

SAC 2019 Theme:

Mass General's Most Valuable Research Resource: Our Faculty

71st Meeting of the
MGH Scientific Advisory Committee

SAC 2019

April 3 & 4, 2019
Simches Auditorium
185 Cambridge Street, 3rd Floor

Poster Session Abstracts



MASSACHUSETTS
GENERAL HOSPITAL

RESEARCH INSTITUTE

Contents

Agenda	3
James P. Timilty Middle School Students	6
Poster Session Floor Plans	7
Poster Listing by Category	9
Abstracts	18
Alphabetical Listing by Poster Presenter	106

Wednesday, April 3, 2019
Simches 3.110

Celebration of SCIENCE

10:00 am - 1:30 pm	POSTER SESSION 10:00 - 11:30 am Session 1 12:00 - 1:30 pm Session 2	<i>Simches, Floor 2</i>
2:00 - 5:00 pm	CELEBRATION OF SCIENCE Welcome and Opening Remarks <i>Peter L. Slavin, MD, President, MGH</i>	<i>Simches, 3.110</i>
2:15 - 2:55 pm	Introduction to SAC 2019 & Research at MGH <i>David E. Fisher, MD, PhD, Chair, Executive Committee on Research (ECOR)</i>	
2:55 - 3:30 pm	2019 Martin Prize for Clinical Research <i>Trial of Fingolimod versus Interferon Beta-1a in Pediatric Multiple Sclerosis</i> <i>Tanuja Chitnis, MD</i>	
3:30 - 3:50 pm	Break	
3:50 - 4:25 pm	2019 Martin Prize for Fundamental Research <i>Engineered Sialylation of Pathogenic Antibodies In Vivo Attenuates Autoimmune Disease</i> <i>Robert M. Anthony, PhD</i>	
4:25 - 5:00 pm	2019 Howard M. Goodman Fellowship <i>Combinations of Molecular Engineering Strategies in Immune Cell Design</i> <i>Marcela V. Maus, MD, PhD</i>	

Agenda

Thursday, April 4, 2019
Simches 3.110

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|---------------------|--|
| 8:00 am | Welcome and Introduction
<i>Peter L. Slavin, MD</i> |
| 8:05 – 8:40 am | Interview of Harvard University President Bacow
<i>Lawrence S. Bacow, JD, MPP, PhD, President, Harvard University</i>
<i>Linda Henry, Managing Director of the Boston Globe and STAT</i> |
| 8:40 – 9:20 am | Current State of MGH Research Faculty Introduction
<i>David E. Fisher, MD, PhD</i> |
| | Research Quality of Life Survey
<i>Karen Donelan, ScD, EdM</i> |
| | CNY Quality of Life Committee
<i>David M. Lengenau, PhD</i> |
| 9:20 - 9:35 am | Discussion |
| 9:35 - 10:15 am | Stories from our Faculty
<i>Yakeel Quiroz, PhD; Miguel N. Rivera, MD; Bakhos A. Tannous, PhD; Karen K. Miller, MD</i> |
| 10:15- 10:30 am | Discussion |
| 10:30- 10:45 am | Break |
| 10:45- 11:40 am | Facilitating Career Development
The Center for Faculty Development (CFD)
<i>Anne Klibanski, MD, Director, CFD</i>
<i>Dennis Brown, PhD, Director, Office for Research Career Development (ORCD)</i>
<i>Nancy A. Rigotti, MD, Director, Office for Women's Careers (OWC)</i>
<i>Rochelle Walensky, MD, MPH, 2002 Claflin Awardee</i> |
| 11:40 am - 12:00 pm | Discussion |
| 12:00 - 1:15 pm | Faculty Lunch |

Agenda

Thursday, April 4, 2019
Simches 3.110

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|----------------|---|
| 1:30– 2:15 pm | Attracting and Engaging a Diverse Research Workforce
The Center for Diversity and Inclusion (CDI)
<i>Winfred Williams, MD, Founding Director, CDI</i>
<i>Elena Olson, JD, Executive Director, CDI</i>
<i>Oluwaseun Johnson-Akeju, MD, 2011 Physician-Scientist Development Awardee</i>
<i>Elsie Taveras, MD, MPH, Pediatrics and the Kraft Center for Community Health</i> |
| 2:15 – 2:30 pm | Discussion |
| 2:30 - 2:45 pm | Faculty Retention in a Soft Money Environment
Bridging the NIH Funding Gap
<i>Robert E. Kingston, PhD</i> |
| 2:45 - 3:00 pm | Discussion |
| 3:00 - 3:30 pm | Sustaining our Talent
<i>Susan A. Slaughaupt, PhD, Scientific Director, Mass General Research Institute</i>
<i>Jennifer S. Temel, MD, Hostetter MGH Research Scholar</i>
<i>Mike McNally, Vice President for Development</i> |
| 3:30 - 3:45 pm | Discussion |
| 3:45 - 3:50 pm | Looking Ahead |
| 3:50 - 4:20 pm | SAC Member Session (<i>closed</i>) |
| 4:20 - 5:00 pm | Open Discussion & Debrief: Hospital/Research Leadership & SAC Members (<i>closed</i>) |

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 mentors are shown in parenthesis.

7th Grade Students

Genesis Anes - *Which liquid will cause the most abundant bean seed germination?*
(Ellen Wheeler & Susannah Phillips, Cancer Center)

Marissa Dossantos - *What is the effect of temperature on fingerprints?*
(Samantha LaPierre, Surgery)

Kyann Hay - *How does music genre affect your heart rate?*
(Katie Hardin, Medicine & Robyn Farrell, Cardiology)

Essence Titus - *What is the effect of different liquids on plant growth?*
(Taryn Cordani, Development)

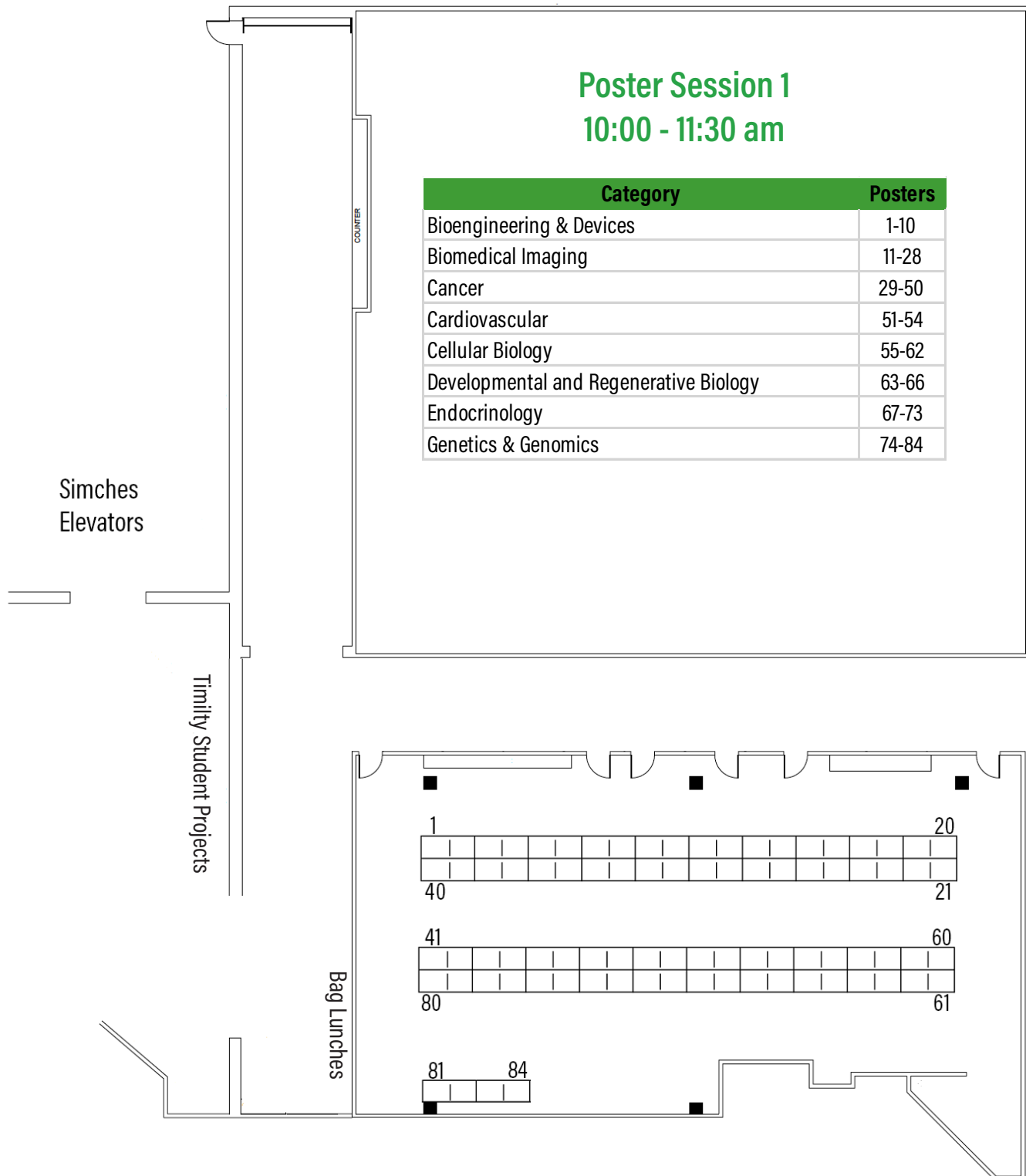
Melanie Monteiro Teixeira - *What is the effect of beverages on teeth staining?*
(Kaitlyn Torres & Rachel Peabody, Medicine)

Chase Noel - *What is the effect of different pH levels on grass?*
(Ramesh Narayanaswamy, Partners Healthcare)

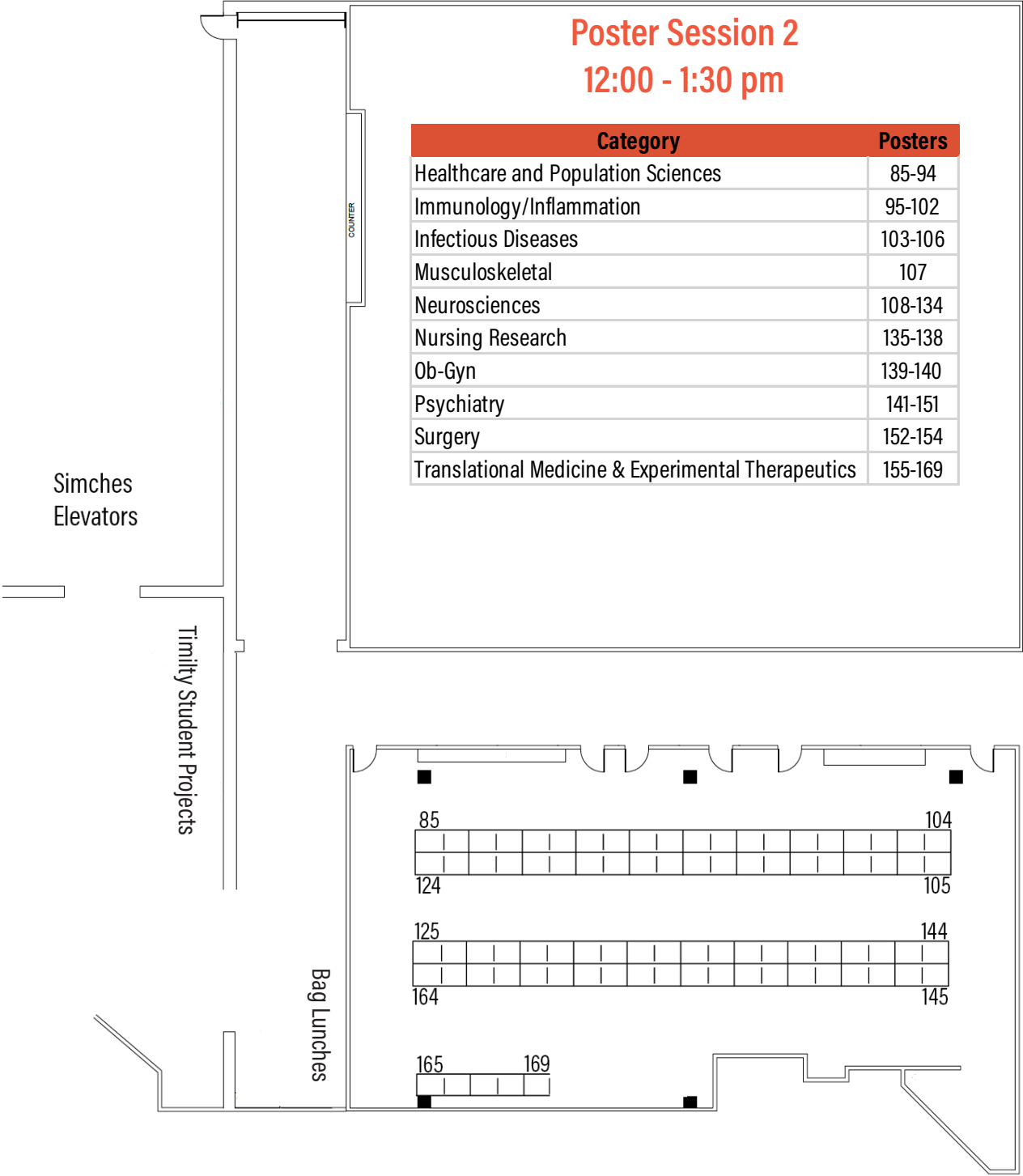
Jehlani President - *Do some materials create more static electricity than others?*
(Bailey Montgomery, Center for Genomic Medicine)

Akalia White - *What is the effect of increasing oxygen to the brain?*
(Ben Isenberg & Caroline Kelberman, Psychiatry)

Session 1 Floor Plan



Session 2 Floor Plan



SESSION 1 POSTERS

Presented 10:00 - 11:30 am

Bioengineering & Devices

Fuenfer, Michael

A Self-Contained Crank-Powered Battery Replacement Module for Critical Diagnostic and Therapeutic Medical Instruments

Jo, Min Joo

Bioengineering of exosomes with in vivo fate controllable, charge-modifiable NIR fluorophores

Ko, Jina

Droplet-based single EV profiling for the improvement of immunotherapy

Lee, Seungwoo

Optimization of micro-coil designs for precise activation of primary visual cortex

Parrish, John

CIMIT Platforms and Processes to Develop Medical Technology for MGH and Across the World

Ryu, Sang Baek

Spatially confined evoked responses of mouse visual cortex by magnetic stimulation using micro-coil

Sabuhi, Wali

Assessing Therapeutic Efficacy in Postoperative Recovery of Rats by Monitoring Pain and Gait

Sajjadi, Amir

Multi-Channel Fluorescence Imaging System for Intraoperative Image-Guided Surgery

