATORS: A. Dechert, W. C. Ho, J. Gilman, Center for Addiction Medicine

In recent years, many states have expanded the availability of medical marijuana (MM). Previous studies have largely examined recreational marijuana users but with wider availability of MM, a profile of patients seeking MM can be established. This ongoing year-long longitudinal study examines participants seeking a MM card for self-reported pain, mood, and/or sleep disorders. Ninety-six participants were enrolled, of which most were adult women (average age 37.82 (15.25); 64.2% female). Of those enrolled, 17.9% were marijuana naïve (no use in past year and <5 lifetime uses). Approximately 61.1% of participants reported marijuana use within the past 3 months, and the average participant used marijuana once per week at the time of enrollment. Most participants (39.19%) reported pain as their primary condition for obtaining MM, followed by mood (35.14%) and sleep (25.68%) disorders. All participants completed the Medical Marijuana Effect Expectancy Questionnaire, a questionnaire designed to assess patient expectations of medical marijuana. Subscales of this questionnaire assessed general expectations and those for physical symptoms, mood symptoms, and emotional state. Results indicate that participants with affective disorders reported significantly higher expectations that MM would improve their emotional state (concentration/energy/anger/sleep/relaxation) and relieve mood symptoms (depression/anxiety/panic) compared to those with sleep or pain disorders ($p<0.05$). Patients with insomnia and pain report no significant differences in their expectations of all symptoms. Men and women also reported no significant differences in medical marijuana expectations. These preliminary results indicate that people with mood disorders may be more optimistic about the efficacy of medical marijuana on their symptoms.

Poster Number 183

Laura Duque, MD
Psychiatry, Research Fellow | lduqueserrano@mgh.harvard.edu

Psychological Well-Being and Type 2 Diabetes

INVESTIGATORS: C. N. Massey, E. H. Feig, L. Duque, J. C. Huffman, Cardiac Psychiatry Research Program

Positive psychological characteristics such as optimism, positive affect, gratitude, and related constructs may play an important role in health. In patients with type 2 diabetes (T2D), positive psychological constructs have been associated with superior medical outcomes, including better glucose control and lower mortality rates. The beneficial effects of positive psychological states in T2D are most likely mediated through health behaviors such as increased physical activity and adherence to a healthier diet. Furthermore, numerous studies with non-diabetic populations have shown that performing various positive psychological exercises (e.g., writing gratitude letters, performing acts of kindness) have led to greater well-being. Compared to other available treatments, these activities are simple and involve constructs that have been associated with superior adherence and diabetes-related outcomes. However, there has been minimal research on the use of positive psychological interventions in T2D, though small studies of related interventions have been linked to improvements in positive affect and, in some cases, greater health behavior adherence and lower blood sugar. Continued work is needed to ascertain whether positive psychology interventions can truly impact functioning, blood sugar, and overall health in this key population.

Poster Number 184

Melanie Freedman, BS
Psychiatry, Research Coordinator | mfreedman2@mgh.harvard.edu

A positive psychology intervention to promote health behaviors in heart failure: a proof-of-concept trial

INVESTIGATORS: M. E. Freedman, E. E. Beale, F. Gomez-Bernal, J. C. Huffman, C. M. Celano

Patients with heart failure (HF) frequently struggle to adhere to health behaviors, like being physically active, reducing sodium intake, and taking medications regularly. Positive psychological constructs may play an important role in patients' abilities to adhere to health behaviors, and positive psychology (PP) interventions can improve well-being and positive affect in patients with and without medical illness. We developed a novel, telephone-delivered, 10-week PP-based health intervention and explored its feasibility and impact in patients with New York Heart Association class I, II, or III HF in a single-arm, proof-of-concept trial (N=10). Participants completed PP-based exercises and physical activity goal-setting and reviewed these activities weekly with a study interventionist. Feasibility was assessed by calculating the number of sessions completed, while acceptability was measured by self-report Likert scales (0-10) of ease and utility. Finally, we assessed the intervention's impact on positive and negative psychological constructs, HF symptoms, quality of life, and health behavior adherence. The intervention was feasible (with 87% of total exercises completed) and acceptable (mean ease = 7.7/10, mean utility = 7.9/10). PP exercises led to a significant increase in happiness (7.8 [pre-exercise] vs. 8.6 [post], t=5.4, p<.001) and a marginally significant increase in optimism (8.2 [pre-exercise] vs. 8.4 [post], t=1.7, p=.09). PP was also associated with improvements in positive and negative psychological outcomes, HF symptoms, quality of life, and adherence to health behaviors. Further testing of this intervention is warranted in a larger, controlled trial to assess its effects on important health outcomes.

Poster Number 185

Taylor Gianangelo, BA
Psychiatry, Clinical Research Coordinator | tgianangelo@mgh.harvard.edu

A positive psychology-motivational interviewing intervention for patients with type 2 diabetes: proof-of-concept trial

INVESTIGATORS: C. M. Celano, T. A. Gianangelo, R. A. Millstein, W. Chung, D. J. Wexler, E. R. Park, J. C. Huffman

Eighteen million Americans with type 2 diabetes (T2D) do not follow recommended guidelines for physical activity. Motivational interviewing (MI) has had modest effects on activity and related behaviors in T2D. Positive psychological attributes (e.g., optimism) are associated with superior medical outcomes in T2D, and positive psychology (PP) interventions promote such attributes. There had been no study in T2D of a combined PP-MI intervention to promote well-being and health behavior adherence. We developed a novel, telephone-delivered, 16-week PP-MI intervention and explored its feasibility and impact in T2D patients in a single-arm, proof-of-concept trial. Participants completed PP-based exercises and MI-based physical activity goal-setting activities and reviewed these activities weekly with a study trainer for 16 weeks. Feasibility and acceptability were assessed via exercise completion rates and post-exercise ratings of ease/utility (0-10 scales). Impact was explored by examining changes in physical activity (via accelerometers and self-report), other health behaviors, psychological measures, and medical outcomes (e.g., hemoglobin A1c [A1C]) from baseline to 16 weeks, using paired t-tests. Twelve participants enrolled, and 10 provided follow-up data. Seventy-eight percent of PP-MI activities were completed, and participants rated the PP-MI content and sessions as easy (mean=8.2/10, SD 1.5) and useful (mean=9.1/10, SD 1.2). PP-MI was associated with improved adherence to health behaviors and overall self-care, variable effects on accelerometer-measured activity and psychological outcomes, and modest beneficial effects on body mass index and A1C. Further testing of this intervention is warranted in a larger, controlled trial to assess its effects on important health outcomes.

Poster Number 186

Maya Hareli, BA
Psychiatry, Clinical Research Coordinator | mhareli@mgh.harvard.edu

No Change in Executive Function with One Month of Cannabis Abstinence

INVESTIGATORS: M. Hareli, Y. Wu, M. Vilme, E. Nip, A. Moser, J. Gilman, A. E. Evins, R. M. Schuster, Center for Addiction Medicine, Department of Psychiatry

Cross sectional studies suggest an association between adolescent cannabis use and poor executive functioning (EF); however, it is unknown whether these deficits resolve with abstinence. Improvement in EF with cannabis abstinence would support a causal link between cannabis use and poor EF. Cannabis users (n=68), aged 19-25, were randomized 2:1 to one month of abstinence (MJ-Abst; n=46), verified by quantitative urinalysis, or monitoring with no abstinence requirement (MJ-Mon; n=22). Participants completed behavioral and self-report measures of EF at baseline and 30 days. The Spatial Working Memory test and One Touch Stockings of Cambridge from the Cambridge Neuropsychological Test Automated Battery were used to behaviorally assess EF. The Behavior Rating Inventory of Executive Function-2nd edition was used as the self-report measure of EF (Behavioral Regulation Index, BRI; Metacognitive Index, MI; and Global Executive Composite, GEC). Models controlled for sex, frequency of cannabis use in the 90 days prior to baseline assessment, and age of cannabis initiation. There was no association between group and change in any measure of working memory or planning (p-values>.05). There was a trend between group and self-reported change in behavioral regulation [F(4,63)=1.21, p=.083], metacognition [F(4,63)=1.45, p=.082], and global EF [F(4,63)=1.55, p=.057], with abstinent cannabis users reporting greater improvement in these domains (BRI: M=-1.91, SD=6.16; MI: M=-3.04, SD=8.49; GEC: M=-2.72, SD=7.48) compared to those who continued using (BRI: M=.68, SD=5.82; MI: M=-.36, SD=4.64; GEC: M=.14, SD=5.18). Future studies may aim to further understand this disparity between self-perceptions and objective results of cognitive functioning.

Poster Number 187

Aura M Hurtado-Puerto, MD
Psychiatry, Research Fellow | auhurta@gmail.com

Volumetric changes in the right hippocampus and the dentate gyrus explain negative, but not positive, affective improvement after ECT

INVESTIGATORS: A. M. Hurtado-Puerto, K. K. Ellard, M. E. Henry, N. Makris, J. A. Camprodon

Electroconvulsive Therapy (ECT) is the most effective treatment in psychiatry and among the most effective in medicine. While a number of studies have described right hippocampal volume increases after ECT, the relationship of these changes with clinical response remains unclear. We study these dynamics by quantifying dentate gyrus volumes beyond the hippocampus with subfield anatomy, and positive vs. negative affect beyond overall depression severity with dimensional assessments. Sixteen patients treated with ECT for depression were studied. Before and after acute treatment, we obtained high-resolution T1-weighted MRI scans, measures of clinical depression severity (QIDS-SR), and of positive and negative affective dimensions (PANAS). We used FreeSurfer 6.0 for the structural reconstruction and segmentation of images, and the hippocampal module for subfield segmentation setting significance levels at $p<0.05$ after familywise error correction for multiple comparisons.