Sun, Haoqi

Sleep Staging from Electrocardiography and Respiratory Effort with a Deep Learning Model

Wu, Tong

Engineering distal pulmonary epithelium from induced pluripotent stem cells (iPSCs) derived alveolar cells

Biomedical Imaging

Adlueu, Eeswar

Developing NIR-II Imaging System for Intraoperative Image-Guided Surgery

Aganj, Iman

Expected label value computation for atlas-based medical image segmentation

Alt, Clemens

Cerebrospinal Fluid Transport to the Retina Enables Detection of CNS Inflammation by Retinal Imaging

Girouard, Vincent

Novel Software Solution for Intraoperative Imaging Procedures with Mesoscale Image Analyses

Goenka, Chhavi

Implantable optical sensor for measuring muscle oxygenation in vivo in elephant seals

Kang, Homan

Tumor Targetability of PEGylated Fluorophores via Small Size EPR Effect

Katagiri, Wataru

Real-Time Imaging of Vaccine Biodistribution Using Zwitterionic NIR Nanoparticles

Mo, Dandan

Combining active learning and deep learning in image classification of blood cells

Montesi, Sydney

Collagen-Targeted Positron Emission Tomography (PET) in Pulmonary Fibrosis: Initial Human Experience

Nomura, Shinsuke

Bioengineered Nanoprobe for Targeting Gastrointestinal Stromal Tumors

Park, Kate

Structure-Inherent NIR Imaging of Brown Adipose Tissue

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2

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6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

Posters

Ramos-Torres, Karla <i>Evaluation of K⁺ channel tracer [18F]3F4AP in human tumor xenografts in mice: a potential biomarker in cancer</i>	22	Goruppi, Sandro <i>Autophagy controls the stability of CAF gene program repressor CSL/RBPJk through an SQSTM1/p62-dependent mechanism</i>	33
Ryu, Jiheun <i>Unsedated diagnosis of eosinophilic esophagitis using spectrally encoded tethered capsule endomicroscopy</i>	23	Guarino, Carla <i>Exploring the role of MTDH/AEG-1 lipidation in cancer progression</i>	34
Sajedi, Salar <i>Limited angle PET imaging with time-of-flight information for intraoperative use</i>	24	Khan, Amjad <i>Design and evaluation of applicators for photodynamic therapy of oral cavity tumors in low resource settings</i>	35
Sepulcre, Jorge <i>Genetic Syndromes and Connectomic-Genetics of the Visuo-Motor Cortical System</i>	25	Kiyokawa, Juri <i>Modification of extracellular matrix improves oncolytic virus immunotherapy in glioblastoma</i>	36
Tetrault, Marc-Andre <i>A multi-modality microscope for single cell radioluminescence imaging studies</i>	26	Kontos, Filippou <i>IL-15/B7-H3 TriKEs-based specific immunotherapy for pancreatic ductal adenocarcinoma (PDAC)</i>	37
Yen, Yi-Fen <i>Hyperpolarized ¹³C Metabolic Imaging</i>	27	Kurokawa, Tomohiro <i>Can intrahepatic delivery of Chimeric Antigen Receptor (CAR) T cells enhance their safety and efficacy against intrahepatic cholangiocarcinoma (ICC)?</i>	38
Zhou, Yu-Peng <i>Development of PET tracers for calcium channel $\hat{I}\pm 2\hat{I}^{-1}$ to image pain</i>	28	Li, Tao <i>Mesenchymal stem cell-derived extracellular vesicles promote proliferation and migration in breast cancer cells through activation of the ERK pathway</i>	39
Cancer		Li, Yanjing <i>Differential inflammatory response dynamics in normal lung following stereotactic body radiation therapy with protons versus photons</i>	40
Bukhari, Syed <i>Role of RNA modifications in quiescent chemo-resistant cells</i>	29	Lipkin, Lev <i>Clinical Machine Learning Model for Mismatch Repair Deficiency Prediction</i>	41
Chen, Ivy <i>Blocking CXCR4 alleviates desmoplasia, increases T-lymphocyte infiltration, and improves immunotherapy in metastatic breast cancer</i>	30	Lv, Xiangmin <i>Yes-Associated Protein 1 (YAP1) Increases Human Papillomavirus (HPV) Infection and Inhibits Innate Immunity to Drives Cervical Carcinogenesis</i>	42
Ferraro, Gino <i>Mechanisms of enhanced drug delivery in brain metastases with focused ultrasound-induced blood-tumor barrier disruption</i>	31		
Ferrer, Christina <i>Identifying Mechanisms Governing Metastatic Outgrowth</i>	32		

Man, Cheuk-Him <i>Proton shifting induces leukemia growth by epigenetic activation of H⁺/lactate-cotransporter MCT4</i>	43	McCurley, Jessica <i>Worksite food purchases and employees' overall dietary quality and health</i>	53
Mohan, Raghav <i>Mullerian inhibiting substance suppresses H19 and regulates epithelial to mesenchymal transition in ovarian cancer ascites cells</i>	44	Zhu, Qiuyu Martin <i>ARHGEF26 is a Novel Genetic Risk Factor for Vascular Inflammation and Coronary Artery Disease</i>	54
Oh, Juhyun <i>Age-related tumor growth in mice is related to integrin β4 in CD8⁺ T cells</i>	45	Cellular Biology Abels, Erik <i>Glioma reprograms microglial cells through extracellular transfer of miR-21</i>	55
Schweiger, Markus <i>EVs Modulate GBM Intratumor Heterogeneity and Treatment Resistance</i>	46	Chiasson-MacKenzie, Christine <i>Growth factor-induced macropinocytosis in tumorigenesis</i>	56
Shi, Lei <i>Leveraging reprogrammed metabolism to define selective vulnerabilities of IDH1 mutated cholangiocarcinoma cells</i>	47	Chindavong, Peter <i>Human iPSC-Derived Neuronal Cell-Based Assays for Evaluating the Therapeutic Potential of Small-Molecule Probes Targeting the WNT/GSK3 Pathway as Regenerative Medicines</i>	57
Su, Mack <i>A novel hapten-based vaccine enhances response to immune checkpoint blockade</i>	48	Kawakami, Akinori <i>MITF specifies melanocytes by repressing neural crest genes</i>	58
Sun, Yao <i>Losartan treatment enhances chemotherapy efficacy and reduces ascites in ovarian cancer models by normalizing the tumor stroma</i>	49	Kokkaliaris, Konstantinos <i>Quantitative bone-marrow imaging reveals niche signatures of distinct types of adult and juvenile hematopoietic stem cells</i>	59
Yin, Kanhua <i>Validation of a More Efficient Approach to The Meta-Analysis of Cancer Susceptibility Gene Penetrance Using Natural Language Processing</i>	50	O'Loughlin, Evan <i>Cell polarity and RTK signaling in biliary morphogenesis and tumorigenesis</i>	60
Cardiovascular He, Shun <i>Gut intraepithelial T cells calibrate metabolism and accelerate cardiovascular disease</i>	51	Santos, Alba <i>Microtubule acetylation promotes myofibroblast mechanoactivation and lung fibrosis</i>	61
McAlpine, Cameron <i>Sleep modulates hematopoiesis and protects against atherosclerosis</i>	52	Sohn, Jihee <i>Unraveling the mechanism of neutropenia in patients with Barth Syndrome</i>	62

Developmental and Regenerative Biology

Bhave, Sukhada

63 *A novel in vivo model of colonic aganglionosis using diphtheria toxin-mediated ablation of the enteric nervous system*

Gustafsson, Karin

64 *Thymus regeneration is dependent on distinct mesenchymal stromal cell populations*

Kaur, Gurbani

65 *Human Induced Pluripotent Stem Cell Derived Pro-Epicardial Cells Enhance Survival of and Facilitate Compaction of Three-Dimensional Cardiac Disc, Spherical Organoids, and Tube Constructs Ex Vivo*

Saatcioglu, H. Duygu

66 *Single cell sequencing of neonatal uterus reveals a subluminal endometrial stromal progenitor indispensable for female fertility*

Endocrinology

Dashti, Hassan

67 *Decreased Oral Glucose Tolerance And Insulin Response During Biological Evening Versus Morning Among Adults Under Free-living Conditions*

Dichtel, Laura

68 *Serum Neuroactive Steroid Levels in Postmenopausal Women with Treatment-Resistant Major Depressive Disorder*

Kaliannan, Kanakaraju

69 *Integrated Metagenomic and Metabolomic Investigation of Host-microbiota interactions Reveals the Beneficial Effects of Combination of Elevated Tissue Omega-3 Fatty Acids and Genistein supplementation on High-fat Diet Induced Obesity and Metabolic Syndrome*

Kerem, Liya

70 *Oxytocin significantly attenuates the functional connectivity between food motivation brain areas in overweight and obese men exposed to high caloric food images*

Kimball, Allison

71 *A randomized placebo-controlled trial of low-dose testosterone therapy in women with anorexia nervosa*

Schorr, Melanie

72 *Tibial and radial bone structure as assessed by HRpQCT may explain differences in peripheral skeletal integrity and fracture risk across the weight spectrum that cannot be explained by areal BMD alone*

Tucker, Chandler

73 *Night eating among night shift workers: preliminary results from the SHIFT Study*

Genetics & Genomics

Feng, Yen-Chen Anne

74 *Ultra-rare genetic variation in the epilepsies: a whole-exome sequencing study of 17,606 individuals*

Lakhani, Chirag

75 *Repurposing large health insurance claims data to estimate genetic and environmental contributions in 560 phenotypes*

Lane, Jacqueline

76 *Genetic and Molecular Basis of Circadian Rhythm Disorders: A Patient-Centered Research Study*

Lowther, Chelsea

77 *Systematic evaluation of prenatal and pediatric diagnostic yields from whole-genome sequencing in 8,954 individuals*

Merino, Jordi

78 *Multi-trait genome-wide association meta-analysis of dietary intake identifies new loci and functional links with metabolic traits*

Murtha, Ryan

79 *Identification of genetic modifiers of somatic CAG repeat instability by in vivo CRISPR-Cas9 genome editing*

Sunwoo, Hongjae

80 *Xist deletional analysis reveals a co-dependency between Xist RNA and Polycomb complexes for spreading along the inactive X*

Tai, Derek <i>CRISPR-mediated molecular dissection of Prader-Willi syndrome</i>	
Walton, Russell <i>High-throughput development of CRISPR genome editing technologies with enhanced properties</i>	
Xu, Wanxue <i>Role of RAB3GAP2 mutations in syndromic and non-syndromic forms of Isolated GnRH deficiency revealed by Whole exome sequencing</i>	
Yu, Dongmei <i>Interrogating the genetic determinants of TS and other tic disorders through genomewide association studies</i>	

SESSION 2 POSTERS

Presented 12:00 - 1:30 pm

Healthcare and Population Sciences

Cloutier, Alison <i>Visualization of Multivariate Stroke Recovery Data using Radar Plots</i>	
Greenwald, Kelsy <i>Assessment of mental health and stigma associations with HIV serostatus disclosure in a refugee settlement in rural Uganda: a cross-sectional study</i>	
Hsu, Yu-Tien <i>Transplant Center Perceptions of Engagement in Organization Health Literacy</i>	
Hyle, Emily <i>Clinical impact and cost-effectiveness of genotype testing at HIV diagnosis in the United States</i>	
Kazemian, Pooyan <i>Persistent Treatment Gaps and Disparities in US Diabetes Mellitus Care in 2005-2016</i>	
Khidir, Hazar <i>Assessing the burden of mental illness among refugees in Uganda</i>	

81 Neilan, Anne <i>Cost-effectiveness of frequent HIV screening for self-identified high-risk young men who have sex with men in the United States</i>	91
82 Reddy, Krishna <i>Cost-effectiveness of urine-based tuberculosis screening in hospitalized patients with HIV in Africa</i>	92
83 Robinson, Ellen <i>Experience with a Hospital Policy on Not Offering Cardiopulmonary Resuscitation When Believed More Harmful than Beneficial</i>	93
84 Sanchez, Angie <i>Five-year Massachusetts General Hospital demographic trends in clinical trial enrollment</i>	94

Immunology/Inflammation

Adiliaghdam, Fatemeh <i>Divergent immunomodulatory capacity of the healthy versus IBD human enteric virome</i>	95
85 Allouche, Jennifer <i>Melanocyte-specific regulation of PD-L1 by MITF confers immune tolerance to UV mutagenesis</i>	96
86 Amatullah, Hajera <i>Crohn's disease-associated epigenetic reader SPI40 orchestrates immune cell identity through repression of chromatin accessibility</i>	97
87 Grabowski, Eric <i>Platelet Thrombus Formation in eHUS is Prevented by Anti-MBL2</i>	98
88 Lion, Mattia <i>Dynamic changes in chromatin accessibility and nucleosome positioning in the control of antigen receptor rearrangement</i>	99
89 Mora Buch, Rut <i>IL-4, a new player in CD8 resident memory T cell formation and persistence</i>	100

Posters

Rodell, Christopher

Re-polarization of Tumor Associated Macrophages for Cancer Immunotherapy

Sokol, Caroline

CCR8 Controls the Stepwise Migration of Dendritic Cells into the Lymph Node and the Initiation and Polarization of the Allergic Immune Response

Infectious Diseases

Dugdale, Caitlin

Risks and benefits of dolutegravir-based ART for women with HIV of childbearing age in South Africa: A model-based analysis

Iyer, Anita

Protective properties of anti-Vibrio cholerae antibodies: implications for future vaccine design

Kuo, Hsiaohsuan

TNFRSFs Upregulation and Survival Pathway Activation in Productive and Latent HIV-1 Infection

Millham, Lucia

Clinical and economic impact of ibalizumab for patients with multidrug-resistant HIV in the United States

Musculoskeletal

Guastaldi, Fernando

Tissue engineered reconstruction of large bicortical defects in minipigs

Neurosciences

Aviolat, Hubert

Assessing somatic CAG repeat instability at the protein level

Balena, Trevor

Delayed post-traumatic neuronal death in the developing hippocampus

Bordt, Evan

Early life immune activation induces sex-biased mitochondrial and behavioral alterations

101

Dujardin, Simon

Heterogeneity of tau seeding in the human brain, a step toward understanding the diversity of Alzheimer's disease?

102

Gokcal, Elif

Structural Brain Alterations and Cognition in Cerebral Amyloid Angiopathy: A Longitudinal Follow-up Study

103

Grange, Robert

Effect of therapeutic chronic hypoxia on cerebral arteriovenous differences in the Ndufs4 knockout mouse model of Leigh Syndrome

104

Guo, Nannan

Dentate granule cell recruitment of feedforward inhibition governs engram maintenance and remote memory generalization

105

Hendriks, William

In Vitro Parkinson's Disease Modeling Reveals PARK7 Mutation-Associated Mitochondrial Dysfunction

106

Kathuria, Annie

Modelling interneuron dysfunction in schizophrenia using patient-derived neurons

107

Kegel-Gleason, Kimberly

Structural and physical aspects of the synaptic compartment are altered in the Q175/Q7 knock-in Huntington's disease mouse model

108

Kovalenko, Marina

The impact of genetic knockout of Hdac2 and Hdac3 in striatal medium spiny neurons of Huntington's disease knock-in mice

109

Lan, Yu

Novel [11C]-labeled Radioligands for Imaging Sigma-1 Receptor in the Brain Using Positron Emission Tomography (PET)

110

Lau, Lauren

Dissecting ictogenesis in a model of post-traumatic epilepsy

111

112

113

114

115

116

117

118

119

120

Leger, Brittany <i>A conserved role for the N-glycosylation pathway in sleep and seizures</i>	121	Yekula, Anudeep <i>Characterization of Plasma EVs Following 5-ALA use in Malignant Gliomas</i>	133
Lopez-Lengowski, Kara <i>Modeling Schizophrenia in a Dish: A Stem Cell Approach</i>	122	Zhang, Yiying <i>Isoflurane causes oogenesis impairment through stress-induced germline apoptosis pathway in C.elegans</i>	134
Mills, Alexandra <i>Histone Deacetylase 3 Localization is Altered in ALS Post-Mortem Motor Cortex</i>	123	Nursing Research	
Mishra, Nibha <i>Targeting FUS mislocalization to mitigate disruption of nuclear integrity in ALS</i>	124	Coakley, Amanda <i>Exploring the experience of Partners Healthcare Agency nurses</i>	135
Pezaris, John <i>Simulations of artificial vision showing the effects of training, and the effects of gaze contingency</i>	125	Feldpausch, Meghan <i>Retention of Research Participants in a Longitudinal HIV Clinical Trial</i>	136
Rahmati, Negah <i>Characterization of neuronal chloride microdomains</i>	126	Jones, Dorothy <i>Psychometric Evaluation of the Power Influencing Professional Practice Changes (PIPPC Scale)</i>	137
Rodriguez-Torres, Axana <i>Hypertension Severity and Stroke Recurrence Risk among Oldest Old Intracerebral Hemorrhage Survivors</i>	127	Sheppard, Kimberly <i>Nurse Comfort and Knowledge with Assessing Firearm Access and Providing Patient Education on Safe Firearm Storage</i>	138
Rogers, Madeline <i>Endogenous estrogen exposure and delay of gratification in females with anorexia nervosa</i>	128	Ob-Gyn	
Sadri-Vakili, Ghazaleh <i>Genetic variants and alterations in Hippo/YAP signaling in post-mortem ALS motor cortex</i>	129	del Carmen, Gabriel <i>Does the Day of the Week Predict a Cesarean Section?: A Statewide Analysis</i>	139
Silva Amaral, Ana Claudia <i>Selective partial decrease of GSK-3 beta reduces tau hyperphosphorylation, aggregation and propagation</i>	130	Edelson, Paula <i>Adverse perinatal and long-term outcomes differ among physiologic subtypes of gestational glucose intolerance</i>	140
van Veluw, Susanne <i>Vasomotion as a driving force for paravascular clearance in the awake mouse brain</i>	131	Psychiatry	
Xia, Ning <i>Assessing urate effects on mutant LRRK2 toxicity in human iPSC-derived dopaminergic neurons</i>	132	Batchelder, Abigail <i>Substance Use Stigma, Avoidance Coping, and Missed HIV Appointments among MSM who Use Substances</i>	141
		Berman, Zohar <i>Neural Correlates of Threat Processing in Women with PTSD Following a Traumatic Childbirth</i>	142

Posters

Feig, Emily

Physical activity and weight loss after bariatric surgery: influences of self-efficacy and internalized weight bias

Fried, Ronna

An Innovative SMS Intervention to Improve Adherence to Stimulants in Children with ADHD

Hoeppner, Susanne

A bridge too far? Happiness exercises in people with low happiness in recovery from substance use

Joshi, Gagan

Predictive Utility of Autistic Traits in Youth with ADHD: A Controlled 10-year Longitudinal Follow-up Study

Seo, Jeehye

Relationships of sleep with cortical thickness among trauma exposed individuals

Uchida, Mai

Cingulum Bundle Anomalies in Children with Emotional Dysregulation: A Diffusion Tensor Imaging Study

Vilsaint, Corrie

Racial Health Disparities in the Prevalence and Persistence of Substance Use Disorders

Watmuff, Bradley

Effects of clozapine and haloperidol on dendritic spine dynamics and function in iPSC-derived cortical neuron-like cells

Yeum, Tae-Sung

A novel method to improve the quality of clinical trials using the computer-administered Montgomery Depression Rating Scale data

Surgery

Hansdorfer, Marek

Occipital Neuralgia and Migraine: Intra-Operative Evidence for Extracranial Pathology

Perez, Numa

Patient Mobility by Race in New York City

143

Sangji, Naveen

Increasing the Value Proposition in Trauma: A Case for an Intermediate Care Unit

144

Translational Medicine & Experimental Therapeutics

Beauchamp, Roberta

145

High throughput kinome and transcriptome analyses reveal novel therapeutic targets in NF2-deficient meningioma

146

Collins, John

CIMIT's Guidance and Impact Tracking System (GAITS)

147

Dubash, Taronish

Blood-based Monitoring in Hormone Receptor Positive Metastatic Breast Cancer Identifies Acquired HER2 Mutations Conferring Susceptibility to Targeted Inhibition

148

Dzhala, Volodymyr

Modulation of KCC2 activity for Control of Anticonvulsant Resistant Seizures

149

Garcia, Ronald

Effects of Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS) in the Modulation of Blood Pressure in Hypertensive Patients

150

Ji, Zhenyu

Regulation of BRAF inhibitor resistance by protein degradation pathway

151

Lariviere, Amanda

Illuminating DA-9803's Potential as a Therapeutic Candidate for Alzheimer's Disease

152

Minikel, Eric

A pathway to primary prevention in genetic prion disease using antisense oligonucleotides

153

Morehouse, Elizabeth

Increasing skin functionality in patients with radiation damaged skin

154

155

156

157

158

159

160

161

162

163

Nguyen, Emily 164

Assessing the incidence of skin and soft tissue infection in patients on biologics

Ptaszek, Leon 165

Epicardial Application of Bioadhesive Hydrogel Improves Survival and Preserves Left Ventricular Function in a Mouse Model of Myocardial Infarction

Scarfo, Irene 166

Development of new strategies to target Peripheral T cell lymphomas with Chimeric Antigen Receptor T cells

Shao, Tuo 167

Prognosis of liver fibrosis via ^{18}F - $\alpha\text{v}\beta_3$ targeted PET imagining studies

Teng, Jian 168

Using low dose of quinoline methanol derivatives as novel treatments for primary brain cancer and brain metastasis

Zazzeron, Luca 169

Extracorporeal Removal of Carbon Monoxide Using Phototherapy in Rats with normal or injured lungs

Poster Number 1

Michael Fuenfer, MD

Pediatrics, Instructor | mfuenfer@mgh.harvard.edu

A self-contained crank-powered battery replacement module for critical diagnostic and therapeutic medical instruments

INVESTIGATORS: M. M. Fuenfer, N. Hanumara, G. Horn, R. Hoffman, K. Wu, A. A. Urpi, P. Duest, J. Lee, A. Slocum

Introduction: Many critical medical instruments are dependent upon disposable or rechargeable batteries as a source of energy. The disadvantages of this traditional means of power include: availability of replacement batteries (dry cell), availability of an electrical power grid (rechargeable batteries), potential for leakage causing damage to the instrument, heavy weight, expense, safe disposal, and a limited and unpredictable lifespan. Patient care in many low-resource practice environments could benefit from an alternate means of powering common diagnostic and therapeutic medical instruments.

Design Specifications: Utilization of proven engineering technology, readily available and inexpensive components, incorporation of all components into a self-contained module that is dependable, durable, and capable of replacing disposable dry cell or rechargeable batteries with no alteration of the instrument, while maintaining fully the functional requirements of the instrument. We used a standard otoscope/ophthalmoscope as our research platform.

Results: We successfully designed and constructed a self-contained crank-powered module, that can be inserted in place of two C-cell batteries into the handle of a standard otoscope. Two minutes of crank rotation is capable of powering the otoscope for 15 minutes. This device has been granted US PATENT NO. 62/647,654

Conclusion: A low cost, dependable, alternate power source for critical battery-powered medical instruments is feasible. Using an otoscope as a model, we have designed and constructed a working prototype utilizing a self-contained rechargeable power module incorporating a geared hand-crank mechanism, internal motor, and lithium battery. This alternative power module concept could be feasibly incorporated into numerous other types of medical devices.

Poster Number 2

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Bioengineering of exosomes with in vivo fate controllable, charge-modifiable NIR fluorophores

INVESTIGATORS: D. Hwang, H. Kang, K. Bao, S. Hu, Y. Baek, G. Fakhri, H. Choi, M. J. Jo

Controlling the physiological behavior of nanomaterials is the key to optimize targetability to the specific tissue and to reduce nonspecific background uptake during biodistribution and clearance. Due to the strong influence of surface chemistry, modification of bioinert exosomes with chargeable and traceable small molecule fluorophores have greatly changed the distribution, stability, and toxicity of the final conjugates. In this study, we designed charge-variable exosomes by conjugating the surface proteins with near-infrared (NIR) fluorophores, possessing different physicochemical properties, to control the fate of exosomes in vivo. Interestingly, zwitterionic fluorophore-labeled exosomes showed rapid renal uptake with minimum nonspecific tissue uptake, whereas anionic fluorophore-labeled exosomes excreted through the hepatobiliary route with high uptake in the liver. The biodistribution and pharmacokinetics of each exosome conjugate were comparable to its corresponding free NIR fluorophores, representing the surface characteristics govern the fate of final conjugates in the living organism. Such unique surface properties of exosomes were confirmed in the lymphatic system after intradermal administration, resulted in distinctive kinetic profiles in the secondary lymphoid tissues. These findings imply that surface protein engineering of exosome may alter the in vivo fate of bioengineered exosomes, which could subsequently lay the foundation of developing exosome-based therapeutics.

Poster Number 3

Jina Ko, PhD

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Droplet-based single EV profiling for the improvement of immunotherapy

INVESTIGATORS: J. Ko, R. Weissleder

Immunotherapy has been highly successful in a subset of cancer patients that were previously incurable while the remainder do not respond to the therapy at all. Several clinical questions underscore the need to develop better predictive diagnostics: who will respond to the therapy and how long can it be administered? Extracellular vesicles (EV) shed into circulation from cancer cells and host cells contain proteins and nucleic acid cargo and can potentially be used as blood-based biomarkers to answer these questions. Although multiple EV-based diagnostic platforms have been developed, most of them are based on bulk measurements, which make it difficult to resolve the heterogeneity of EVs and discover rare EV subsets that can indicate molecular signatures of response to therapy. To solve these challenges, we have developed a droplet-based single EV profiling platform that enables analysis of individual EVs by compartmentalizing each EV using droplets. We encapsulate single EVs into droplets and multiplex protein measurements to profile hundreds of proteins simultaneously using DNA barcoded antibody conjugates. Our goal is to profile immune cell EVs derived from the tumor immune microenvironment and to monitor the Gene Mediated Cytotoxic Immunotherapy for glioblastoma treatment. Using the droplet-based single EV profiling platform, we will be able to identify rare immune EV subtypes in the peripheral blood, which would otherwise be impossible to detect due to the co-presence of abundant normal EVs. This cutting-edge technique has the potential to revolutionize treatment monitoring of high-cost immunotherapies and enhance personalized medicine capabilities.

Poster Number 4

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Optimization of micro-coil designs for precise activation of primary visual cortex

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

Direct activation of neurons of the primary visual cortex (V1) via implantable electrodes has the potential to restore vision to patients suffering from a wide range of visual impairments. Despite the initial success in eliciting visual perception in human subjects, the effectiveness remains limited in part due to the inability to selectively activate specific types of cortical pyramidal neurons (PNs) as well as the intricate biological reactions that diminish the stability of the electrode-neuron interface. Recent demonstrations that magnetic stimulation from a micro-coil can selectively activate vertically oriented PNs while avoiding horizontal passing axons suggest the possibility that a coil-based approach may overcome some of those limitations. Here we describe novel micro-coil designs for enhancing selectivity and demonstrate the effectiveness via a series of electrophysiology experiments. Computational modeling using a finite element method was conducted to compare the strengths of the field gradients, the driving force for neuronal activation, induced by different coil designs. The most promising designs (V- vs. W-coils) were fabricated for use in electrophysiological experiments. Patch-clamp recordings using mouse brain slices revealed that all fabricated coils could activate the L5PNs of V1, and also that double loop coils had enhanced strength of stimulation. Calcium imaging using GCaMP6f transgenic mice revealed that both V- and W-coils better confine activation than electrodes. Our results show how coil design influences the response of cortical neurons to stimulation and are an important step towards the development of next-generation cortical prostheses.

Poster Number 5

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CIMIT platforms and processes to develop medical technology for MGH and across the world

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

Background: The Consortia for Improving Medicine with Innovation & Technology (CIMIT; <http://cimit.org/>) was founded in 1998 by MGH, BWH, MIT, and Draper Laboratory as a “center-without-walls” to foster multidisciplinary collaborations that bridge silos of medicine and technology to improve patient care. Early on, CIMIT supported teams primarily with grants to traverse the Healthcare Innovation Cycle. More recently, CIMIT has expanded its platforms and processes to support other granting organizations within and outside Boston and in this way has greatly scaled its impact on improving patient care.

Rationale: CIMIT took the platforms and processes that it had developed for Boston-area CIMIT consortium members and has made them available to other organizations outside Boston in support of their med-tech translational activities, including a secure cloud-based application submission and review platform (CoLab), a commercialization boot camp (CRAASH), and a guidance and tracking platform for monitoring team progress through the Healthcare Innovation Cycle (GAITS).

Results: Two examples are CIMIT’s work with the Translational Research Institute for Space Health (TRISH) at Baylor College of Medicine to run their grant solicitations, and CIMIT being funded in 2018 as a Coordinating Center for the NIH Point-of-Care Technologies Research Network (POCTRN). CoLab, CRAASH, and GAITS will assist each of the 4 national POCTRN Centers to create multidisciplinary partnerships necessary to move technologies from an early stage of development into clinical testing.

Conclusions: The CIMIT platforms and processes that were developed locally have been adapted and adopted by organizations globally to improve patient care through med-tech development and commercialization.