ECT led to right but not left increase in hippocampal and dentate gyrus volumes. These changes did not explain the syndromal clinical improvement of depression as measured by the QUIDS-SR (categorical). Nevertheless, we identified a significant relationship between right hippocampal volume change and improvement of negative (but not positive) affective dimensions (p=0.015). This relationship also existed in the dentate gyrus (p= 0.002). These results confirm ECT-mediated right hippocampal volume increase, and report a parallel increase in dentate gyrus volume. These volumetric changes do not explain changes in overall depression severity. More nuanced relationships between volume change and improvement in affective dimensions may pave the way for a dimensional explanation of ECT effects.

Poster Number 188

Melissa Maravic, PhD, MPH
Psychiatry, Project Director | mmaravic@partners.org

Baseline Findings from the PCORI Pragmatic Trial Integrated Smoking Cessation Treatment for Smokers with Serious Mental Illness

INVESTIGATORS: M. C. Maravic, G. N. Pachas, C. Cather, S. Reyering, E. M. Manghis, A. E. Evins

Nicotine addiction is highly prevalent among those with serious mental illness (SMI). Despite extensive evidence that most want to quit smoking and that first-line pharmacotherapies for smoking cessation are efficacious and well tolerated in this population, few are offered smoking cessation advice or effective treatments by their primary care providers (PCP). This 3-year study enrolled smokers with SMI. Participants completed a 5-minute survey about their smoking habits and PCP care during the last year, and a carbon monoxide (CO) breath test. Participants (n=1,166) most frequently reported smoking 11-20 tobacco products per day (33.2%), and on average had an expired CO of 21.3 ± 16.0. Many (65.9%) reported one or more smoking-related health condition. Most (92.1%) reported that their PCP is aware they smoke tobacco products. While 69.7% reported that their PCP recommended cessation, only 36.4% report that their PCP prescribed treatment for cessation. Most common treatment recommended or prescribed was nicotine patch (71.2%), followed by nicotine gum/lozenge (50.8%), varenicline (16.8%) and bupropion (3.8%). Baseline data indicate that participants are moderate smokers with more than half reporting a current or past smoking related illness. While PCPs are largely aware that their patients smoke, as previously reported few offer first-line pharmacological treatment for smoking cessation.

Poster Number 189

Caitlin Ravichandran, PhD
Psychiatry, Lecturer | cravichandran@mclean.harvard.edu

Accuracy of Self-Reported History of Autoimmune Disorders

INVESTIGATORS: C. Ravichandran, J. A. O'Rourke, Y. Howe, J. Mullett, C. Keary, S. Golas, A. Rao, J. Chung, C. J. McDougle

Research providing evidence for an immune-mediated subtype of autism spectrum disorder (ASD), which has the potential to identify new avenues for treatment and prevention, requires accurate ascertainment of family history of autoimmune diseases. To characterize the accuracy of self-report diagnosis of autoimmune diseases, we conducted a study comparing diagnosis using self-report questionnaire to diagnosis using medical record review in 1013 adult (age 18-70 years) patients. Participants were identified and contacted through the Partners Research Patient Data Registry at Massachusetts General Hospital. For diseases with adequate prevalence, we estimated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for self-report diagnosis under the assumption that medical records based diagnoses were accurate. Only one (celiac disease) of 11 diseases had sensitivity and PPV above 80%. Most diseases had either sensitivity or PPV below 50%, with the lowest PPV for skin diseases. Common errors included reporting type 2 diabetes when type 1 was present, and reporting rheumatoid arthritis when osteoarthritis was present. Specificity and NPV were high for all diseases. Results suggest that self-report assessment contributes to inconsistencies in studies of autoimmune disease history in relatives of patients with ASD. Future studies investigating an immune-mediated subtype of ASD should avoid relying exclusively on self-report diagnosis.

Poster Number 190

Alexander Rockhill, BS
Psychiatry, Clinical Research Coordinator | arockhill@mgh.harvard.edu
Bayesian State-Space Model for Learning Applied to EEG Lempel-Ziv Complexity

INVESTIGATORS: T. Deckersbach, A. Rockhill, A. S. Widge

Learning is a process that is often impaired in psychiatric disorders, and provides insight into consciousness via implicit memory. One interesting example representative of the relationship between consciousness and learning is HM learning a difficult drawing task in a mirror but not remembering that he had learned this skill. We studied this association by using a well-replicated learning reversal task where participants are shown shapes and learn the associations of a button on a button box with a particular shape, then subsequently relearn these associations when the correct answers are reshuffled. A Bayesian state-space model using reaction time and the success of the choice was used to estimate the learning state for each trial. This learning state was then correlated with the Lempel-Ziv complexity of binarized EEG source space matrices where binarization was determined by the relation of voltage at each time point to a threshold based on a cluster permutation test of baseline data. The Lempel-Ziv complexity has been shown to correlate with consciousness in TMS-EEG experiments giving rise to the theory that the complexity of neural response to a perturbation indexes consciousness. In previous fMRI studies, the cognitive processes associated with learning have been shown to have greater involvement of executive cognition areas compared to the greater recruitment of basal areas associated with retrieving implicit memories. This shift away from executive function once the task is learned has been shown in preliminary data to be associated with a decrease in the complexity of the EEG data.

Poster Number 191

Carl E. Schwartz, MD
Psychiatry, Assistant Professor | carl_schwartz@hms.harvard.edu
High-reactive Temperament in 4 Month-Old Infants Predicts Reduced Amygdala Volume and Increased Amygdala Reactivity in Adults

INVESTIGATORS: J. M. Felicione, P. S. Kunwar, R. Franklin, D. N. Greve, C. E. Schwartz

Infant temperament is defined as a biologically based predilection for distinctive patterns of emotions, cognitions, and behaviors. High-reactive (HR) infants at 4 months of age show vigorous limb activity, arching of the back, and crying to unfamiliar visual, olfactory, and auditory stimuli. Low reactive (LR) infants show both low activity and distress to the same stimuli. High reactivity is shown to be a risk factor for social anxiety and depression in children, adolescents, and young adults. In individuals who are already diagnosed with social anxiety disorder and depression, reduced amygdala volumes are reported. Amygdala volume was determined with MRI in 135 adolescents (age 18.20 ± 0.07), who had been HR (n=55) or LR (n=80) infants. Effects of infant classification (HR, LR) on amygdala volume in adulthood were analyzed with a MANCOVA, with age, sex, handedness, and ICV as covariates. Left amygdala volumes in subjects who had been HR (1652 ± 20 mm3) were smaller than left amygdala volumes in subjects who had been LR (1720 ± 25 mm3; $F(1,118)=4.7$, $p=0.03$). Right amygdala results trended in the same direction (HR=1741 ± 24 mm3, LR=1784 ± 20 mm3, $p=0.16$). We show that infant phenotypes observed in the first months of life predicted differences in amygdala volume and function in the brains of young adults 18 years later, suggesting that volumetric and functional differences reported in cross-sectional studies of anxiety disorders and depression could be attributable to a shared phenotype rooted in early infancy that cuts across diagnostic categories.

Poster Number 192

Jeehye Seo, PhD
Psychiatry, Research Fellow | jseo6@mgh.harvard.edu

Delayed Fear Extinction in Individuals with Primary Insomnia

INVESTIGATORS: J. Seo, K. N. Moore, S. Gazecki, R. M. Bottary, E. F. Pace-Scott

Insomnia increases risk for anxiety disorders - conditions that are also associated with fear extinction deficits. We compared functional activation of brain regions implicated in fear and extinction between individuals with Primary Insomnia (PI) and good-sleeping controls (GS). 23 GS age- and sex-matched to 23 PI completed 14 days of actigraphy and sleep diaries and three nights of ambulatory polysomnography. They then completed a 2-day, fear conditioning and extinction paradigm. Fear conditioning and Extinction Learning occurred on the first day, followed 24h later by Extinction Recall. During all phases, blood-oxygen level dependent fMRI signal and skin conductance level were simultaneously recorded. Beta weights were extracted from regions where activation differed between PI and GS and regressed against sleep quality and psychophysiological variables. During Fear Conditioning, both PI and GS activated fear-related structures, however, they activated different sub regions of the left insula. Comparing the end to the beginning of Extinction Learning, PI demonstrated little change, whereas GS activated both fear and extinction-related areas. During Extinction Recall, while GS demonstrated limited activation, PI activated regions similar to those previously activated in GS. Better sleep quality predicted less activation of insula and amygdala at Extinction Recall. In conclusion, across Extinction Learning, GS activated elements of both fear and extinction-related networks, perhaps reflecting their dynamic competition, whereas PI did not. At Extinction Recall, PI engaged similar regions whereas GS no longer did so. Individuals with PI may show a delay in their acquisition of fear extinction memories.