Poster Number 6

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Spatially confined evoked responses of mouse visual cortex by magnetic stimulation using micro-coil

INVESTIGATORS: S. Ryu, A. C. Paulk, J. C. Yang, M. Ganji, S. A. Dayeh, S. S. Cash, S. I. Fried, S. Lee

Electrical stimulation of the cortex has been suggested as a potential treatment for a wide range of neurological disorders. Despite the success in some clinical trials, it has limitations such as unintended activation of the passing axons from distal cortical regions. Magnetic stimulation with micro-coils implanted in the cortex has the potential to overcome the limitations because coils can selectively avoid activation of axons, and thus confine activation to a focal region.

To test the efficacy of the magnetic stimulation, in-vivo experiments were performed using anesthetized mice. For the recording of the cortical activity (ECoG) in response to electric or magnetic stimulation, a 128-channel recording array was positioned on the surface of the mouse visual cortex (V1). For the stimulation, a micro-coil (or an electrode) was inserted through the hole in the center of the recording array using a micromanipulator. This arrangement allowed us to quantitatively evaluate and compare the spread of activation.

High frequency (200 Hz) trains of both electric and magnetic stimulation elicited cortical responses, although the spatial extent of activation was different for the two approaches. Electric stimulation activated a spatially expansive area of visual cortex, often more than 1 mm from the stimulation site, while the region activated by magnetic stimulation was confined to a focal area around the stimulation site (approximately 300 μ m in diameter).

Our findings suggest that magnetic stimulation from an implantable micro-coil can improve the ability to focally activate cortex and thus may offer advantages over conventional electric stimulation from micro-electrodes.

Poster Number 7

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Assessing therapeutic efficacy in postoperative recovery of rats by monitoring pain and gait

INVESTIGATORS: W. A. Sabuhi, C. P. Leape, Y. Fan, Y. Xiao, S. C. Grindy, D. Gil, R. J. Mort, H. Bedair, J. E. Collins, O. K. Muratoglu, M. Randolph, E. Oral

At the Harris Orthopaedic Laboratory, we develop implantable materials to address orthopaedic challenges. Currently, we are working to release therapeutics from implant bearing surfaces to reduce postoperative pain and curtail periprosthetic infection risk.

Here, we describe a rat model for total knee replacement with gait analysis to study functional recovery from pain and infection. Our surgical model consists of insertions of a tibial titanium screw and a transcondylar cylindrical polyethylene implant. The first comparison was between a group receiving postoperative buprenorphine and another receiving no postoperative medication. A second characterization was conducted on a group receiving an injection of *S. aureus* at implantation site. Spatiotemporal gait, toe spread, weight-bearing, and Von Frey measurements were all taken at baseline and at multiple timepoints in the weeks post-procedure. Rats without postoperative pain medication offloaded their operated limb significantly more than did rats receiving buprenorphine. They also took longer to recover their toe spread, gait temporal symmetry, and duty factor balance. Limb weight-bearing, a proxy for pain, was statistically correlated with functional measurements such as duty factor imbalance and toe spread. The data suggest that these methods for assessing animal well-being can quantify pain and functional restoration outcomes not just as a binary before-after defect comparison but also over a multi-week postoperative timeframe. Our goal is to study the efficacy of local pain medication regimens using therapeutic-eluting implants.

Our gait analysis system is a shared resource through the Preclinical Research Support Core for other researchers who wish to use rodent models of locomotion.

Poster Number 8

Amir Sajjadi, PhD

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Multi-channel fluorescence imaging system for intraoperative image-guided surgery

INVESTIGATORS: A. Y. Sajjadi, H. Kang, P. Das, G. El Fakhri, H. S. Choi

Optical imaging is getting more attention in clinical settings due to its low cost, safety and ease of use, especially during surgical operation. Near-infrared imaging has relatively high tissue penetration and minimal autofluorescence. Combination of appropriate contrast agents and efficient imaging systems helps surgeons increasing the efficacy of surgery.

Mesoscale Imaging System (MIS) allows real-time concurrent color and two NIR imaging channels. LED light source (400-650 nm) and two diode lasers (660 and 760 nm) are used as excitation sources. A custom-made prism split the light into three channels: color (400-650 nm), NIR1(660-750 nm) and NIR2 (750-1000 nm) to be distributed on three CCD sensors in the camera (Condor3) after further filtration of NIR1 (660 ± 20 nm) and NIR2 (760 ± 10 nm) channels. Custom software was developed to capture images and to stream all three channels on three screens along with the superimposed image.

Experiments were performed on leaf phantoms with various concentrations of both 700 nm (ZW700-1) and 800 nm (ZW800-1) fluorophores in saline. Real-time biodistribution of the fluorophores was performed in 25 g CD-1 mice to study the effects of illumination power distribution and imaging exposure time. The signal-to-background ratio of the MIS was compared with the K-FLARE in terms of different exposure times and sensitivity.

MIS provided real-time simultaneous multi-channel fluorescence imaging with great SBR and offers clinicians a powerful diagnostic and monitoring tool. It can be utilized for monitoring tissue-specific drug delivery and tumor-to-background diagnosis in the current surgical setting.

Poster Number 9

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Sleep staging from electrocardiography and respiratory effort with a deep learning model

INVESTIGATORS: H. Sun, W. Ganglberger, E. Panneerselvam, S. A. Qader Quadri, M. Leone, B. Goparaju, R. J. Thomas, M. Bianchi, B. Westover

Sleep staging with the four sleep stages N1, N2, N3, and REM sleep is important to understand sleep quality and to help diagnose and manage sleep disorders. The electroencephalogram is usually the primary signal used for staging sleep in a polysomnography study. Since the brain controls the heart and lungs, information about staging sleep is present in signals obtained from respiration and heartbeat. The latter signals have the advantage of being easily applicable, allowing us to stage sleep at home and in the ICU. Here, we developed a deep learning model based on a large-scale dataset containing 8,682 polysomnograms. Retrospective analysis of the de-identified dataset was performed after the approval from Partners IRB. The dataset is heterogeneous with a variety of health conditions. The model's inputs are one or more of the raw ECG and respiratory effort signals from the chest and abdomen, the output is a probability distribution of the sleep stages for every 30 seconds. Testing the models on 1000 subject-nights yielded performance of Cohen's kappa between 0.49-0.60 for the four sleep stages and wake, and between 0.65-0.76 for NREM, REM and wake, depending on the choice of input signals. The performance tends to be better for the younger population group (<60 years old) as well as for the non-severe sleep apnea group (apnea-hypopnea index < 30 events/hour). Our work has demonstrated the feasibility to infer sleep stages from heartbeat and respiration. It gives insight into the management of sleep disorders and delirium in critically ill patients.

Poster Number 10

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Engineering distal pulmonary epithelium from induced pluripotent stem cells (iPSCs) derived alveolar cells

INVESTIGATORS: T. Wu, D. E. Gorman, L. Zhu, K. Ohata, S. E. Jeffs, D. Evangelista Leite da Silva, M. Vedaie, D. N. Kotton, S. E. Gilpin, H. C. Ott

Background: Lung transplantation remains the only treatment for many end-stage lung diseases. However, donor organ shortage and the need for immunosuppression limit its clinical impact. We had shown that cadaveric lungs can be decellularized to yield scaffolds that provide a suitable platform for lung tissue regeneration. Directed differentiation of human iPSCs toward a lung epithelial fate is a promising approach to facilitate the goal of whole organ regeneration with patient-specific cells.

Methods: We directed iPSCs carrying two fluorescent reporters, Nkx2.1-GFP and SPC-TdTomato, towards a distal lung alveolar fate following established protocols. We seeded alveolar cells into decellularized rat lungs (n=6) to repopulate the distal niche. We subsequently induced epithelial formation through extended ex vivo biomimetic culture. We analyzed the regenerated rat lungs for their tissue architecture and cell fate. Acellular human lung scaffolds were dissected into slices and repopulated with iPSC derived cells. Seeded lung slices (n=12) were cultured then the tissue structure and cell identity were analyzed.

Results: We successfully induced the formation of early distal lung epithelium with mature type II alveolar cell phenotype that expressed lung distal epithelial cell marker genes Nkx2.1, E-Cadherin, SPC, SPB. We also observed the presence of AQP5 positive cells, which may identify a type I alveolar fate. Similar findings were seen in human lung slices.

Conclusions: Human iPSC-derived alveolar epithelial cells can form distal lung epithelium on both rodent and human acellular native lung matrix. This pilot research demonstrates the potential for human lung regeneration at a transplantable scale in the future.

Poster Number 11

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Developing NIR-II imaging system for intraoperative image-guided surgery

INVESTIGATORS: A. Sajjadi, H. Kang, E. Adluru, G. E. Fakhri, H. S. Choi

Despite limited penetration of light in biological tissues, fluorescence imaging is getting popular in animal research and surgical settings. To enhance penetration depth and resolution of fluorescence imaging, second-near infrared (NIR-II) window (1,000-1,700 nm) was used. In this window light scattering is minimal in most of biological tissues and penetration depth is maximum. When combined with appropriate contrast agents, it provides higher penetration and signal to noise ratio (SNR) compared to NIR-I window, hence, increasing the efficacy of reflectance-based fluorescence imaging.

To prove this concept, NIR fluorescent Indocyanine Green (ICG; $\lambda_{abs} = 805$ nm; $\lambda_{em} = 830$ nm) with tail emission in the NIR-II window was used for in vitro and in vivo vasculature imaging. A 1280 x 1024 InGaAs VIS-SWIR sensor (OWL1280, Raptor Photonics) was utilized with a tunable diode laser (600-800 nm) as an excitation source in conjunction. The camera has intrascene dynamic range of 68dB and cooled to -15°C, which enables highly sensitive imaging and simultaneous capture of bright and dark portions of an image. A custom variable focusing lens (Navitar; 0-6X), coated for NIR-II wavelength, was used for focusing and magnification. First, in vitro experiments were performed on capillary lipid phantoms at different depths and concentrations to study the penetration depth and sensitivity. Then, in vivo imaging was followed by using 25 g CD-1 mice to observe vasculature under the skull and skin noninvasively.

NIR-II imaging provides great SNR which offers clinicians a powerful tool for monitoring tissue-specific drug delivery and tumor diagnosis in surgical setting.

Poster Number 12

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Expected label value computation for atlas-based medical image segmentation

INVESTIGATORS: I. Aganj, B. Fischl

Supervised automatic image segmentation is often a central step in medical imaging studies, enabling the analysis of specific regions of interest (ROIs). In atlas-based segmentation of a new image, atlas images are deformably registered to the new image. The manual labels are then propagated into the new image space using the computed transformations and fused to form the new labels.

Being computationally very demanding, deformable image registration of the atlas images to the new image is the bottleneck of atlas-based segmentation. Also, registration is prone to entrapment in local optima, potentially leading to erroneous propagation of the labels, and even choosing the single correct transformation would mean disregarding valuable information provided by other potentially valid transformations.

In this work, we present a new atlas-based image soft-segmentation method that produces the expected value of a label at each voxel of the new image, while considering the probability of possible transformations, without explicitly sampling from the transformation distribution. Although accounting for deformations, we do not run deformable registration in the training or testing stages. We create a single image from the training data, which we call the key. Then, for a new image (after affine alignment), we compute the expected label value (ELV) map simply via a convolution with the key, which is efficiently performed using the fast Fourier transform. The soft segmentation provided by the ELV map can be further used to initiate a subsequent hard-segmentation procedure. We validate our approach through liver segmentation experiments on abdominal computed tomography (CT) images.

Poster Number 13

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Cerebrospinal fluid transport to the retina enables detection of CNS inflammation by retinal imaging

INVESTIGATORS: J. C. Cruz Hernandez, M. W. Zeller, F. Herisson, J. M. Runnels, P. Baranov, E. C. Klawiter, J. W. Chen, C. P. Lin, C. Alt

Neuro-inflammation is increasingly identified as a key component in many neurological-diseases. However, incomplete detection of subclinical CNS inflammation limits detection of early disease development, although sensing of inflammatory mediators in the CSF could predict impending disease. We introduce retinal imaging as a non-invasive method for detecting CNS inflammation that is based on the quantification of leukocyte endothelial interaction (LEI). LEI is absent in the healthy CNS, but is observed when the inflamed CNS releases pro-inflammatory mediators. We demonstrate by injection of fluorescent tracer directly into the cerebrospinal fluid (CSF), that the retina is a destination for CSF transport. Presence of pro-inflammatory mediators in the CSF, exemplified by lipopolysaccharide (LPS, a bacterial endotoxin), caused LEI in retinal vasculature that we quantified by live retinal imaging. We evaluated retinal and meningeal LEI by ophthalmoscopy and confocal intravital microscopy in the same mice, respectively, after LPS injection into the cisterna magna as a model of meningitis. Retinal LEI mirrors the inflammatory status of the meninges and closely tracks the expression of CSF cytokines. We also tested the method in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. LEI coincides with active recruitment of peripheral leukocytes into the CNS that precedes EAE disease symptoms and peak at disease onset, raising the interesting possibility that this method has potential to predict MS disease activity and relapse of clinical symptom. Retinal LEI imaging can be performed noninvasively, without fluorescent dyes, and repeatedly to monitor the CSF for signs of CNS inflammation, underscoring its translational potential.

Poster Number 14

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Novel software solution for intraoperative imaging procedures with mesoscale image analyses

INVESTIGATORS: V. Girouard, M. Lalonde, H. Kang, A. Sajjadi, M. Tetrault, H. S. Choi

Near-infrared (NIR) cameras have shown their usefulness in several surgical applications, such as vein targeting and intraoperative imaging. NIR imaging systems are also essential in testing and characterizing new fluorophores. Existing commercial systems do not support many functional features expected for multidisciplinary research in a single package. We are building a mesoscale system with aiming to provide real-time imaging of living organisms in various scales, and this presentation focuses on its software control. To stand out, the system must be intuitive and limit the need for user intervention. Menial tasks such as camera configuration, image capture sequence, and area of interest quantification should be automated. Currently our mesoscale imaging system is implemented with integrated camera settings, image capture presets with automatic on-image settings annotation as well as video recording and flexible area of interest quantification. Our end goal is to provide a simple and complete solution to enhance user's productivity including an object tracking function with motion correction and automatic area of interest delimitation for efficiency.

Poster Number 15

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Implantable optical sensor for measuring muscle oxygenation in vivo in elephant seals

INVESTIGATORS: C. Goenka, M. A. Escandon, T. Dent, A. Hindle, W. Franco

Elephant seals undergo hypoxia during deep dives induced by external disturbances perceived by them as a threat. Their bodies undergo various physiological adjustments during these hypoxic conditions. One way to understand these physiological changes is to measure the oxygen saturation changes in their muscles while they are freely diving in their natural environment. Our group is designing an optical probe based on the diffusion model of light transport to make these measurements. One challenge we face in designing this probe is the lack of information about the optical properties of the muscles of marine mammals, leading us to determine these properties through modeling and experiments. We have made spectroscopic measurements of different tissues in the body of an elephant seal in vivo using a commercially available spectrometer. In this work we use the data from these measurements to create a Monte Carlo model and determine the optimum source to detector distance in the probe. In this poster, we will discuss this model and present the design of an implantable, biocompatible and autonomous probe based on this model.