Poster Number 193

Amy Yule, MD
Psychiatry, Instructor | ayule@partners.org

Evidence of the Diagnostic Utility of the Child Behavior Checklist for Identifying Pediatric Bipolar I Disorder

INVESTIGATORS: A. Yule, M. Fitzgerald, T. Wilens, J. Wozniak, K. Y. Woodworth, A. Pulli, M. Uchida, S. V. Faraone, J. Biederman

The objective is to assess the diagnostic utility of a unique profile derived from the Child Behavior Checklist (CBCL), the CBCL-BP profile, to identify children at high risk for BP-I in the clinical setting. This study examined the ability of the CBCL-BP profile to identify children with and without a structured interview diagnosis of BP-I disorder using receiver operating characteristic (ROC) curves. Subjects were derived from four independent datasets of children with and without attention deficit hyperactivity disorder (ADHD) and BP-I evaluated at baseline. All subjects had structured clinical interviews with raters blinded to subject ascertainment status. The sample of 661 subjects had an average age of 11.7 ± 3.3 years, was 57% male, and 94% Caucasian. 130 (19.7%) subjects had a structured interview derived diagnosis of BP-I disorder. The ROC analysis of the CBCL-BP profile in children with and without BP-I disorder yielded an area under the curve of 0.91. A score of ≥195 on the CBCL-BP profile correctly classified 86% of subjects with BP-I disorder with 80% sensitivity and 87% specificity. The CBCL-BP profile is an efficient tool to identify children at high risk for pediatric BP-I disorder who need to be prioritized for further clinical assessment.

Poster Number 194

David Alagpulinsa, PhD
Medicine, Research Fellow | davida_alagpulinsa@dfci.harvard.edu

Enhanced and long-term immune isolation and function of human type 1 diabetes patients' stem cell-derived β cells in a murine model of type 1 diabetes as a result of microencapsulation with clinical grade alginate and CXCL12

INVESTIGATORS: D. A. Alagpulinsa, J. J. Cao, R. K. Driscoll, R. F. Sirbulescu, M. F. Penson, M. Sremac, A. Veres, E. N. Engquist, T. Brauns, J. Oberholzer, D. A. Melton, M. C. Poznansky, JDRF

Functional insulin producing β cells are now routinely produced from human pluripotent stem cells (SC-β cells). This advancement makes β-cell replacement a potential practical definitive treatment for type 1 diabetes (T1D). To obviate the need for systemic immunosuppression, microencapsulation of islets in alginate has been widely explored as a delivery vehicle for β-cell replacement. However, microencapsulated islet grafts retrieved from patients are characterized by dense pericapsular fibrotic overgrowth that causes islet cell death and graft failure. Here, we use a novel approach to prevent the pericapsular fibrotic foreign body response to achieve long-term replacement therapy with SC-β cells without immunosuppression in a murine model of T1D. This involves co-encapsulation of human T1D patients' SC-β cells with CXCL12, an immune-modulating chemokine known to support β-cell survival, in clinical grade alginate microcapsules. We first demonstrate that CXCL12 enhances glucose-stimulated insulin secretion of SC-β cells, while inducing the expression of genes associated with β-cell function and survival, and protecting them against cytokine-induced apoptosis. Subsequently, we demonstrate in sensitized, immunocompetent streptozotocin treated diabetic C57BL/6 mice, that co-encapsulation of SC-β cells with CXCL12 in alginate microcapsules accelerates the reduction of hyperglycemia and prevents the pericapsular fibrotic response, leading to long-term functional competence and glycemic control over 150 days without systemic immunosuppression. This is the first study to our knowledge, using human β cells microencapsulated in clinical grade alginate to achieve long-term glycemic control in immunocompetent mice and enables further preclinical translational step to explore the safety and efficacy of this novel approach in non-human primates.

Poster Number 195

Sukhada Bhave, PhD
Pediatrics, Research Fello | smbhave@mgh.harvard.edu

Neuronal cell therapy to restore colorectal motility in a novel animal model of enteric neuropathy

INVESTIGATORS: S. M. Bhave, R. Hotta, E. Arciero, N. Nagy, A. M. Goldstein

Cell therapy offers an innovative approach for treating Hirschsprung disease (HSCR), a congenital disorder characterized by lack of enteric nervous system (ENS) in the distal colon, resulting in functional colonic obstruction. Testing the effect of cell therapy on gut motility in HSCR is hampered by the poor survival of existing mouse models of HSCR, which die within the first few weeks of life, limiting the time for analysis after cell transplantation. Our goal was to develop a novel, non-lethal, and highly specific model of colonic aganglionosis and use it to demonstrate restoration of gut function following enteric neural stem/progenitor cell (ENSC) transplantation.

We crossed Wnt1-Cre mice with R26R-iDTR reporter mice, generating a Wnt1-iDTR transgenic line in which active Cre recombination renders Wnt1-expressing neural crest cells sensitive to diphtheria toxin (DT). Treatment of neural crest cells isolated from Cre+ Wnt1-iDTR mice with DT (10 ng/ml) resulted in a marked increase in apoptosis. Similarly, intraperitoneal administration of DT (40 µg/kg) to Cre+ Wnt1-iDTR mice resulted in pronounced intestinal dilatation, absence of coordinated contraction patterns and enteric neuronal loss. To limit neural crest cell injury to a focal region of the ENS, we injected DT into the wall of the mid-colon of these mice via laparotomy. DT (1 ng/µl) resulted in ENS loss that was maintained at 3 weeks. This focal aganglionosis was associated with abnormal gut motility.
Current studies involve transplanting Wnt1-tdT+ ENSCs into the aganglionic segment and performing functional analyses to assess for restoration of intestinal motility following cell delivery.

Poster Number 196

William Hendriks, PhD
Neurology, Instructor | whendriks@partners.org

Human iPSC-based DJ1-Parkinsonism Disease Modeling

INVESTIGATORS: B. F. Braendl, S. Schneider, D. C. Bragg, J. T. Teo, J. Bras, F. J. Mueller, W. T. Hendriks

Mutations in PARK7 have been associated with Parkinson's disease (PD). PARK7 encodes DJ1, a protein involved in transcriptional regulation, kinase activity regulation, protein ubiquitination and oxidative stress. Although pathogenic contributions of DJ1 mutations to PD remain elusive, evidence obtained from animal and in vitro studies suggests that these mutations result in oxidative stress and mitochondrial dysfunction. We have characterized a novel homozygous DJ1 mutation as the cause of a severe, early onset sporadic atypical complex dystonia-parkinsonism syndrome. We have generated and characterized induced pluripotent stem cells (iPSCs) from the DJ1 mutation patient and his unaffected sibling. In addition, we have gene-edited the patient mutation in an unrelated control iPSC line using CRISPR/Cas9 to generate isogenic patient mutation iPSC lines. These iPSC lines were differentiated towards dopaminergic fate and generated floorplate progenitors (FPPs) and mature dopaminergic neurons. Dopaminergic neuronal cells were screened for general health and neuronal morphology and expressed dopaminergic neuronal markers (OTX2, FOXA2, TH). Gene expression analysis in iPSCs, FPPs, and mature neurons revealed a near absence of DJ1 transcript(s) and absence of DJ1 protein was confirmed by immunocytochemistry and Western Blotting. Additional ongoing studies using this cellular DJ1-PD model involve 1) RNA-seq and transcriptional profiling; and 2) mitochondrial function assays. The generation and use of this human DJ1-PD disease model in-a-dish will prove invaluable for elucidating the contribution of DJ1 mutations to PD pathogenesis.

Poster Number 197

Baboucarr Lowe, MS
Oral and Maxillofacial Surgery, Research Fellow | blowe3@mgh.harvard.edu

Diatom biosilica nanoparticles for intra-articular regeneration

INVESTIGATORS: B. Lowe, F. Guastaldi, M. J. Troulis

Ostoeartheritis (OA) is a degenerative joint disease affecting all of the body joints including the Temporomandibular Joint (TMJ). Tissue engineering and stem cell based therapies have become an alternative approach for the treatment of joint disorders. Diatoms are inexpensive biogenic silica with natural three-dimensional form and nanoscopic properties suitable for the delivery of biomolecules. The goal of this study was to develop a nano-carrier system capable of modulating the recruitment of cells and serve as a regenerative matrix for articular tissue. A cost-efficient method for processing diatom biosilica of nanoparticles capable of being an intra-articular carrier biomaterial was developed. The morphology and elemental composition of the particles were characterized by Scanning Electron Microcoscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX). Results confirmed the grafted amine group and the intricate nanoscopic properties of the particles. Our ongoing investigations are using this system for the intra-articular regeneration of the TMJ components in mouse model.
Keywords: Osteoarthritis, Temporomandibular Joint, Tissue Engineering, Stem Cells, Biosilica Nanoparticles

Poster Number 198

Junjie Lu, PhD
Pediatrics, Instructor | junjie.lu@mgh.harvard.edu

Tracheal aspirate-derived mesenchymal stromal cells reveal transcriptional dynamics of lung development in preterm infants

INVESTIGATORS: J. Lu, R. Spadafora, C. Zhang, H. Li, P. H. Lerou

Bronchopulmonary dysplasia (BPD) is the most frequent adverse outcome for infants born less than 30 weeks gestational age and the most common chronic lung disease in infancy. Due to a lack of experimentally accessible human tissue, limitation of animal models, and the complex nature of prematurity, BPD has proven very challenging to study. To overcome these limitations, we have focused on tracheal aspirates, which are readily collected during routine suctioning of intubated newborns, and established protocols to derive mesenchymal stromal cells (MSCs) from those aspirates. Here we show that patient-specific tracheal aspirate-derived MSCs reflect development and pathology of the premature lung. We performed RNA sequencing on 20 MSC lines from infants born between 23 and 42 weeks. Weighted Gene Co-expression Network Analysis identified one gene module strongly correlated with infant's gestational age. Genes in critical developmental signaling pathways were enriched in this module, suggesting the cells retain developmental signatures of the premature lung. We identified another gene module correlated with the degree of BPD. Within this module, there was a strong enrichment of extracellular matrix components, which are known to be part of an interconnected network perturbed in BPD. Expression of these genes shows heterogeneity in BPD patients, reflecting the clinical heterogeneity, which may be useful to define disease endotypes. Our tracheal-aspirate derived MSCs represent a novel ex-vivo system to study the developing human lung, the effects of prematurity and clinical interventions. Further studies are necessary to validate it as a biomarker of BPD for prevention, diagnosis, and precision medicine.