Poster Number 16

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Tumor targetability of PEGylated fluorophores via small size EPR effect

INVESTIGATORS: H. Kang, S. Hu, Y. Baek, G. El Fakhri, H. Choi

The enhanced permeability and retention (EPR) effect is a crucial concept for solid tumor targeting in cancer nanomedicine. There is, however, a trade-off between the long-term blood circulation of nanoparticles (NPs) and their nonspecific tissue uptake. To define this size-dependent tumor targetability, we report here the EPR effects of small size polymeric NPs in terms of their molecular weight/hydrodynamic diameter (HD), passive tumor targeting, biodistribution, pharmacokinetics, and renal clearance. The elimination half-life (24.37 to 224.14 min) and AUC values (990 to 24,806 %ID·min/g) of PEGylated fluorophores increased gradually with the molecular weights of PEGs (1-60 kDa). PEGs smaller than 11 nm showed minimal uptake in major organs except for the kidneys, while PEGs larger than 13 nm started to accumulate in major organs such as the lungs, liver, and pancreas, and stayed longer in blood vasculature. In addition, size-dependent renal clearance was observed that renal clearance of PEGs exponentially decreases (~85 %ID to ~5 %ID) with the HD increase of PEGs. PEG 20 kDa with an HD of 11 nm showed the best performance in tumor targeting with maximized TBR and minimized potential toxicity. We found that the small size of polymeric NPs (<10 nm) can target the tumor site by the EPR effect, and the total body elimination of administered NPs is vital to enhance TBR and reduce toxicity. Our results lay the foundation of design considerations of NPs for tumor diagnostics and therapeutics.

Poster Number 17

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Real-time imaging of vaccine biodistribution using zwitterionic NIR nanoparticles

INVESTIGATORS: J. H. Lee, M. A. Tétrault, H. Kang, J. Sinyoung, S. Yokomizo, S. Santos, S. Hu, G. E. Fakhri, M. Henary, S. Kashiwagi, H. S. Choi, W. Katagiri

Efficient and timely delivery of vaccine antigen to the secondary lymphoid tissue is a crucial event to induce the protective immune response by vaccination. However, there are insufficient methodologies to determine longitudinal biodistribution of the injected vaccine in vivo. A zwitterionic near-infrared (NIR) fluorophore ZW800-1C has been shown to offer a real-time intravital imaging with a high signal-to-background ratio (SBR) and minimal fluorophore-tissue interactions.

We conjugated an N-hydroxysuccinimide (NHS) ester form of ZW800-1C to three sizes of model vaccines and characterized their physicochemical properties including the hydrodynamic diameter. We determined biodistribution of the intradermally administered vaccines using a multispectral real-time fluorescence imaging (FLARE) system in mice. We were able to observe time-course trafficking through the lymphatics and size-dependent accumulation of the vaccine in the lymph node (LN) with high SBR. Flow cytometry analysis of LN consistently confirmed that uptake of the vaccine by antigen presenting cells (APCs) were dependent on the hydrodynamic diameter of the vaccine. Furthermore, image analysis revealed that a significant fraction of the injected vaccine remained in the injection site regardless of the size.

These results demonstrate that the FLARE system using a zwitterionic fluorophore is a powerful tool to determine the biodistribution of vaccine antigen in vivo in a real-time manner. Since vaccine accumulation in an unexpected site is connected to side effects, this method is not only useful for optimization of vaccine design, but also for evaluation of the safety of clinical vaccines.

Poster Number 18

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Combining active learning and deep learning in image classification of blood cells

INVESTIGATORS: D. Mo, M. Pacula, C. Mow, J. Higgins, L. Le

Deep learning has sparked a revolution in computer vision, giving rise to applications like self-driving cars, facial recognition and robust image retrieval. Unsurprisingly, deep learning also holds a promise for medical imaging, with applications ranging from quantitative analysis to disease classification.

However, the lack of high-quality human annotated data sets poses a significant challenge for training when applying deep learning to biomedical images. Unlike natural images where several well-organized & annotated databases exist, a universal repository of medical images does not exist because of the complexity of medical image formats and the specialized expertise required for their annotation.

We propose an active learning approach to improve the procedure of annotating medical images for training deep learning models. We apply our methods on segmented image tiles with individual cells from hematology blood smear slides. We use a pre-trained deep learning model (ResNet50) to extract image features which are then fed into a Logistic Regression classifier to predict the cell type. We start from a small labeled data set, iteratively selecting the most informative unlabeled data points for annotation. We compare the learning curves of random selection and active learning, finding that active learning leads to improved classification in a more efficient manner. We demonstrate that with active learning we can achieve peak accuracy with only 1/3 of the training data, highlighting our approach as a sensible solution for efficient large-scale annotation of medical images and model building.

Poster Number 19

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Collagen-targeted positron emission tomography (PET) in pulmonary fibrosis: initial human experience

INVESTIGATORS: S. B. Montesi, D. Izquierdo-Garcia, E. Abston, P. Désogère, R. Seethamraju, M. Lanuti, C. Catana, P. Caravan

We invented a type I collagen targeted PET probe, 68Ga-CBP8, and showed that it could detect and stage pulmonary fibrosis in mouse models and measure treatment response. Here we present the first in-human studies of 68Ga-CBP8 to assess its safety, tracer distribution, and ability to detect increased collagen in idiopathic pulmonary fibrosis (IPF) patients.

We performed 68Ga-CBP8 PET and simultaneous MRI in 5 healthy volunteers and 9 IPF subjects. Test re-test was performed in 4 IPF subjects. Preliminary results demonstrate that 68Ga-CBP8 is safe, well-tolerated, and rapidly renally cleared. The whole lung PET signal in IPF subjects was significantly increased compared to healthy volunteers with standardized uptake values, $SUV = 0.71 \pm 0.06$ vs 0.42 ± 0.15 , $p = 0.002$ at 1-hour post-injection. Subjects scanned twice showed excellent reproducibility with less than 5% average variation between tests, suggesting 68Ga-CBP8 can be utilized to detect small changes with disease progression and treatment response. In contrast to healthy volunteers where lung PET signal was low and uniform, in IPF subjects PET signal was higher and heterogeneous. High PET signal intensity was seen in lung regions that were fibrotic as determined by computed tomographic (CT) chest imaging, but also in regions where CT showed "normal" lung. These findings suggest that 68Ga-CBP8 PET may detect active collagen deposition not yet visible on CT. Ongoing studies will determine if these abnormal PET signals predict disease progression. In summary we have developed a PET probe that can sensitively and reproducibly detect small changes in lung fibrosis.

Poster Number 20

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Bioengineered nanoprobes for targeting gastrointestinal stromal tumors

INVESTIGATORS: Z. Wang, H. Kang, H. Tsujimoto, H. Ueno, Y. Morimoto, G. E. Fakhri, H. S. Choi, S. Nomura

The surgical principle of non-metastatic gastrointestinal stromal tumors () treatment is complete resection and avoidance of perforation. would be resected more reliably with real-time image navigation. In this study, ZW800-1C, zwitterionic near-infrared (NIR) fluorophore, was conjugated with a CD117 ligand stem cell factor (SCF) for targeting for intraoperative imaging. 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 -T1 (receptor-positive) and -5R (receptor-negative) cell lines. We confirmed the specific targeting of SCF800 on the surface of -T1 cells using the NIR fluorescence microscopy, while negligible fluorescence signals were observed in receptor-compromised -5R cells. Intraoperative imaging and image-guided tumor resection will be followed in -bearing xenograft mice and genetically engineered animal models. This result suggests that SCF800 can be used for highly sensitive, rapid, and non-radioactive imaging for early diagnosis and intraoperative tumor imaging.

Poster Number 21

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Structure-inherent NIR imaging of brown adipose tissue

INVESTIGATORS: G. K. Park, Y. Baek, G. E. Fakhri, H. S. Choi

It was recently discovered that brown adipose tissue (BAT) persists into adulthood and is responsible for maintaining metabolism, playing a vital role in the propensity to gain weight and develop obesity. Clinical significance of BAT in human health and disease is being unraveled, but the mechanism of transitioning back and forth between brown and white fat remains unclear due to scarce detection methods for the longitudinal monitoring. Engineering contrast agents that specifically target BAT remains a challenging research endeavor due to the unknown targetable molecular receptor. We focused on the development of structure-inherent brown fat targeted near-infrared (NIR) fluorophores by strategically targeting mitochondria that are abundant in BAT cells. After a systematic screening of small NIR molecules, we were able to define a generic pharmacophore that showed appealing BAT localization. The NIR region is unique in that it features minimal human tissue absorbance and fluorescence characteristics—this offers an appealing potential for imaging with low background, reduced tissue attenuation, and high signal given the contrast agent that exists for a particular tissue.

Poster Number 22

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Evaluation of K⁺ channel tracer [18F]3F4AP in human tumor xenografts in mice: A potential biomarker in cancer

INVESTIGATORS: K. Takahashi, M. Wilks, E. McDonald, G. El Fakhri, P. Brugarolas, K. Ramos-Torres

Potassium channels are involved in a wide variety of cellular functions. While most commonly studied regarding cell excitability, assessment of their roles in angiogenesis, cell proliferation and migration has propelled their emergence as potential biomarkers for cancer. Altered expression of voltage-dependent K⁺ (Kv) channels is found in several types of cancers and has been correlated to tumorigenic state and malignancy, suggesting the possibility of potassium channels as tumoral biomarkers.

[18F]3-fluoro-4-aminopyridine ([18F]3F4AP) is a radiofluorinated analog of the general K⁺ channel blocker 4-aminopyridine, recently developed to image changes in K⁺ channel expression in neurological disorders like multiple sclerosis. Binding of [18F]3F4AP to channels in the Kv1 family led us to investigate this novel radioligand as a tool to image K⁺ channels as potential tumor markers.

To this end, [18F]3F4AP was produced with molar activity (>1 Ci/μmol) and high radiochemical purity (>95%). Nude mice were subcutaneously inoculated with glioblastoma (U-118), squamous lung cancer (H-520) or melanoma (A-375) cells on the right or left flank. Dynamic PET combined with computed tomography (PET/CT) imaging was performed for 60 min after tail vein injection of 200 μCi of [18F]3F4AP. Preliminary results show low but differential tumor uptake of the radiotracer when comparing the different lines: U-118 tumors showed homogenous distribution of the tracer whereas H-520 and A-375 showed lower uptake in the core than in the periphery. Time-activity curves show slow tumoral uptake of the tracer when compared to other tissues. Studies are ongoing to characterize tracer binding to compare with K⁺ channel levels.

Poster Number 23

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Unsedated diagnosis of eosinophilic esophagitis using spectrally encoded tethered capsule endomicroscopy

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Eosinophilic esophagitis (EoE) is a chronic inflammatory disease caused by food allergy. EoE is characterized by the infiltration of eosinophils in the esophagus, and EoE patients need to be treated with either dietary restriction or topical corticosteroid therapy. The diagnosis of EoE is made by endoscopically acquiring biopsies of the esophagus and histopathologically identifying >15 eosinophils per microscopic high-power field. Since EoE patients may need this assessment to be conducted many times throughout the course of their treatment, the current approach is associated with a high diagnostic cost and poor patient acceptance. We have developed a tethered, swallowable capsule microscope that can identify and count individual eosinophils in a much less invasive manner. The capsule implements a high-speed form of reflectance confocal microscopy called spectrally-encoded confocal microscopy (SECM) that images the entire esophagus at the cellular level in a few minutes. The size of the capsule is similar to that of a small dietary supplement pill; thus, patients can easily swallow the capsule without sedation. To date, we have imaged 9 EoE patients with this capsule, aged 14-41. SECM readers, blinded to biopsy results, rendered a diagnosis of EoE positive or negative. These results were compared to EoE diagnosis from endoscopic biopsies. Results on a per patient basis demonstrated that SECM has an overall diagnostic accuracy of 88.9% (95% Confidence Interval: 68.4-100%). These results suggest that tethered capsule SECM endomicroscopy may become a viable, less invasive alternative to endoscopic biopsy for EoE diagnosis in the future.

Poster Number 24

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Limited angle PET imaging with time-of-flight information for intraoperative use

INVESTIGATORS: S. Sajedi, L. Blackberg, G. El Fakhri, H. Choi, H. Sabet

Objectives: It has been shown that radio-guided probes can be used to identify sentinel lymph node and to delineate tumor margins. In this regard, we are developing a platform consisting of a PET-like detector system having a high-resolution Probe Detector (PD) with time-of-flight (TOF) information, in coincidence with a half-ring detector system (HDS) in which the PD is placed in various locations while HDS is fixed underneath the patient bed.

Methods: We modeled the intraoperative system and a reference PET using Monte-Carlo code GATE. Detector blocks in HDS and PD are 13x13 array of 3.6x3.6x20 mm³ LYSO:Ce pixels with 3.8 mm pitch. A PD placed in proximity of the FOV moving laterally in coincidence with detectors in the bottom HDS.

We are investigating how fast coincidence resolving time (CRT), and location of PD can improve image quality of 1-5 mm radius hot-spheres.

Results: Results show that with a 2-minute acquisition and full-ring detector blocks, one can clearly resolve 3-5 mm spheres but as expected, 2 mm sphere is only marginally resolved when TOF with 150 ps CRT is used. We observed that placing the PD close to the object with 3 PD positions and CRT of 150 ps, one can partially detect 3-5 mm hot spheres.

Conclusion: The results suggest that to improve the image quality with minimum number of PD positions, detectors with fast CRT such as 150 ps is needed. It is apparent that utilizing PD and HDS detectors with smaller crystals is necessary to resolve smaller lesions.

Poster Number 25

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Genetic syndromes and connectomic-genetics of the visuo-motor cortical system

INVESTIGATORS: E. Bueicheku, I. Diez, F. Doleire-Uquillas, L. Ortiz-Teran, J. Sepulcre

Visuo-motor impairments characterize many neurodevelopmental syndromes such as Autism Spectrum Disorder, Dravet, Fragile-X, Prader-Willi and Williams. Despite great advances in neural science, the brain biological basis underlying visuomotor functional impairments in these different neuro-clinical conditions is still unknown. In this study we used task and resting-state fc-MRI data to first map the human visuomotor integration system in the human brain, and then investigated how those functional maps spatially relate to the Allen Human Brain Atlas, a whole-brain transcriptome-wide cortical genetic expression atlas. Finally, we studied if the genes spatially associated to the visuomotor integration network were functionally related to the wide spectrum of developmental neurogenetic syndromes. We found that 21 genes associated with prominent genetic syndromes were related to visual and motor functional impairments, in which TBR1 and MAGEL2 showed a highly significant association. TBR1 is an Autism Spectrum Disorder related gene whose expression is related to neural development of the cortex, the hippocampus, and different subcortical areas, as well as regulation of Auts2 gene-related frontal cortex differentiation. MAGEL2 has been found to be insufficiently expressed in Prader-Willi Syndrome, and has been linked to neural differentiation and maintenance. All together we shed light on genotype-phenotype relationships with different neurodevelopmental syndromes and clinical conditions by describing how the topology of brain systems that support cognitive and motor functions is associated with the aberrant cortical expression of protein coding genes.

Poster Number 26

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A multi-modality microscope for single cell radioluminescence imaging studies

INVESTIGATORS: M. A. Tetrault, Y. Sung, K. Takahashi, G. Pratz, G. El Fakhri, M. Normandin

Radioluminescence microscopy (RLM) is a relatively recent autoradiography imaging modality that, unlike most existing methods, combines single cell-level spatial resolution and good quantitative measurement capabilities. The radio-labeled cells under study are placed in direct contact with a scintillating crystal, where the beta radiation scintillation tracks are captured by an EMCCD camera. RLM thus circumvents the positron range as the spatial resolution limit for positron emission tomography (PET) tracers, which makes it an attractive method to study PET tracers at the cell level. However, a current challenge is to understand FDG uptake disparities in cell populations observed in existing studies. We propose to combine RLM with Quantitative Phase Imaging (QPM) to include the cell dry mass into the image analysis. To explore the concept, we built a multi-modal microscope which sequentially supports bright field, fluorescence, beta-decay radio-luminescence and quantitative phase imaging.

Experimental validation involves imaging FDG uptake in living HeLa cells. First, they are cultured to reach 80% confluence, transferred onto a CdWO₄ crystal in a culture dish and left to rest overnight. Cells are then starved for one hour, then incubated with FDG (2 mCi/mL) for one hour. The data acquisition begins by selecting a field of view, acquiring the QPM image (single-shot) and acquiring RLM signals for 8 minutes. Cell dry mass, cell contours and RLM counts are extracted from the data. The results show that FDG uptake is correlated to the cell dry mass, an observation previously unattainable by existing methods at this granular range.

Poster Number 27

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Hyperpolarized ^{13}C metabolic imaging

INVESTIGATOR: Y. Yen

Until a decade ago, metabolism could only be imaged in vivo by Positron Emission Tomography (PET) from the uptake of radioactive glucose analogs. Recent breakthroughs enabling the hyperpolarization of biologically interesting compounds containing ^{13}C , are revolutionizing the ways we image metabolism. This novel technique uses ^{13}C -enriched metabolites as imaging contrast agents and uses a hyperpolarizer to boost the ^{13}C signal by 10,000 to 100,000-fold. Unlike PET, hyperpolarized ^{13}C imaging not only detects the injected ^{13}C -labeled metabolite but also the downstream products through changes in the measured ^{13}C magnetic resonance spectra. This new molecular imaging technique allows assessment of flux along various metabolic pathways and hence, can detect disease-altered metabolism in vivo. Hyperpolarized ^{13}C metabolic imaging has shown promise in elucidating metabolic changes in cancer and treatment effect. This young field is progressing rapidly in recent years with > 30 clinical trials and > 200 patients already scanned worldwide. Although still new to MGH, hyperpolarized ^{13}C metabolic imaging is coming to the Athinoula A. Martinos Center for Biomedical Imaging at MGH this year, made possible by an NIH funded High-end Instrumentation Grant for a state-of-the-art clinical ^{13}C polarizer. This polarizer will be an invaluable resource for the rich body of clinical translational research at the Martinos Center and the larger MGH research community, as well as other institutions in New England. This poster will showcase this novel technology and its applications in cancer research.

Poster Number 28

Yu-Peng Zhou, PhD

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Development of PET tracers for calcium channel $\alpha 2\delta$ -1 to image pain

INVESTIGATORS: Y. P. Zhou, P. Brugarolas

Chronic pain affects over 25 million Americans. In addition to the debilitating effects that neuropathic pain causes, pain has contributed to the current opioid epidemic. Thus, developing new non-addictive pain drugs is now a major area of interest in biomedical research. An important challenge for developing new pain treatments is how to objectively measure their effects. Positron emission tomography (PET) has the potential to provide specific and quantitative biochemical information on whether drugs are engaging their targets in vivo or whether a disease relevant target has increased or decreased as a result of a treatment. The goal of this investigation is to develop a novel PET tracer for pain.

We hypothesized that $\alpha 2\delta$ -1 subunit of voltage-gated calcium channels (the target of the pain drugs pregabalin and gabapentin) could serve as a biomarker of neuropathic pain. $\alpha 2\delta$ -1 protein has been shown to be highly upregulated in the spinal cord and dorsal root ganglia in animal models of pain. Therefore, we propose that ^{11}C -labeled gabapentin could be useful for imaging chronic changes associated with pain. Autoradiography experiments in tissues from mice with neuropathic pain (spared nerve injury (SNI) model) showed a 1.5-2x increase in $[^3\text{H}]$ gabapentin binding in tissue sections from SNI animals vs. control. Gabapentin does not contain O-methyl or N-methyl groups amenable for standard ^{11}C -methylation. Therefore, we are currently exploring strategies for labeling with $[^{11}\text{C}]$ HCN. In summary, we are developing a novel PET tracer for pain based on the drug Neurontin.

Poster Number 29

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Role of RNA modifications in quiescent chemo-resistant cells

INVESTIGATORS: S. I. Bukhari, S. S. Truesdell, E. Plotsker, M. Granovetter, J. Lee, S. Lee, S. Kollu, J. Kreuzer, Y. Lin, E. Zeme, W. Haas, S. Vasudevan

Recent advances in high-throughput sequencing have helped to decipher the role of “epitranscriptome” in post-transcriptional gene regulation. With over 100 types of chemical modifications reported in eukaryotic RNA species, one of the most prevalent RNA modifications is N6- methyl adenosine (m6A). m6A RNA modification is mediated by RNA methyltransferase METTL3, that has been implicated in mRNA metabolism, decay and translation. We found that in the low mTOR activity cells such as quiescent and chemo-surviving leukemic cells where canonical translation is compromised, one alternative non-canonical translation mechanism is mediated by FXR1a-microRNP of its targets through interactions with p97/DAP5, a non-canonical translation factor. We show that in these low mTOR activity cells, METTL3 levels increase and promote chemo-resistance. Upon knockdown of METTL3, these cells become sensitive to chemotherapy, indicating a role for METTL3 in chemo-resistance. We show that FXR1 and p97 associate with m6A and promote microRNA dependent non-canonical translation of reporter mRNAs, that is reduced upon METTL3 knockdown. microRNA mediated activation targets fail to interact with FXR1 in the absence of METTL3. Global profiling studies of METTL3 knockdown cells showed that genes involved in tumor progression, cell growth, adhesion and migration are down-regulated, whereas cell cycle genes are up-regulated. Furthermore, our studies showed that m6A associated readers in these chemotherapy-treated cells include export, processing and translation factors. Our data suggest that m6A RNA modification regulates post-transcriptional steps of RNA stability, export and translation, to enable specific gene expression in quiescent and chemo-resistant cells.

Poster Number 30

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Blocking CXCR4 alleviates desmoplasia, increases T-lymphocyte infiltration, and improves immunotherapy in metastatic breast cancer

INVESTIGATORS: I. X. Chen, V. P. Chauhan, J. Posada, M. R. Ng, M. W. Wu, P. Adstamongkonkul, P. Huang, N. Lindeman, R. Langer, R. K. Jain

Metastatic breast cancers (mBCs) are largely resistant to immune checkpoint blockade, but the mechanisms remain unclear. Primary breast cancers are characterized by a dense fibrotic stroma, which is considered immunosuppressive in multiple malignancies, but the stromal composition of breast cancer metastases and its role in immunosuppression are largely unknown. Here we show that liver and lung metastases of human breast cancers tend to be highly fibrotic, and unlike primary breast tumors, they exclude cytotoxic T-lymphocytes (CTLs). Unbiased analysis of the TCGA database of human breast tumors revealed a set of genes that are associated with stromal T-lymphocyte exclusion. Among these we focused on CXCL12 as a relevant target based on its known roles in immunosuppression in other cancer types. We found that the CXCL12 receptor CXCR4 is highly expressed in both human primary tumors and metastases. To gain insight into the role of CXCL12-CXCR4 axis, we inhibited CXCR4 and found that AMD3100 decreases fibrosis, alleviates solid stress, decompresses blood vessels, increases CTL infiltration, and decreases immunosuppression in murine mBC models. We confirmed that these immunosuppressive effects are dependent on cancer-associated fibroblast (CAF) signaling through genetic deletion of CXCR4 in aSMA+ cells. Accordingly, CXCR4 inhibition more than doubles the response to immune checkpoint blockers in mice bearing mBCs. These findings demonstrate that CXCL12/CXCR4-mediated desmoplasia in mBC promotes immunosuppression and is a potential target for overcoming therapeutic resistance to immune checkpoint blockade in mBC patients.

Poster Number 31

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Mechanisms of enhanced drug delivery in brain metastases with focused ultrasound-induced blood-tumor barrier disruption

INVESTIGATORS: G. B. Ferraro, C. Arvanitis, V. Askoxylakis, Y. Guo, M. Datta, J. Kloepper, M. O. Bernabeu, D. Fukumura, N. McDannold, R. Jain

Blood-brain/blood-tumor barriers (BBB and BTB) and interstitial transport may constitute major obstacles to the transport of therapeutics in brain tumors. In this study, we examined the impact of focused ultrasound (FUS) in combination with microbubbles on the transport of two relevant chemotherapy-based anticancer agents in HER2-positive breast cancer brain metastases at cellular resolution: the non-targeted chemotherapeutic doxorubicin and the antibody-drug conjugate ado-trastuzumab emtansine (T-DM1).

Using an orthotopic xenograft model of HER2-positive breast cancer brain metastasis and quantitative microscopy we demonstrate multifold increases in the extravasation of both agents (7-fold and 2-fold for doxorubicin and T-DM1, respectively) and we provide evidence of increased drug penetration ($>100\mu\text{m}$ vs. $<20\mu\text{m}$ and $42\pm7\mu\text{m}$ vs. $12\pm4\mu\text{m}$ for doxorubicin and T-DM1, respectively) after application of FUS as compared to control (non-FUS). Integration of experimental data with physiologically based pharmacokinetic (PBPK) modeling of drug transport reveals that FUS in combination with microbubbles alleviates vascular barriers and enhances interstitial convective transport via increase in hydraulic conductivity. Combination of experimental data and PBPK modeling suggests that FUS in combination with microbubbles increases the endothelial cell transmembrane transport and uptake. PBPK modelling indicates selective increase in transvascular transport of the non-targeted small chemotherapeutic doxorubicin through small vessel-wall pores size with a narrow range (Diameter: 10-50nm). Our work provides a quantitative framework for the optimization of FUS-drug combinations to maximize intratumoral drug delivery and facilitate the development of novel therapeutic strategies against brain metastases.

Poster Number 32

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Identifying mechanisms governing metastatic outgrowth

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Background: Only 0.01% cells enter circulation, survive & produce metastasis, however, metastatic disease accounts for 90% of cancer-related deaths. The molecular mechanisms that primary tumor cells acquire to recruit blood vessels and invade/intravasate has been well studied, however, the molecular changes that these cells acquire in transit and during the colonization and proliferation in the secondary metastatic site still remains widely unknown.

Goal: We are interested what characteristics confer metastatic cells with a survival and growth advantage once in the metastatic niche, and how can these vulnerabilities be exploited to treat metastatic disease.

Strategy: Perform unbiased RNA-Seq using established models of PDAC and BC metastasis to identify a gene signature unique to metastatic lesions. Using gene expression data, establish a targeted functional shRNA screen to identify genes conferring anoikis-resistance, thus exploiting vulnerabilities unique to metastatic lesions.

Poster Number 33

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Autophagy controls the stability of CAF gene program repressor CSL/RBPJk through an SQSTM1/p62-dependent mechanism

INVESTIGATORS: S. Goruppi, A. Clocchiatti, V. Neel, G. Dotto

Cancer associated fibroblasts (CAFs) are critical for tumor initiation, recurrence and progression. CSL/RBPJ, a transcriptional repressor that mediates Notch signaling, suppresses the gene expression program leading to CAF activation and associated metabolic reprogramming, as well as autophagy. Surprisingly little is known on control of CSL protein turnover especially in the tumor microenvironment.

Here we report that in human dermal fibroblasts (HDFs), conditions inducing autophagy - often found in tumor stroma - down-regulate CSL protein levels while not affecting its mRNA levels. Genetic or pharmacologic targeting of the autophagic machinery blocks CSL down modulation. Mechanistically, endogenous CSL associates to the autophagy and signaling adaptor p62/SQSTM1, which is required for CSL down modulation by autophagy. This is of functional significance since both CSL and p62 levels are lower in skin SCC-derived CAFs, in which autophagy is increased. Increasing CSL cellular levels stabilize p62 and interrupts the autophagic process. We uncover here an autophagy-initiated mechanism for CSL down modulation, which could be targeted for stroma-focused cancer prevention and treatment.

Poster Number 34

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Exploring the role of MTDH/AEG-1 lipidation in cancer progression

INVESTIGATORS: C. Guarino, X. Wu

Metadherin (MTDH) is a potent oncogene involved in the progression of many tumors. MTDH is frequently amplified in multiple cancers, and its high expression level correlates with poorer outcome in patients of uveal melanoma (UM), the most common ocular cancer. Using metabolic labeling with bioorthogonal chemical probes, we recently identified that MTDH is posttranslationally modified by 18-carbon fatty acid at a conserved cysteine residue (S-stearoylation). Our preliminary data show that MTDH lipidation is strongly associated with more aggressive tumour behaviour. Here, we propose that MTDH tumor-promoting functions are regulated by fatty acid through a unique regulatory process. Misregulation of lipid metabolism and MTDH fatty acylation is involved in progression and metastasis of UM. Our work combined chemical biology and cancer biology and reveal novel insights into how fatty acylation is involved in cancer metastasis, ultimately leading to new therapeutics.