Poster Number 199

Yonggang Pang, MD, PhD
Orthopedics, Instructor | ypang1@mgh.harvard.edu

3D Bioprinted Native-comparable Micro-cartilages: Repairing Large Articular Cartilage Defect Minimally Invasively

INVESTIGATORS: B. E. Grottkau, Z. Hui, Y. Pang

Articular cartilage has very limited capability of self-healing and osteoarthritis is the leading cause of disability. 3D live cell bioprinting is an emerging technology which has strong potential to regenerate damaged tissues. Previously, we have successfully bioprinted micro cartilages using our novel 3D bioprinting system and accomplished the implantation to the cartilage defect through a minimally invasive approach. In the current study, we further validated the clinical feasibility of the bioprinted micro-cartilage using a large articular cartilage defect model, which approximates the defect size that is normally observed clinically. The bioprinted micro cartilages show standard morphology and biochemical and molecular profiles comparable to the native cartilage. The micro-cartilages were successfully implanted arthroscopically and completely covered the cartilage defect. An orthopedic surgeon can accomplish the implantation of the bioprinted micro-cartilages using the standard arthroscopic surgical techniques. Only a few days post implantation, the implanted micro-cartilages show effective adhesion to the surrounding cartilage and subchondral bone remaining in place even in the present of shear force. The self-assembly of the micro cartilages started hours after implantation and assembled cartilage shows continuous growth and mature. Histology and immunohistology results show good integration of the bioprinted cartilages to the surrounding cartilage and subchondral bone. The results from this study demonstrate that our bioprinted micro-cartilage is promising for clinical application.

Poster Number 200

Ruxandra Sirbulescu, PhD
Medicine, Research Fellow | rsirbulescu@mgh.harvard.edu

Mature B cells accelerate wound healing after acute and chronic diabetic skin lesions

INVESTIGATORS: R. F. Sirbulescu, C. K. Boehm, E. Soon, M. Q. Wilks, I. Ilies, H. Yuan, B. Maxner, N. Chronos, C. Kaittanis, M. D. Normandin, G. El Fakhri, D. P. Orgill, A. E. Sluder, M. C. Poznansky

Chronic wounds affect 12-15% of patients with diabetes and are associated with a drastic decrease in their quality of life. Here we show that purified mature naïve B220+/CD19+/IgM+/IgD+ B cells improve healing of acute and diabetic murine wounds after a single topical application. B cell treatment significantly accelerated acute wound closure by 2-3 days in wild-type mice and 5-6 days in obese diabetic mice. The treatment led to full closure in 43% of chronic diabetic wounds, as compared to only 5% in saline-treated controls. Applying equivalent numbers of T cells or disrupted B cells failed to reproduce these effects, indicating that live B cells mediated pro-healing responses. Topically-applied B cell treatment was associated with significantly reduced scar size, increased collagen deposition and maturation, enhanced angiogenesis and increased nerve growth into and under the healing wound. β-III tubulin+ nerve endings in scars of wounds treated acutely with B cells showed increased relative expression of growth-associated protein 43. The improved healing associated with B cell treatment was supported by significantly increased fibroblast proliferation and decreased apoptosis in the wound bed and edges, altered kinetics of neutrophil infiltration, as well as an increase in TGF-β and a significant reduction in MMP2 expression in wound granulation tissue. Our findings indicate that the timeline and efficacy of wound healing can be experimentally manipulated through the direct application of mature, naive B cells, which effectively modify the balance of mature immune cell populations within the wound microenvironment and accelerate the healing process.

Poster Number 201

Yiying Zhang, MD
Anesthesia, Critical Care and Pain Medicine, Instructor | yzhang37@mgh.harvard.edu

Cyclophilin D mediates neurogenesis and cognition in young mice

INVESTIGATORS: Y. Zhang, L. Pan, N. Liufu, Y. Dong, Z. Xie

Cyclophilin D (CypD) is a modulatory factor for mitochondrial permeability transition pore (mPTP) and may regulate cognition in Alzheimer's disease transgenic mice. However, the effects of CypD in neurogenesis in developing brain and cognition in young mice are unknown. Anesthesia would be associated with cognitive impairment in children, but the underlying mechanisms also remain largely unknown. We therefore used sevoflurane to evaluate the effects of CypD on anesthesia-induced neurotoxicity and cognition in young mice and explore the underlying mechanisms. We treated wild-type and CypD knockout young mice and neural progenitor cells (NPCs) harvested from the wild-type or CypD knockout mice with sevoflurane. We used immunofluorescence staining, flow cytometry, Western blot, and Morris Water Maze to assess the interaction of sevoflurane and CypD on mitochondria function, neurogenesis, apoptosis, synapse and cognition in young mice and NPCs. Here we showed that sevoflurane increased CypD levels and induced mPTP opening, mitochondrial dysfunction, inhibition of neurogenesis, apoptosis, synapse loss and cognitive impairment. These effects were mitigated by CypD deficiency. These data demonstrate the novel role of CypD in neurogenesis in developing brain and cognition in young mice, and suggest CypD as a target of anesthesia neurotoxicity in young mice. Blockade of CypD may be a therapeutic strategy for the anesthesia/surgery-induced cognitive impairment, pending further investigation in young animals and children.

Poster Number 202

Ya Wen, PhD
Neurology, Research Fellow | ywen3@mgh.harvard.edu

BCL2 as a potential major player in autism and cancer

INVESTIGATORS: Y. Wen, M. R. Herbert

We sought possible common mechanisms between autism and cancer regarding genes, signaling pathways and mitochondria. Genes in four categories, autism, cancer, mitochondrial localized, and signaling pathways, were examined. 990 Autism genes were downloaded from SFARI (Simons Foundation Autism Research Initiative) Gene Human Module, and 699 cancer genes from Cancer Gene Census. 1158 genes encoding proteins with strong support for localization in mitochondria were downloaded from MitoCarta2.0. Seven signaling pathways, Calcium, Wnt, MAPK, PI3K-Akt, mTOR, Ras and insulin signaling pathways were included in the study, chosen based on studies linking them all to both autism and cancer; we downloaded the 981 genes that participate in these pathways from KEGG (Kyoto Encyclopedia of Genes and Genomes) Pathways.

We looked for overlaps among the genes from the four categories. Numbers of genes shared: Autism/cancer 101; mitochondria/ signaling pathways: 24; autism/mitochondria: 40; cancer/mitochondria: 25; autism/signaling pathways: 113; cancer/signaling pathways: 130. We then noted that the group of genes shared between autism and cancer has 28 genes in common with signaling pathway genes, and 3 genes (BCL2, FHIT, and SND1) in common with mitochondrial genes. BCL2 is the only gene that presents in both the "genes shared between autism and cancer" and the "genes shared between mitochondria and signaling pathways" groups. Identifying how, when and where BCL2 and other shared genes from different groups that contribute to autism and cancer may help us to understand the cause of the conditions.

Poster Number 203

Laura Burlage, MD
Surgery, Research Fellow | lburlage@mgh.harvard.edu

Ex-vivo Subnormothermic Oxygenated Machine Perfusion of Rodent Hind Limb: Feasibility Study to Elongate Preservation Time of Vascularized Composite Allograft

INVESTIGATORS: L. C. Burlage, A. G. Lellouch, S. N. Tessier, C. A. Pendexter, S. E. Cronin, I. M. Schol, M. A. Randolph, R. J. Porte, C. L. Cetrulo, K. Uygun

Over 900.000 Americans are living with limb loss due to traumatic amputation, including thousands of U.S. soldiers who lost a limb in combat. Today, vascularized composite allotransplantation (VCA) is the only viable treatment option to restore aesthetic and motor function after limb loss. However, widespread application of VCA is still held back by the limited preservation time of VCA grafts. The study investigates the utility of subnormothermic oxygenated machine perfusion (SNMP) to extend preservation time of rodent hind limbs. Five rat hind limbs were procured and immediately flushed with a heparin/saline mixture. During 3 hours of SNMP, limbs were perfused using a pressure-controlled system through the femoral artery with free venous outflow. Arterial flow and vascular resistance were monitored. Lactate levels and oxygen consumption were evaluated as markers of muscle viability. At the end of SNMP, biopsies were taken for energy level analysis and immunohistochemistry.

Arterial flow and vascular resistance remained stable throughout perfusion, between 0.5-2.0 mL/min and 20-40 mmHg/mL/min respectively. After an initial rise, median lactate levels decreased. Oxygen consumption increased during the first hour and stabilized thereafter. Energy levels were comparable with fresh tissue samples (n=2). H&E and TUNEL staining showed normal muscle architecture and no cell apoptosis. This study shows that 3 hours of ex vivo SNMP of rat hind limb is feasible. SNMP has the potential to preserve and even improve the quality of limbs prior to transplantation, thereby enhancing clinical outcome and application of VCA.