Poster Number 35

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Design and evaluation of applicators for photodynamic therapy of oral cavity tumors in low resource settings

INVESTIGATORS: A. P. Khan, S. Mallidi, H. Liu, L. Daly, G. Rudd, M. B. Hussain, S. Khan, S. A. Siddiqui, S. A. Hasan, K. Akhtar, M. August, M. Troulis, F. Cuckov, J. P. Celli, T. Hasan

Oral cancer represents over 30% of cancers reported in low middle-income countries (LMIC), like India and is the leading cause of cancer death among Indian men. Surgery, radiation and chemo therapies are the mainstay of management but are either too expensive, unavailable for people or have extensive side effects. An alternate effective therapy for oral cancer is photodynamic therapy (PDT), a light based spatially targeted cytotoxic therapy that has shown excellent healing of the oral mucosa post treatment. We here combined engineering, optics and biochemistry to produce a low-cost, mobile LED-based light source with 3D printed light applicators for smart phone-based, image-guided PDT. After validating the devices in preclinical models, we performed an ergonomics study on 10 healthy volunteers at the MGH, where the comfort level of the applicators (anterior buccal cheek, posterior buccal cheek and retromolar positions) and presence of fatigue or numbness in the mouth due to the applicators was evaluated. We found that the retromolar and posterior applicators were the most comfortable and well tolerated. After these initial steps, the device was tested in clinical studies of early oral cancer in India. We observe in subjects with T1N0M0 oral lesions that our applicator and light system combination delivered light to cover the entire lesion area and yielded effective PDT response. Of the 18 treatments so far, 14 subjects have responded, with no residual/recurrent disease in follow-up biopsy. The significance of this work is that it offers an alternative treatment modality for early disease without associated morbidities.

Poster Number 36

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Modification of extracellular matrix improves oncolytic virus immunotherapy in glioblastoma

INVESTIGATORS: E. Barcin, R. L. Martuza, S. D. Rabkin, K. Shah, H. Wakimoto, J. Kiyokawa

Complex interplays between heterogenous cells and extracellular matrix (ECM) in the tumor microenvironment drive the pathogenesis and therapeutic resistance of glioblastoma (GBM). We hypothesized that modification of ECM would enhance oncolytic virus immunotherapy of GBM by overcoming the immunosuppressive functions of GBM ECM. Using stem-like cells-generated GBM model in immunocompetent mice, we show that intratumoral injections of ICOVIR17, an oncolytic adenovirus armed with hyaluronidase PH20 cDNA, promotes degradation of hyaluronan, the major component of GBM ECM, and increases animal survival by recruiting CD4+ and CD8+ T cells and macrophages to the tumor sites. ICOVIR17-induced upregulation of immune checkpoint PD-L1 on GBM and immune cells enables us to effectively combine ICOVIR17 with systemic anti-PD1 antibody therapy to further improve therapeutic outcomes.

Poster Number 37

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IL-15/B7-H3 TriKEs-based specific immunotherapy for pancreatic ductal adenocarcinoma (PDAC)

INVESTIGATORS: F. Kontos, T. Kurokawa, S. Ferrone, C. R. Ferrone

Background: The lack of effective therapies for PDAC has prompted us to develop immunotherapeutic strategies. In this study, we have selected as effectors NK-cells which are expected to be less toxic than T-cells because of their shorter life span. To target NK-cells to tumor cells we use bispecific antibodies which consist of an antibody specific for tumor cells and an antibody specific for CD16 on NK-cells. To activate and expand the NK-cells, we have added IL-15 to the conventional bispecific NK-cell engagers (BiKEs) converting them into IL-15 trispecific NK-cell engagers (TriKEs). B7-H3 has been selected as a target since it is expressed on both differentiated PDAC cells and PDAC cancer initiating cells, is up-regulated on tumor associated fibroblasts and stroma and has a restricted distribution in normal tissues.

Methods: B7-H3 expression was tested by FACS analysis of cells stained with the B7-H3-specific monoclonal antibody 376.96. The latter was used to generate an IL-15/B7-H3-specific TriKE. The antitumor activity of IL-15/B7-H3-specific TriKEs combined with NK-cells was tested in vitro with co-culture experiments. FACS analysis was utilized to assess the mitogenic effect of IL-15/B7-H3-specific TriKEs on NK-cells.

Results: B7-H3 is highly expressed on PDAC-2, PDAC-3 and PDAC-6 cell lines. IL-15/B7-H3-specific TriKEs can stimulate the proliferation of NK-cells without affecting the proliferation of T-cells. Moreover, PDAC cell lines were almost 100% eliminated when co-cultured with IL-15/B7-H3-specific TriKEs and NK-cells.

Conclusion: These results suggest that IL-15/B7-H3 TriKEs combined with NK-cells are a useful immunotherapeutic strategy against PDAC.

Poster Number 38

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Can intrahepatic delivery of Chimeric Antigen Receptor (CAR) T cells enhance their safety and efficacy against intrahepatic cholangiocarcinoma (ICC)?

INVESTIGATORS: T. Kurokawa, F. Kontos, S. Ferrone, C. R. Ferrone

Background: The need for an effective therapy for ICC has prompted us to develop a novel immunotherapeutic strategy which targets differentiated ICC cells and ICC cancer initiating cells (CICs). The effector mechanism we have selected is represented by B7-H3 CAR T-cells. B7-H3 has been selected as a target since it is expressed on both differentiated ICC cells and ICC CICs, is up-regulated on tumor associated fibroblasts and stroma and has a restricted distribution in normal tissues.

Methods: B7-H3 expression was tested by FACS analysis of cells stained with the B7-H3-specific monoclonal antibody 376.96. The latter was used to generate a B7-H3 CAR. The antitumor activity of B7-H3 CAR T-cells was tested in vitro with co-culture experiments and in vivo with NSG mice orthotopically grafted with human ICC cells.

Results: B7-H3 is expressed on ICC2 and ICC3 cell lines and on the 10 surgically resected human ICCs. B7-H3 CAR T-cells eliminate almost 100% of ICC cells when co-cultured with ICC-2 and ICC-3 cell lines in vitro. In contrast, intravenous systemic administration of B7-H3 CAR T-cells to NSG mice orthotopically grafted with ICC2 and ICC3 cell lines prolonged their survival but were not able to control tumor growth.

Conclusions: These results suggest that B7-H3 CAR T-cells may be useful for immunotherapy of ICC. The inadequate elimination of ICC in NSG mice is likely due to the low number of B7-H3 CAR T-cells infiltrating the tumor. We are testing whether B7-H3 CAR T-cell injection through the portal vein improves tumor infiltration.

Poster Number 39

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Mesenchymal stem cell-derived extracellular vesicles promote proliferation and migration in breast cancer cells through activation of the ERK pathway

INVESTIGATORS: T. Li, Y. Zeng, X. Li, H. Chen

Mesenchymal stem cells (MSCs) have been demonstrated to be involved in tumor progression and modulation of the tumor microenvironment, partly through their secretome. Extracellular vesicles (EVs) are membranous nanovesicles secreted by multiple types of cells and have been demonstrated to mediate intercellular communication in both physiological and pathological conditions. However, numerous questions still remain regarding the underlying mechanisms and functional consequences of these interactions. The purpose of this study was to investigate the effect of human umbilical cord mesenchymal stem cell-derived EVs (hUC-MSC-EVs) on proliferation, migration, and invasion of human breast cancer cells. We successfully generated and identified hUC-MSCs and hUC-MSC-EVs which were used in this study. Our results showed that hUC-MSC-EVs treatment significantly enhanced proliferation, migration, and invasion of human breast cancer cells MDA-MB-231 and MCF-7 in vitro. hUC-MSC-EVs treatment reduced E-cadherin expression and increased N-cadherin expression thus promoted epithelial-mesenchymal transition (EMT) of the breast cancer cells. Pretreatment of the breast cancer cells with ERK inhibitor prior to interaction with hUC-MSC-EVs significantly reversed hUC-MSC-EVs-enhanced proliferation, migration and invasion as well as hUC-MSC-EVs-promoted EMT of the breast cancer cells. These data indicated that hUC-MSC-EVs could promote the invasive and migratory potential of breast cancer cells through induction of EMT via the ERK pathway, leading to malignant tumor progression and metastasis. Taken together, our results suggest that targeting pathways to reverse EMT may lead to development of novel approaches to combat breast cancer.

Poster Number 40

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Differential inflammatory response dynamics in normal lung following stereotactic body radiation therapy with protons versus photons

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Background and Rationale: To compare time-dependent changes in lung parenchyma of early-stage non-small cell lung carcinoma (NSCLC) patients after stereotactic body radiation therapy with protons (SBPT) or photons (SBRT).

Materials and Method: We retrospectively identified NSCLC patients treated with SBPT and matched each one with a SBRT patient by patient, tumor, and treatment characteristics. Lung parenchyma on serial post-treatment chest computer tomography (CT) scans was deformably registered with the treatment plan to analyze lung density changes as function of dose, quantified by Hounsfield Unit (HU)/Gy. A thoracic radiologist also evaluated the CTs using an established grading system.

Results: We matched 23 SBPT/SBRT pairs, including 5 patients treated with both modalities (internally matched cohort). Normal lung response following SBPT significantly increased early (median, 3 months) post-treatment compared to SBRT for which changes kept increasing up to later time points (median, 9 months, $p=0.003$). These differences were most pronounced in sensitive (response $>6\text{HU/Gy}$) patients and in the internally matched cohort. However, there was no difference in the maximum observed response in the entire cohort over all time points, median $3.1[\text{IQR}, 1.1-5.9]\text{HU/Gy}$ (SBPT) versus $2.9[1.7-5.2]\text{HU/Gy}$ (SBRT). Qualitative radiological evaluation was highly correlated with the quantitative analysis ($p<10^{-13}$). Maximum lung responses between SBPT and SBRT tended to differ more ($p=0.05$) in low-dose regions ($<15\text{ Gy}$).

Conclusion: While there was no difference in maximum response after SBPT versus SBRT, dose-defined lung inflammation occurred earlier after proton irradiation. Further investigation is warranted into the mechanisms of inflammation and therapeutic consequences after proton versus photon irradiation.

Poster Number 41

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Clinical machine learning model for mismatch repair deficiency prediction

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DNA mismatch repair (MMR) is critical for maintaining fidelity of genome during replication. MMR-deficient (MMR-D) cancers correlate with a high rate of response to immunotherapy making MMR-D testing essential to personalized cancer treatment. Unfortunately, routine MMR-D testing is only feasible in certain tumor sites (colorectal, endometrium), while others (PAC) do not get routine testing due to low incidence of MMR-D and limited tissue availability. Regardless, MMR-D remains a strong treatment prognostic indicator thus motivating the development of a robust test with broader applicability. Here we present a Machine Learning-based approach which uses routine genotyping results to predict MMR-D with high accuracy.

Our classifier works with variant data from the SNAPSHOT-NGS-V2 assay (MGH CID). The classifier first extracts expert features including counts of insertions & deletions in repetitive regions, number of variants from a curated "hotspot" list, among others, for a total of seven (7) indicators total. We then train a Logistic Regression classifier to combine those features into a single probability score, where higher scores correspond to a higher predicted likelihood of MMR-D.

We train the classifier on a combined cohort of colorectal and endometrial carcinoma cases reviewed by a molecular pathologist. In validation the classifier shows 70% sensitivity (9/13) and 97% specificity (184/190) for other cancer sites. Our classifier exhibits promising utility in identifying cases with MMR-D for further workup and with more data could be clinically validated for patient use. Future work includes validating the classifier on individual tumor sites, pending acquisition of more MMR-D positives.

Poster Number 42

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Yes-Associated Protein 1 (YAP1) increases human papillomavirus (HPV) infection and inhibits innate immunity to drives cervical carcinogenesis

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Although human papillomavirus (HPV) is believed to be the major causative agent for cervical cancer, accumulating evidence indicates that HPV alone is insufficient to induce cervical cancer. The molecular mechanisms underlying cervical cancer development remain unclear. In the present study, we provide evidence showing that the Hippo/YAP pathway plays a critical role in cervical carcinogenesis. We found that genetic alteration of the Hippo/YAP pathway frequently occurred in human cervical cancer patients and was associated with poor prognosis of these patients. Keratin 14 promoter-driven expression of constitutively active Yes-Associated Protein 1 (YAP1) in mouse cervical epithelial cells induced invasive cervical squamous cell carcinoma (CVSCC), suggesting that hyper-activation of YAP1 is sufficient to induce cervical cancer. Hyper-activation of YAP1 in cervical epithelial cells not only increased the putative HPV receptor molecules and cellular susceptibility to HPV infection, but also disrupted the host cell innate immune system, resulting in failure of HPV viral recognition, suppression of type I IFN production, inhibition of the IFNRs/JAKs/STATs pathway, and blockage of production of antiviral interferon-stimulated genes. Cervical epithelial specific YAP1 and HPV double knockin mouse models showed that YAP1 synergizes with HPV to promote the initiation and progression of CVSCC. The present study demonstrates that the Hippo/YAP pathway plays a central role in the development of CVSCC and the synergism between hyper-activated YAP1 and high-risk HPV may be the key driver of cervical cancer development. The Hippo/YAP signaling pathway may represent a promising target for developing novel strategies to prevent and treat cervical cancer.

Poster Number 43

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Proton shifting induces leukemia growth by epigenetic activation of H⁺/lactate-cotransporter MCT4

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Ion balance is critical for maintaining membrane polarity, signaling and energy transfer in cells. Here, we report that proton distribution can reset carbon metabolism and alter growth characteristics in hematopoietic cells. Multiple distinct oncogenic mutations in acute myeloid leukemia (AML) utilize proton partitioning to enhance their growth advantage. They do so by epigenetically increasing expression of the H⁺/lactate co-transporter, MCT4, effectively shuttling protons extracellularly to increase intracellular pH (pHi). Secondly, enzymatic activity of select metabolic regulators is increased including hexokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase. This increases carbon flux through glycolysis and the pentose phosphate pathway (PPP) necessary for macromolecule generation. Overexpression of MCT4 in normal hematopoietic stem and progenitor cells (HSPC) increases growth without inducing malignant transformation. Yet, inhibition of MCT4 in AML decreases pHi, enzymatic activity and PPP carbon flux leading to improved animal survival and, unexpectedly, elimination of leukemic initiating cells (LIC) in vivo. AML cell lines with increased MCT4 expression have the activating histone mark, H3K27ac, in the MCT4 promoter region where MLL-AF9 and, JQ-1-inhibitable, BRD4 also directly bind. These data demonstrate the sequential alteration of metabolism through epigenetic activation of an intracellular proton regulator and point to cytoplasmic alkalization as a growth promoting strategy exploited by malignant cells. Inhibiting this process may diminish the competitive advantage of leukemic cells and potentially improve AML treatment.