Poster Number 204

Esa Farkkila, MD, DMD
Oral and Maxillofacial Surgery, Research Fellow | efarkkila@mgh.harvard.edu

Association of Craniomaxillofacial Fractures and Cervical Spine Injuries

INVESTIGATORS: E. Farkkila, Z. S. Peacock, A. Gervasini, R. J. Tannyhill, L. Petrovick, G. Velmahos, L. B. Kaban

The reported incidence of cervical spine injury (CSI) in patients with craniomaxillofacial fractures (CMF) is 1-4 percent. With improved diagnostic techniques (CT and MRI), the incidence of CSI may be higher. The presence of CSI in patients with CMF is crucial for pre-operative management and surgical treatment planning. Aims of this study are to determine: 1) Incidence of CSI in patients admitted with CMF, 2) incidence of CMF in patients admitted for CSI, 3) type of CMF most frequently associated with CSI, and 4) most common injury mechanism. This was a retrospective cohort study of adults evaluated at Massachusetts General Hospital (MGH) for CMF and CSI, from May 2007-June 2017. Potential subjects were identified using ICD-9 (2007-2014) and ICD-10 (2015-2017) codes within the MGH Trauma Registry. Demographic, clinical and radiologic variables were recorded. Uni and multivariate statistics were used to identify risk factors for CSI in CMF patients. During the study period, 4435 of 23,394 patients (18.9%) had CMF: Mandible (n=1156/4435, 26%), Midface (n=3412, 77%), Mandible + Midface (n=134, 3%). CSI was present in 1822 patients (7.8%) while both CMF and CSI were found in 297, 6.7% of CMF and 16.3 % of CSI patients. CSI occurred in 1.8% of subjects with isolated mandible and 10% of those with mandibular plus any midfacial factures. Fall was the most frequent mechanism of injury followed by automobile and motorcycle crashes respectively. Patients with a combination of mandibular and any midface fracture are at highest risk for CSI.

Poster Number 205

Amy Fiedler, MD, MS
Surgery, Resident | afiedler@partners.org

Surgical Aortic Valve Replacement Is Associated with Survival in Severe Aortic Regurgitation and Low Ejection Fraction

INVESTIGATORS: A. G. Fiedler, V. Bhambhani, E. Laikhter, M. H. Picard, M. M. Wasfy, G. Tolis, S. Melnitchouk, T. M. Sundt III, J. H. Wasfy

Although guidelines support aortic valve replacement (AVR) in patients with severe aortic regurgitation (AR) and left ventricular ejection fraction (LVEF) < 50%, severe left ventricular dysfunction (LVEF < 35%) is thought to confer high surgical risk. We sought to determine if a survival benefit exists with AVR compared to medical management in this high-risk population. Our institutional echocardiography database was queried to identify patients with severe AR and LVEF < 35%. Manual chart review was performed. Due to small sample size and population heterogeneity, corrected group prognosis method was applied, which calculates the adjusted individual survival curve using fitted Cox proportional hazard model. Average survival adjusted for co-morbidities and age was calculated using the weighted average of the individual survival curves. 254,614 echocardiograms were considered, representing 145,785 patients. 40 met inclusion criteria. Of those, 18 underwent AVR and 22 were managed medically. Mortality was 27.8% in the AVR group, and 91.2% in those medically managed. After multivariate adjustment, end stage renal disease (HR=17.633, p=0.0335) and peripheral arterial disease (HR= 6.050, p=0.0180) were associated with higher mortality. AVR was associated with lower mortality (HR=0.143, p=0.0490). Mean follow-up time was 6.58 years, and mean survival for AVR was 6.31 years. Even after adjustment for clinical characteristics and age, AVR is associated with higher survival for patients with low LVEF and severe AR. Although treatment selection bias cannot be completely eliminated, these results provide evidence that surgery may be associated with prolonged survival in this high-risk group.

Poster Number 206

Rajshri Mainthia, MD
Surgery, Resident | rmainthia@mgh.harvard.edu

What have we learned from malpractice claims after cholecystectomy? A 128 million dollar question

INVESTIGATORS: R. Mainthia, J. Bloom, K. Lillemoe, E. Mort

Cholecystectomy complications carry significant morbidity and rank among the leading sources of surgical malpractice claims. We aimed to study the contributing factors and costs of these claims given their emotional, physical, and financial toll on patients, providers, and the healthcare system. Using the CRICO Strategies' CBS database, representing ~30% of all paid and unpaid malpractice claims in the US, 4,081 claims filed against general surgeons from 1995-2015 were reviewed to isolate 745 cholecystectomy-related claims. A multivariable model was used to determine factors associated with claim outcome. The most common associated complications included bile duct injury (n=397), bowel perforation (n=96), and hemorrhage (n=78). The total cost for all claims over the study period was $128,496,004 and the average time from event to case close was over 3 years. 40% of claims resulted in patient payout; of these, all but one claim were settled out of court and the average cost per claim was $264,650. For claims not resulting in patient payout, most cases were denied, dropped, or dismissed (n=443), yet still averaged over $10,000 per claim in legal and administrative fees. On multivariable analysis, bile duct injury, bowel perforation, and high clinical severity were associated with patient payout, while a resident or fellow being named in a claim was negatively associated with patient payout ($p<0.05$). Cholecystectomy-related claims are costly and time-consuming. Strategies that reduce the risk and aid in recognition of cholecystectomy complications, as well as advance support of patients after poor outcomes, can improve clinical care and reduce litigation burden.

Poster Number 207

Thomas O'Shea, BA
Pathology, Research Technician | toshea@mgh.harvard.edu

The role of pDCs in formation of Treg-rich organized lymphoid structures in spontaneously accepted murine kidney allografts is dependent on mismatching of MHC Class II molecules at the H2-I-Ab locus

INVESTIGATORS: T. F. O'Shea, C. Yang, I. Rosales, N. Oh, D. K. Ndishabandi, P. S. Russell, J. C. Madsen, R. B. Colvin, A. Alessandrini

Our lab has demonstrated that murine kidney allografts are accepted in specific donor-recipient strain combinations. Histologic examination of spontaneously accepted DBA/2J (DBA) kidneys into C57BL/6 (B6) recipients reveal organized lymphoid structures around the renal cortex that are dense in CD3+Foxp3+ T-regulatory cells, which we term Treg rich organized lymphoid structures (TOLS). Here we characterize the composition of TOLS, and provide a potential mechanism for their induction. Analysis of spontaneously accepted DBA kidney allografts revealed the presence of PDCA-1+ cells in TOLS. We demonstrated that in vitro, DBA H2d pDCs can induce a high level of FoxP3 expression when co-cultured with naïve B6 H2b CD4+ T cells. This induction of FoxP3 was not observed in co-cultures using pDCs from donor strains that resulted in kidney allograft rejection. pDCs from bm12 mice, which are congenic to B6 except for an MHC class II mismatching through the H2-I-Ab locus, can induce Tregs from naïve B6 T cells in vitro, and, when transplanted into a B6 mouse, bm12 kidneys were spontaneously accepted with the development of TOLS. In contrast, bm1 MHC class I mismatched kidneys are accepted, but did not form TOLS. pDCs from B6.NOD mice have H2d genetics except for a g7 mutation at the H2-I-Ab locus and induce less Foxp3 positivity from naïve H2b T cells compared to fully H2d DBA pDCs. Spontaneous murine renal allograft tolerance may be mediated by plasmacytoid dendritic cells in TOLS, that induce functional Tregs upon recognition of an allogeneic MHC Class II locus.

Poster Number 208

Maggie Westfal, MD, MPH
Surgery, Research Fellow | mwestfal@partners.org

A Population-Based Analysis of Pediatric Breast Cancer

INVESTIGATORS: M. L. Westfal, D. C. Chang, C. M. Kelleher

The purpose is to evaluate trends in demographics and outcomes of pediatric breast cancer from 1973-2014 in a US population-based cohort. The Surveillance, Epidemiology, and End Results database was utilized to identify all pediatric patients (19 years old and younger) with malignant breast tumors diagnosed between 1973 and 2014. Analysis was performed using Stata Statistical Software (v13.1). Univariate survival analysis was completed using the log-rank test. Kaplan-Meier analysis investigated five-year survival rates across several variables. Correlations between categorical variables were made using the X2 test. 135 patients with breast malignancies were identified. The majority (85.19%) were 15-19 years old at the time of diagnosis, with a median age of 17 years old. Evaluation of race showed that the majority was white (67.91%). Carcinoma was the most prevalent histologic type (48.51%), followed by fibroepithelial tumors (FETs) (35.07%), and sarcoma (14.18%). FETs were twice as common in non-whites compared to whites ($p<0.05$). Analysis of histology by stage revealed that 100% of FETs were early stage disease ($P<0.0001$). Nearly half (46.67%) of the tumors tested were ER/PR negative, over twice as many when compared to the published adult estimate of 20%. Survival analysis revealed significant survival differences by stage, grade, and histology, but not race. Breast cancer remains a rare malignancy among pediatric patients. Although non-white patients had the majority of non-carcinomatous tumors with less advanced disease, this did not confer a survival advantage. In addition, this data suggests that adolescents may have higher rates of ER/PR negative breast cancer than adults.

Poster Number 209

Sherif Ahmed, PhD
Anesthesia, Critical Care and Pain Medicine, Research Fellow | sherif.ahmed@mgh.harvard.edu

Adeno-associated virus delivery of apoptosis-associated speck-like protein (ASC), a newly described schwannoma tumor suppressor, inhibits schwannoma growth in vivo

INVESTIGATORS: S. G. Ahmed, A. Abdelanabi, M. Doha, C. Maguire, A. Rachamimov, K. Stankovic, G. Fulci, G. Brenner

Schwannomas are benign tumors that cause a variety of severe neurological deficits and can also lead to death. Their treatment includes debulking procedures that can cause additional neurologic damage and are not always possible due to tumor location. Other therapeutic options include γ-irradiation and antiangiogenic therapy (bevacizumab), but both are limited in scope and γ-irradiation may induce malignant transformation of schwannomas. Overall, there is a paucity of therapeutic options for these tumors, partially due to limited knowledge of their genetic and biologic composition. Given their homogenous Schwann-lineage cellular composition, schwannomas are appealing targets for gene therapy. We have previously generated an adeno-associated serotype 1 virus (AAV1)-based vector delivering cytotoxic genes under the control of the Schwann-cell specific promoter, P0, specifically to schwannoma cells. We have now discovered that the pro-apoptotic intracellular signaling protein ASC is expressed in normal human nerves, but inhibited in schwannomas, and that ASC overexpression induces schwannoma cell apoptosis by activating caspase-3, caspase-9, and BH3-interacting domain death agonist (BID). We further demonstrate that an AAV1-P0 vector delivering ASC to intrasciatic schwannomas debulks these tumors and resolves tumor-associated pain without causing neurologic damage. These findings support ASC as a schwannoma tumor suppressor that acts as an upstream regulator of multiple apoptotic pathways. We have identified for the first time a tumor suppressor with high potential clinical utility for schwannoma gene therapy, and generated a vector that treats schwannomas via a novel mechanism that does not overlap with current treatments.

Poster Number 210

Lieselot Carrette, PhD
Molecular Biology, Research Fellow | carrette@molbio.mgh.harvard.edu

Applying knowledge of X chromosome inactivation for the treatment of X-linked diseases

INVESTIGATORS: L. L. Carrette, C. Wang, C. Wei, W. Press, R. Blum, W. Ma, R. J. Kelleher III, J. T. Lee

For over 20 years, our lab has been studying X chromosome inactivation, the process of silencing one of the two female X chromosomes for dosage compensation with males that only have one. In this project, we aim to apply the gathered knowledge for the treatment of X linked diseases. Within the inactive X chromosome (Xi) lays potentially a silenced cure. Our goal has been to unlock the Xi and restore expression of the good gene copy. We focus on Rett Syndrome (RTT), a severe neurodevelopmental disorder caused by a mutation in the X-linked Methyl-CpG-binding protein 2 (MECP2) that affects 1 in 10.000 females and for which there is currently no disease-specific treatment. Experiments in male mice have showed that re-expression of MECP2 can reverse the symptoms. However, reactivation of the stably silent Xi in females has been proven challenging. It is also presently unknown how much MECP2 expression is required for phenotypic improvement and on the other hand how much X reactivation will be toxic. Moreover, progress in RTT research has been hampered in part by the lack of reliable strong phenotypes in female mouse models. In this poster, we begin to address these questions in vitro and in vivo with a new bimodal drug combination and an improved female mouse model.

Poster Number 211

Reinier de Vries, BS
Surgery, Graduate Student | rjdevries@mgh.harvard.edu

Supercooling of human livers to extend the preservation time for transplantation

INVESTIGATORS: R. J. De Vries, S. N. Tessier, P. D. Banik, S. Ozer, S. Napgal, S. Cronin, H. Yeh, K. Uygun

Optimizing preservation of donor organs has the potential to dramatically improve the outcome of organ transplantation. Static cold storage (SCS) at +5°C is the current standard and allows for s maximum liver preservation time of 12 hours. We hypothesized that the preservation duration of human livers can be doubled to 24 hours, by storing the organ at -4°C in an ice-free, supercooled state. Marginal human livers were recovered from SCS using sub-normothermic machine perfusion (SNMP) before they were loaded with cryoprotectant agents (CPAs). The livers were supercooled and stored for 20 hours at -4°C. After supercooling the CPAs were washed out and the livers were recovered with SNMP. Pre- and post-supercooling SNMP conditions were identical. Vascular resistance, blood gas parameters, electrolytes, urea, liver enzymes and bile production were measured every 30 minutes during pre- and post- supercooling SNMP. Bilateral wedge biopsies for the measurement of energy status and conventional histology were also sampled throughout the protocol. As compared to pre-supercooling SNMP conditions, post-supercooling livers showed preserved energy status, which is correlated to graft viability. Also, no differences were observed in arterial resistance and oxygen consumption. Viable livers produced bile an urea post-supercooling. Histology shows minimal necrosis, no edema and limited endothelial injury. ALT and AST, were slightly elevated post-supercooling. Preliminary results demonstrate the feasibility of storing whole human livers in the supercooled state to double the preservation duration, as compared to clinical standards. Moreover, we identified important CPAs and perfusion conditions which are critical for success.

Poster Number 212

Haichuan Hu, MD
Cancer Center, Research Fellow | hhu5@partners.org

Decoding tumor microenvironment to enhance NSCLC targeted therapy

INVESTIGATORS: H. Hu, L. Sequist, Z. Piotrowska, D. Kodack, A. Hata, M. Niederst, C. Benes, J. Engelman

Tyrosine kinase inhibitors (TKI) have yielded great responses in non-small-cell lung cancer (NSCLC) with EGFR mutations and ALK translocations. However, these and other targeted therapies are limited by intrinsic and acquired drug resistance. The previous study from our group was looking into tumor autonomous resistance mechanisms by developing patient-derived cancer models (PDCs). In this study, we aimed to decipher the non-autonomous resistance mechanisms via tumor microenvironment by developing patient-derived fibroblast (PDF) cell lines. Cancer-associated fibroblast cells are isolated directly from EGFR mutant and ALK translocated NSCLC biopsies. Over 30 PDFs models have been established, which represent different clinical features and response profiles. By co-culturing the PDCs with PDFs, we found that there is considerable variability in both models for their magnitude and mechanism by which the TKI treatment is desensitized. Both HGF dependent and HGF independent resistance mechanisms can be overcome by specific therapeutic combinations. Together, our results indicate that PDFs are clinically relevant models for deciphering non-autonomous resistance mechanisms, that they are heterogeneous in protecting cancer cells from TKI treatment, and that the resistance mediated by PDFs can be overcome by specific therapeutic combinations.

Poster Number 213

Dennis Jones, PhD
Radiation Oncology, Research Fellow | dennisjones@steele.mgh.harvard.edu

Methicillin-resistant Staphylococcus aureus causes sustained collecting lymphatic vessel dysfunction

INVESTIGATORS: D. Jones, E. F. Meijer, C. Blatter, S. Liao, E. R. Pereira, E. M. Bouta, K. Jung, S. M. Chin, P. Huang, L. L. Munn, B. J. Vakoc, M. Otto, T. P. Padera

Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of morbidity and mortality worldwide and is a frequent cause of skin and soft tissue infections (SSTIs). Lymphedema—fluid accumulation in tissue caused by impaired lymphatic vessel function—is a strong risk factor for SSTIs. SSTIs also frequently recur in patients and sometimes lead to acquired lymphedema. However, the mechanism of how SSTIs can be both the consequence and cause of lymphatic vessel dysfunction is not known. Intravital imaging in mice revealed both an acute reduction in lymphatic vessel contractility and lymph flow after localized MRSA infection. Moreover, chronic lymphatic impairment is observed long after MRSA is cleared and inflammation is resolved. Associated with decreased collecting lymphatic vessel function was the loss and disorganization of lymphatic muscle cells (LMCs), which are critical for lymphatic contraction. In vitro, incubation of MRSA-conditioned supernatant led to LMC death. Proteomic analysis identified several accessory gene regulator (agr)-controlled MRSA exotoxins that contribute to LMC death. Infection with agr mutant MRSA resulted in sustained lymphatic function compared to animals infected with wild type MRSA. Our findings suggest that agr is a promising target to preserve lymphatic vessel function and promote immunity during SSTIs.

Poster Number 214

Junghyun Lim, BA
Medicine, Graduate Student | jklim@mgh.harvard.edu

Environmental risk factors according to genetic susceptibility for symptomatic gallbladder disease

INVESTIGATORS: J. Lim, J. Wirth, K. Wu, E. Giovannucci, A. T. Chan, A. D. Joshi

We and others have identified genetic susceptibility for gallstone disease through genome-wide association studies (GWAS). However, no study has examined gene-environment interactions (GxE) using GWAS-identified SNPs for gallstone risk. 4784 cholecystectomy cases were matched to 14,344 controls within three prospective cohorts: NHS, HPFS, NHS2. We assessed gallstone risk factors prospectively from questionnaires prior to case diagnosis, and constructed genetic risk score (GRS) of six GWAS SNPs. GxE were tested using likelihood ratios comparing two conditional logistic models: ±product terms of continuous GRS and categorical exposure variables, adjusted for other gallstone risk factors and Bonferroni corrected(α=2x10-3). Higher BMI, use of menopausal hormones, hypertension, and rapid weight loss increased gallstone disease risk(P<10-4), whereas healthy dietary score, physical activity, and coffee intake were inversely associated(P<10-3). The association of BMI with gallstone disease appeared to differ according to GRS in men(P=2.9x10-3), but not among women. This effect modification was strongest according to variation at CYP7A1 SNP rs6471717 (Pinteraction=3.7x10-4). Among those with low-risk CYP7A1 A/A genotype, obese men had an OR for gallstone disease of 3.2(2.2-4.6) compared with normal-weight men. In contrast, among men with high-risk G/G genotype, obese men were not at higher risk, OR=0.7(0.4-1.5). The association of BMI with gallstone risk in men appears to differ according to genetic predisposition owing primarily to variation in CYP7A1, which catalyzes rate-limiting step for hepatic conversion of cholesterol to bile acids. These results suggest that lifestyle modifications may be less effective in preventing symptomatic gallstone disease among those with high genetic susceptibility.

Poster Number 215

Akito Nakagawa, PhD
Anesthesia, Critical Care and Pain Medicine, Instructor | anakagawa@mgh.harvard.edu

A triazole disulfide compound increases the affinity of hemoglobin for oxygen and reduces the sickling of red blood cells

INVESTIGATORS: A. Nakagawa, M. Ferrari, G. Schleifer, M. K. Cooper, C. Liu, B. Yu, L. Berra, E. S. Klings, M. K. Safo, O. Abdulmalik, D. B. Bloch, W. M. Zapol

Sickle cell disease is an inherited disorder of hemoglobin (Hb). During a sickle cell crisis, deoxygenated sickle hemoglobin (HbS) polymerizes to form fibers in red blood cells (SS RBCs), causing the cells to adopt a "sickled" shape. Consequences of RBC sickling include loss of RBC deformability, hemolysis, vaso-occlusion, severe pain, and organ damage. Increasing the affinity of Hb for oxygen could be a successful strategy for reducing sickling of SS RBCs, because oxygenated HbS does not form polymers and is therefore unlikely to sickle. To identify potential new treatments for sickle cell disease, we screened a library of small molecules and identified a triazole disulfide (TD-3) that markedly increased the affinity of Hb for oxygen and reduced sickling of SS RBCs in vitro. Covalent modification of the Hb thiol β-Cys93 by TD-3 is shown to increase the affinity of Hb for oxygen. The crystal structure of Hb complexed with TD-3 revealed that the covalent modification of the Hb thiol β-Cys93 stabilized the R-state of Hb. Injection of TD-3 (100 mg/kg) into the tail vein of C57BL/6 mice increased the affinity of murine Hb for oxygen and increased the percentage of TD-3 modified murine Hb. All of the mice treated with TD-3 appeared normal for at least 24 h after the injection. The results of this study show that TD-3, and possibly other triazole disulfide compounds that bind to Hb β-Cys93, may prove to be new treatments for patients with sickle cell disease.

Poster Number 216

Amanda Ramos, MD
Obstetrics and Gynecology, Clinical Research Fellow | aramos7@mgh.harvard.edu

Immune Checkpoint Signatures Across Endometrial Cancer Histologies

INVESTIGATORS: A. Ramos, S. Fortin, V. Melchert, D. Jenkins, W. Growdon, D. Borger

Therapies targeting immune checkpoints have revolutionized cancer treatment. Subsets of endometrial carcinoma harbor microsatellite instability (MSI) that has been associated with response to PD-1 inhibition. The purpose of this study was to investigate the immune checkpoint landscape across multiple endometrial cancer subtypes and ascertain whether more complex signatures indicate directions for combination immune therapies. Sixty endometrial tumor samples were obtained consisting of MSI low grade endometrioid (n=11), microsatellite stable (MSS) low grade endometrioid (n=11), MSI high grade endometrioid (n=16), carcinosarcoma (n=12), and uterine serous carcinoma (n=10) histologies. Immune checkpoint RNA expression signatures were evaluated using a Luminex platform and confirmed at the protein level through immunohistochemistry.

Expression for CTLA-4, PD-1, PD-L1, LAG-3, and TIM-3 were compared across the histologies. Levels of TIM-3 and CTLA-4 expression were significantly elevated in endometrioid carcinoma ($P<0.01$, $P<0.001$ respectively) relative to carcinosarcoma or serous carcinoma. More distinct signatures were observed across the endometrioid subtypes. High grade endometrioid tumors were associated with elevated checkpoint expression (LAG-3 ($P=0.04$), PD-L1 ($P=0.04$), and CTLA-4 ($P=0.04$)), as was the presence of MSI (TIM-3 ($P=0.02$), PD-L1 ($P=0.01$), and CTLA-4 ($P=0.014$)). When grade and MSI were factored together, PD-1 was significantly elevated in MSI high grade versus MSI low grade cancers ($P=0.04$). PD-L1 and PD-L2 expression was also significantly elevated in endometrioid cancers. A complex landscape of regulatory immune receptors was found across endometrial cancer histologies, particularly in endometrioid carcinomas that were both grade and MSI-dependent. These data suggest directions for combined immune blockade therapy in endometrial carcinoma.

Poster Number 217

Yin Ren, MD, PhD
Otolaryngology, Resident | yren@partners.org

Tumor-Penetrating Delivery of siRNA Therapeutics to Human Vestibular Schwannomas to Ameliorate Hearing Loss

INVESTIGATORS: Y. Ren, J. E. Sagers, L. D. Landegger, S. N. Bhatia, K. M. Stankovic

Vestibular schwannoma (VS) is the most common tumor of the cerebellopontine angle and the fourth most common intracranial tumor. They are associated with significant morbidity including asymmetric sensorineural hearing loss (SNHL) which affects 95% of patients. Recently, tumor necrosis factor alpha (TNFa) was identified as an ototoxic molecule secreted by VS. Nevertheless, the genomic landscape of VS is complex and many of the molecules implicated in VS pathogenesis or VS-associated SNHL represent targets not amenable to traditional antibody-based or small molecule therapeutics. Tumor-targeted delivery of small interfering RNA (siRNA) therapeutics provides a direct and effective means to interrogate targets while minimizing off-target effects. To establish a pre-clinical model for therapeutic inhibition of targets in VS for the treatment of hearing loss, archived tumor specimens, an established schwannoma cell line, and fresh tumor cells derived from patients with sporadic VS were screened. A novel tumor-penetrating nanoparticle, directed by the tumor-homing peptide iRGD, was selectively taken up by primary VS cultures in vitro via interactions with alpha-v/beta-3/5 integrins and neuropilin-1 receptors. Cellular uptake was inhibited by a neutralizing antibody against alpha-v integrin in a dose-dependent fashion. When applied to primary VS cultures, iRGD-targeted nanoparticles delivered siRNA therapeutics against TNFα in a receptor-specific fashion to potently silence gene expression and protein secretion. Taken together, our results provide a first proof of principle for tumor-targeted, nanoparticle-mediated delivery of siRNA therapeutics to VS and establish a novel platform for the development and pre-clinical screening of molecular therapeutics against VS.

Poster Number 218

Jessica Sagers, BA
Otolaryngology, Graduate Student | jsagers@g.harvard.edu

Computational repositioning and preclinical validation of mifepristone for human vestibular schwannoma

INVESTIGATORS: J. E. Sagers, A. S. Brown, S. Vasilijic, R. Lewis, M. I. Sahin, L. D. Landegger, R. H. Perlis, I. S. Kohane, D. B. Welling, C. J. Patel, K. M. Stankovic

The computational repositioning of existing drugs represents an appealing avenue for identifying effective compounds to treat diseases with no FDA-approved pharmacotherapies. Here we present the largest meta-analysis to date of differential gene expression in human vestibular schwannoma (VS), a debilitating intracranial tumor, and use these data to inform the first application of algorithm-based drug repositioning for this tumor class. We apply an open-source computational drug repositioning platform to gene expression data from 80 patient tumors and identify eight promising FDA-approved drugs with potential for repurposing in VS. Of these eight, mifepristone, a progesterone and glucocorticoid receptor antagonist, consistently and adversely affects the morphology, metabolic activity, and proliferation of primary human VS cells and HEI-193 human schwannoma cells. Mifepristone treatment reduces VS cell viability more significantly than cells derived from patient meningiomas, while healthy human Schwann cells remain unaffected. Our data recommend a Phase II clinical trial of mifepristone in VS.

Poster Number 219

Baehyun Shin, PhD
Center for Genomic Medicine, Research Fellow | bhshin@mgh.harvard.edu

Novel mutant huntingtin-specific DNA aptamers ameliorate metabolic defect in Huntington's disease derived neuronal progenitor cells

INVESTIGATORS: B. Shin, R. Jung, H. Oh, G. Owens, S. L. Cotman, M. E. MacDonald, R. Vijayvargia, I. Seong

The Huntington's disease (HD) CAG repeat expansion, that is the root genetic cause of this neurodegenerative disorder, elongates the polyglutamine tract in huntingtin. This seemingly slight change to the primary amino acid sequence alters the physical structure of the mutant protein and enhances its activity. Here, we have identified a set of G-quadruplex-forming DNA aptamers (MS1, MS2, MS3, MS4) that bind mutant huntingtin proximal to lysines K2932/K2934 in the carboxyl-terminal CTD-II domain. Aptamer-binding to mutant huntingtin, abrogated the enhanced polycomb repressive complex 2 (PRC2) stimulatory-activity conferred by the expanded polyglutamine tract. In HD, but not normal, neuronal progenitor cells (NPC), MS3 aptamer co-localized with endogenous mutant huntingtin and was associated with significantly decreased PRC2 activity. Furthermore, MS3 transfection protected HD NPC against starvation-dependent stress with increased ATP. Therefore, DNA aptamers can preferentially target mutant huntingtin and modulate a gain of function endowed by the elongated polyglutamine segment. These mutant huntingtin binding aptamers provide novel molecular tools for delineating the effects of the HD mutation and encourage mutant huntingtin structure-based approaches to therapeutic development.

Poster Number 220

Jordan Smoller, MD
Psychiatry, Professor | jsmoller@partners.org

The New England Precision Medicine Consortium of the All of Us Research Program

INVESTIGATORS: N.M. Allen, H. Hemley, E. W. Karlson, S. T. Weiss, S. N. Murphy, G. T. O'Connor, C. R. Clark, S. M. Edgman-Levitan, D. C. Xerras, T. A. Battaglia, J. W. Smoller

The All of Us Research Program (AoURP) was established by the National Institutes of Health (NIH) with the goal of building a research program of one million or more U.S. volunteers who are engaged as partners in a longitudinal cohort study. AoURP is a key element of the Precision Medicine Initiative (PMI) to prevent disease and develop individualized treatments that account for differences in lifestyle, environment and biology through collaborations between researchers, health care providers and patients. Partners HealthCare System (PHS) and Boston Medical Center (BMC) are collaborating as AoURP's New England Precision Medicine Consortium (NEPMC). Funding for the NEPMC began in October 2016 with alpha phase launching on September 20, 2017 and beta phase on October 2, 2017. Participants provide lifestyle and medical history surveys, mobile health data, and consent to link blood and urine samples to electronic health record data. Scientific opportunities include developing quantitative estimates of risk for a range of diseases by integrating environmental exposures and genetic factors; identifying the causes of individual variation in response to commonly used therapeutics; discovering biological markers that signal increased or decreased risk of developing common diseases; developing solutions to health disparities; use of mobile health technologies to correlate activity, physiological measures, and environmental exposures with health outcomes; empowering study participants with data and information to improve their own health; and creating a platform to enable trails of targeted therapies. NEPMC will cohere to enroll 93,000 participants, with a target of 46% participants historically underrepresented in biomedical research (UBR).

Poster Number 221

Jian Teng, PhD
Neurology, Instructor | jteng2@mgh.harvard.edu

Repurposing mefloquine at low dose in treatment of malignant and diffuse brain tumors in children

INVESTIGATORS: J. Teng, G. Lashgari, E. Tabet, L. Carvalho, B. A. Tannous

Diffuse intrinsic pontine gliomas (DIPG), the leading cause of death by brain tumors in children, are highly aggressive tumors found in an area of the brainstem. Despite intensive multimodality treatment, refractory disease and relapses are frequent events. Using DIPG cells established from newly diagnosed and recurrent patients, we performed a repurposing drug screen aiming at repositioning known drugs against DIPG and identified the FDA-approved drug mefloquine as a promising candidate. This extensively used anti-malaria drug typically causes adverse neurological effects because it crosses the blood-brain barrier (BBB) and accumulates in the brain at relatively high concentration. In this study, we took advantage of these unique characteristics of mefloquine and use low dose of this compound to achieve enough amount in the brain and target the infiltrative DIPG reservoir, while avoiding the neurological side effects. Our results showed that low dose (300 nM - 1 μM or 2.5 - 7.5 mg/kg, 1/30 to 1/10 of concentration that being used in anti-malaria settings) of mefloquine could cross the BBB, achieved the desired local concentration, stimulated ROS production in vitro and in vivo, which halted DIPG cells infiltration and/or metastasis by initiating cell apoptosis; thus, halted tumor growth and improved the overall survival in DIPG mouse brainstem models. The anticancer effects of mefloquine was at least partially mediated by inhibition of Akt phosphorylation, c-Jun N-terminal kinase (JNK) activation, extracellular signal-regulated kinase (ERK) activation, and adenosine monophosphate-activated protein kinase (AMPK) signaling. These results provide necessary evidence to move this drug into a clinical trial.

Poster Number 222

Satoshi Yoda, MD, PhD
Cancer Center, Research Fellow | syoda@mgh.harvard.edu

Sequential ALK inhibitors select for lorlatinib-resistant compound ALK mutations in ALK-positive lung cancer

INVESTIGATORS: S. Yoda, J. J. Lin, M. S. Lawrence, L. Dardaei, K. Prutisto-Chang, I. Dagogo-Jack, J. F. Gainor, A. N. Hata, A. T. Shaw

The cornerstone of treatment for advanced ALK-positive lung cancer is sequential therapy with increasingly potent and selective ALK inhibitors. The third-generation ALK inhibitor lorlatinib has demonstrated clinical activity in patients who failed previous ALK inhibitors. Here, we integrated experimental modeling of lorlatinib resistance with genomic analysis of paired pre-treatment and progression biopsies for patients. To define the spectrum of ALK mutations that confer lorlatinib resistance, we performed accelerated N-ethyl-N-nitrosourea (ENU) mutagenesis screening of Ba/F3 cells expressing EML4–ALK. Under comparable conditions, ENU mutagenesis generated numerous crizotinib-resistant but no lorlatinib-resistant clones harboring single ALK mutations. In similar screens with EML4-ALK containing single ALK resistance mutations, numerous lorlatinib-resistant clones emerged harboring compound ALK mutations, including a highly refractory ALK G1202R/L1196M double mutant. To determine the clinical relevance of these mutations, we analyzed repeat biopsies from lorlatinib-resistant patients. Four of thirteen samples (31%) harbored compound ALK mutations, including ALK G1202R/L1196M. Whole exome sequencing confirmed the stepwise accumulation of ALK mutations during sequential treatment. Thus, treatment with sequential ALK inhibitors can select for compound ALK mutations that confer high-level resistance to all available ALK targeted therapies. A more efficacious long-term strategy may be upfront treatment with third-generation ALK inhibitors in order to prevent the emergence of on-target resistance.

Poster Number 223

Binglan Yu, PhD
Anesthesia, Critical Care and Pain Medicine, Assistant Professor | byu1@mgh.harvard.edu

Development of a portable mini-generator to safely produce nitric oxide for the treatment of infants with pulmonary hypertension

INVESTIGATORS: B. Yu, M. Ferrari, G. Schleifer, M. Wepler, W. M. Zapol, D. B. Bloch

To test the safety of a novel miniaturized device that produces nitric oxide (NO) from air by pulsed electrical discharge, and to demonstrate that the generated NO can be used to vasodilate the pulmonary vasculature in rabbits with chemically-induced pulmonary hypertension. A miniature NO (mini-NO) generator was tested for its ability to produce therapeutic levels (20-80 parts per million (ppm)) of NO, while removing potentially toxic gases and metal particles. We studied healthy 6-month-old New Zealand rabbits weighing 3.4±0.4 kg (mean±SD, n=8). Pulmonary hypertension was induced by chemically increasing right ventricular systolic pressure to 28-30 mmHg. The mini-NO generator was placed near the endotracheal tube. Production of NO was triggered by a pediatric airway flowmeter during the first 0.5 seconds of inspiration. In rabbits with acute pulmonary hypertension, the mini-NO generator produced sufficient NO to induce pulmonary vasodilation. Potentially toxic nitrogen dioxide (NO2) and ozone (O3) were removed by the Ca(OH)2 scavenger. Metallic particles, released from the electrodes by the electric plasma, were removed by a 0.22 μm filter. While producing 40 ppm NO, the mini-NO generator was cooled by a flow of air (70 ml/min) and the external temperature of the housing did not exceed 31°C. The mini-NO generator safely produced therapeutic levels of NO from air. The mini-NO generator is an effective and economical approach to producing NO for treating neonatal pulmonary hypertension and will increase the accessibility and therapeutic uses of life-saving NO therapy worldwide.

Poster Number 224

Martin Zhang, PhD
Neurology, Assistant Professor | Zhang.Can@mgh.harvard.edu

Curcumin and select analogs reduce levels of Alzheimer's disease-associated amyloid-β proteins and deposition of amyloid-β plaques

INVESTIGATORS: X. Shen, J. Yang, C. Ran, R. Tanzi, C. Zhang

Alzheimer's disease (AD) is a devastating neurodegenerative disease and the primary cause of dementia. Currently there is no available cure. The pathology of AD is characterized by two hallmarks: β-amyloid plaques primarily comprised of the small protein, amyloid-β (Aβ), and neurofibrillary tangles composed of aggregated tau protein. Aβ is produced by the cleavages of amyloid-β precursor protein (APP) by β- and γ-secretase. The aggregation-prone Aβ42 peptide is essential to AD pathology and more prevalent than other Aβ species in cerebral β-amyloid plaques. Curcumin is a compound in the widely used culinary spice, turmeric, which possesses potent biological activities, including anti-inflammatory and anti-oxidant properties. Here we investigated the mechanisms of actions of curcumin in AD pathology and showed that curcumin potently lowers Aβ levels by attenuating the maturation of APP in the secretory pathway. Next, we found that curcumin markedly decreased the levels of intracellular oligomeric Aβ that tend to aggregate using a nanoplasmonic fiber tip probe technology. Furthermore, we established a library of over 100 curcumin analogs and found that select analogs acted in curcumin-like mechanisms that reduced β-amyloid pathology in a transgenic AD mouse model. Particularly, one compound may have higher therapeutic potential in AD owing to its effects in upregulating microglia phagocytosis of Aβ42 peptide. Collectively, our data provide cellular mechanisms of action for curcumin/analogs' ability to attenuate β-amyloid pathology, which, together with in vivo data, suggest that curcumin/analogs may prove useful in developing pharmacological agents for the effective treatment and prevention of AD-related β-amyloid pathology.

ECOR Administrative Offices | 125 Nashua Street, Suite 822 | Boston, MA 02114 | ecor@mgh.harvard.edu

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