Poster Number 44

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Mullerian inhibiting substance suppresses H19 and regulates epithelial to mesenchymal transition in ovarian cancer ascites cells

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Mullerian inhibiting substance (MIS) has been shown to inhibit the growth of a stem-like population of ovarian cancer cells both in-vivo and in-vitro. We have shown that its receptor, MISRII, is expressed in a majority of ovarian serous adenocarcinomas. However, the determinants of response and the pathways elicited by MIS treatment have been difficult to elucidate due to the heterogeneity of tumors. To address this, we have developed an assay in which autologous patient cancer cells and immune cells are isolated from ascites of recurrent chemoresistant ovarian cancer and incubated in cleared ascites supernatant for 24h in presence of drug (MIS 10ug/ml) or vehicle control (N=3). This protocol allows for short-term culture in conditions nearly identical to the peritoneal environment. The effect of treatment on cancer cell states was interrogated by clustering analysis of single cell RNA sequencing (inDROP). A parallel experiment was conducted using matching pure primary cancer cell lines (derived from the ascites samples) treated in media (MIS 10ug/ml or vehicle for 24h). We observed that MIS treatment was associated with a consistent inhibition of H19 in cancer cells associated with changes in epithelial to mesenchymal transition (EMT) markers and TGF- β signaling markers. The cell lines had varied epithelial and mesenchymal characteristics across patients and MIS regulates EMT in a patient-specific manner. The effect of MIS treatment on EMT and cell motility was also compared in monolayer cultures by Scratch "wound" assays. We aim to further dissect how MIS may change EMT cell states to modulate tumor growth, metastasis, and chemoresistance.

Poster Number 45

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Age-related tumor growth in mice is related to integrin α 4 in CD8+ T cells

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Cancer incidence increases with age, but paradoxically, cancers have been found to grow more quickly in young mice compared with aged ones. The cause of differential tumor growth has been debated and, over time, attributed to faster tumor cell proliferation, decreased tumor cell apoptosis, and/or increased angiogenesis in young animals. Despite major advances in our understanding of tumor immunity over the past 2 decades, little attention has been paid to comparing immune cell populations in young and aged mice. Using mouse colon adenocarcinoma model MC38 implanted in young and mature mice, we show that age substantially influences the number of tumor-infiltrating cytotoxic CD8+ T cells, which control cancer progression. The different tumor growth pace in young and mature mice was abrogated in RAG1null mice, which lack mature T and B lymphocytes, and upon selective depletion of endogenous CD8+ cells. Transcriptome analysis further indicated that young mice have decreased levels of the Itga4 gene (CD49d, VLA-4) in tumor-infiltrating lymphocytes when compared with mature mice. Hypothesizing that VLA-4 can have a tumor-protective effect, we depleted the protein, which resulted in accelerated tumor growth in mature mice. These observations may explain the paradoxical growth rates observed in murine cancers, point to the central role of VLA-4 in controlling tumor growth, and open new venues to therapeutic manipulation.

Poster Number 46

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EVs modulate GBM intratumor heterogeneity and treatment resistance

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Glioblastoma (GBM) is the most common primary malignant brain tumor and despite optimal treatment, long-term survival remains uncommon. GBM can be roughly divided into three different molecular subtypes, each varying in aggressiveness and treatment resistance. Interestingly, recent evidence shows plasticity between these subtypes in which the proneural (PN) glioma stem-like cells undergo transition into the more aggressive mesenchymal (MES) subtype leading to therapeutic resistance. Extracellular vesicles (EVs) are membranous structures secreted by nearly every cell and have been shown to play a key role in GBM progression by acting as multifunctional signaling complexes.

Here we show that EVs derived from MES cells educate PN cells to increase stemness, cell proliferation, tumor aggressiveness, and therapeutic resistance by modulating mesenchymal transition through NF- κ B activation.

Our findings could potentially help to explore new treatment strategies for GBM and indicate that EVs may also play a role in the mesenchymal transition of different tumor types.

Poster Number 47

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Leveraging reprogrammed metabolism to define selective vulnerabilities of IDH1 mutated cholangiocarcinoma cells

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Cholangiocarcinoma is an epithelial malignancy of the biliary tract with poor prognosis that has been steadily rising over the past decades. Gain-of-function hot-spot mutations in the isocitrate dehydrogenase gene (IDH) are among the most common genetic alterations in these tumors. The mutant IDH1 enzyme produces high levels of metabolite 2-hydroxyglutarate (2HG), which perturbs epigenetic control and cellular metabolism. We sought to further explore the metabolic processes altered by mutant IDH1 and to utilize this information to define selective vulnerabilities of IDH mutant cholangiocarcinoma cells that may inform novel therapeutic strategies. We show evidence that 2HG broadly reprograms cellular metabolism in cholangiocarcinoma cells, including prominent suppression of mitochondrial respiration, and selective hinderance of the de novo pyrimidine synthesis pathway, which requires mitochondria. We integrated these findings with results from a series of screens that compared the sensitivity of IDH1 mutant and IDH1 wild type cholangiocarcinoma cells to extensive drug libraries. Among the most selective and potent hits in mutant IDH1 cells was an orphan compound that we found promoted an imbalanced nucleotide pool in IDH1 mutant cells, with overwhelming CTP levels compared to other nucleotides. In keeping with defective DNA replication and the generation of DNA damage associated with this imbalance, we observed activation of the ATM-TP53 DNA checkpoint pathway followed by apoptosis in these cells. Thus, here we show alterations in nucleotide metabolism as central to the unique metabolic state of IDH1 mutated cholangiocarcinoma cells and demonstrate the potential of leveraging reprogrammed metabolism as a selective therapeutic strategy.

Poster Number 48

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A novel hapten-based vaccine enhances response to immune checkpoint blockade

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In metastatic melanoma, both tumor neoantigen load and development of vitiligo have been associated with favorable response to immunotherapy. We hypothesize that in the context of immune checkpoint blockade (ICB), neoantigens facilitate epitope spreading and immune targeting of tumor-lineage self-antigens. We also aim to harness this process with a hapten-based vaccine to improve ICB response.

Previously, our lab demonstrated in a murine model of melanoma that tumors with high neoantigen load respond significantly better to ICB than syngeneic tumors with low neoantigen load. We show that this response is associated with increased immune recognition of a melanocyte self-antigen (gp100) and that long-term survivors develop a durable immune response against tumor-lineage self-antigens. We leverage the understanding that neoantigens can promote epitope spreading into a novel vaccine therapy by exogenously introducing "neoepitopes" into tumor cells with hapten treatment. Mice bearing melanomas with low neoantigen load responded significantly better to hapten vaccine plus anti-PD-1 compared to unhaptenated control vaccine plus anti-PD-1. Bulk tumor RNA-Seq revealed enhanced immune and T cell signatures with hapten vaccine treatment. Immunohistochemistry showed increased CD8+ T cells and decreased Foxp3+ Tregs intratumorally, and flow cytometry demonstrated elevated functional CD8+ T cells targeting the melanocyte self-antigen gp100. Depletion of specific immune cell populations confirmed that CD8+ T cells are required for treatment efficacy. Hapten vaccine treatment also increased the efficacy of combination immunotherapies and improved ICB response in a model of pancreatic ductal adenocarcinoma. This novel hapten-based vaccine may have broad clinical applications as a strategy to enhance ICB response.

Poster Number 49

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Losartan treatment enhances chemotherapy efficacy and reduces ascites in ovarian cancer models by normalizing the tumor stroma

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In ovarian cancer patients, tumor fibrosis and angiotensin-driven fibrogenic signaling have been shown to inversely correlate with survival. We sought to enhance drug delivery and therapeutic efficacy by remodeling the dense extracellular matrix in two orthotopic human ovarian carcinoma xenograft models. We hypothesized that targeting the angiotensin signaling axis with losartan, an approved angiotensin system inhibitor, could reduce extracellular matrix content and the associated "solid stress," leading to better anti-cancer therapeutic effect. We report here four translatable findings: i) losartan treatment enhances the efficacy of paclitaxel – a drug used for ovarian cancer treatment – via normalizing the tumor microenvironment resulting in improved vessel perfusion and drug delivery; ii) losartan depletes matrix via inducing anti-fibrotic miRNAs that should be tested as candidate biomarkers of response or resistance to chemotherapy; iii) although losartan therapy alone does not reduce tumor burden, it reduces both the incidence and the amount of ascites formed; and iv) our retrospective analysis revealed that patients receiving angiotensin system inhibitors concurrently with standard treatment for ovarian cancer exhibited 30 months longer overall survival compared with patients on other antihypertensives. Our findings provide the rationale and supporting data for a clinical trial on combined losartan and chemotherapy in ovarian cancer patients.

Poster Number 50

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Validation of a more efficient approach to the meta-analysis of cancer susceptibility gene penetrance using natural language processing

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Background: Quantifying the cancer risk for germline cancer susceptibility genes (i.e. penetrance) enables the personalization of preventive management strategies. As the literature is growing exponentially, conducting a meta-analysis is the best way to obtain robust penetrance estimates. We previously developed a natural language processing (NLP)-based abstract classifier, which classifies abstracts as penetrance, prevalence, both, or neither. Using this classifier, we can partially automate the literature review process required for a meta-analysis. This study evaluates the performance of this procedure.

Methods: The semi-automated procedure, which involved automated abstract classification and text mining, followed by human review, was compared to the traditional approach requiring human review of all studies. Ten high-quality gene-cancer penetrance meta-analyses were used for performance evaluation. The number of abstracts requiring human review (workload) and the ability to identify final included papers in these meta-analyses (coverage) were our primary measures.

Results: Compared with the traditional approach, the semi-automated procedure was associated with lower workload across all ten meta-analyses, with an overall 84% reduction (2,774 vs 17,434 abstracts), translating into saving over 730 hours of human efforts. The overall coverage was 93% (132/142). The main reasons for missed studies included blank abstracts and poorly written abstracts. After additionally reviewing references of the final studies, the coverage improved to 99%.

Conclusions: We demonstrated that a semi-automated NLP-based procedure can significantly reduce the abstract review workload, without compromising the ability to identify relevant studies. NLP algorithms have promising potential in reducing human efforts in the literature review process for gene-cancer penetrance meta-analyses.

Poster Number 51

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Gut intraepithelial T cells calibrate metabolism and accelerate cardiovascular disease

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The biochemical response to food intake must be precisely regulated. Because ingested sugars and fats can feed into many anabolic and catabolic pathways, how our bodies handle nutrients depends on strategically-positioned metabolic sensors that link a meal's intrinsic nutritional value with intermediary metabolism. Here we show that a subset of immune cells, specifically integrin $\beta 7^+$ natural gut intraepithelial T lymphocytes (natural IELs), dispersed throughout the small intestine's enterocyte layer, modulate systemic metabolism. $\beta 7^-$ mice lacking natural IELs are metabolically hyperactive and, when fed a high fat and sugar diet, resist obesity, hypercholesterolemia, hypertension, diabetes, and atherosclerosis. Protection from cardiovascular disease in the absence of natural IELs, we further show, depends on the enteroendocrine cell-derived incretin GLP-1, which is normally under control of IELs via GLP-1 receptor expression. In this metabolic control system, IELs oppose nutrient-taking enterocytes by acting as gatekeepers that limit GLP-1 bioavailability. While its function may prove advantageous when food is scarce, present overabundance of diets high in fat and sugar render this metabolic checkpoint inimical to health.

Poster Number 52

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Sleep modulates hematopoiesis and protects against atherosclerosis

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Sleep is integral to life, and its insufficiency or disruption increases the risk of multiple pathological conditions, including cardiovascular disease. Despite these associations, we know little about the cellular and molecular mechanisms by which sleep maintains cardiovascular health. Here we report that sleep regulates hematopoiesis and protects against atherosclerosis. Mice subjected to sleep fragmentation produce more Ly-6Chi monocytes, develop larger atherosclerotic lesions, and produce less hypocretin, a stimulatory and wake-promoting neuropeptide, in the lateral hypothalamus. Hypocretin controls myelopoiesis by restricting CSF1 production by hypocretin-receptor expressing pre-neutrophils in the bone marrow. Consequently, hypocretin-null and hematopoietic hypocretin-receptor-null mice develop monocytosis and accelerated atherosclerosis, which can be mitigated in sleep-fragmented mice via hypocretin supplementation. Together, these results identify a neuro-immune axis that links sleep causally to hematopoiesis and atherosclerosis.

Poster Number 53

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Worksite food purchases and employees' overall dietary quality and health

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Introduction: Most Americans spend half their waking hours at work. We hypothesized that the healthfulness of worksite food purchases would be associated with employees' overall dietary quality and health.

Methods: Participants were 602 hospital employees who used worksite cafeterias and enrolled in a health promotion study in 2016-18. All cafeterias used traffic-light labels (green=healthy, yellow=less healthy, red=unhealthy). We calculated a Healthy Purchasing Score (HPS) for each participant using the prior 3 months of cafeteria purchases and weighting the proportion of items purchased (red=0, yellow=0.5, green=1). A Healthy Eating Index (HEI) score (range 0-100, higher=healthier diet) was estimated using 24-hour dietary recalls. Body mass index (BMI), blood pressure, and HbA1c were measured; hypertension and prediabetes/diabetes (pre-DM/DM) diagnoses were determined by self-reported and clinical data. Regression analyses examined employees' dietary quality and diagnoses by tertile of HPS (T1=least healthy, T3=most healthy), adjusting for demographics and purchases.

Results: Mean age was 43.6 years; 79% were female and 81% white. Mean BMI was 28.3 kg/m² (SD: 6.5), 21% had hypertension, and 27% had pre-DM/DM. Mean HEI was 60.4 (range 28.2 - 92.4, SD: 12.5); mean HPS was 0.66 (range 0.13 - 0.98, SD: 0.15). HEI increased with each HPS tertile (T1=55.6, T2=61.0, T3=64.5; p for trend < 0.001). Obesity prevalence decreased with each HPS tertile (T1=38%, T2=29%, T3=24%, p for trend < 0.001). Similar patterns were observed for hypertension and pre-DM/DM.

Conclusions: Worksite food purchases reflected employees' overall diet quality and cardiometabolic risk factors. Results suggest interventions promoting healthier worksite food choices could improve employees' health.

Poster Number 54

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ARHGEF26 is a novel genetic risk factor for vascular inflammation and coronary artery disease

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The genetic risk underlying coronary artery disease (CAD) is not well understood. We performed a genome-wide association study in UK Biobank testing 9 million DNA variants for association with CAD. We identified ARHGEF26 as a novel locus significantly associated with CAD (OR=1.08, 95% CI 1.06-1.11, $P=1.02 \times 10^{-9}$). We hypothesized that ARHGEF26 regulates vascular cell function and inflammation. We performed eQTL and allele-specific expression analyses, and found the CAD-risk haplotype, tagged by the lead variant rs12493885 (ARHGEF26 p.Val29Leu), had no significant association with allelic imbalance or local transcription in human arterial samples. Promoter luciferase assay showed no significant difference between the reference and alternative haplotypes. In contrast, overexpression of exogenous Leu29 mutant ARHGEF26 after depletion of endogenous ARHGEF26 led to rescued phenotypes consistently exceeding those observed with overexpression of wild-type ARHGEF26, including increased leukocyte transendothelial migration, leukocyte adhesion on endothelial cells, and smooth muscle cell proliferation. These data suggest that the CAD-risk allele (Leu29) produces a gain-of-function ARHGEF26 in vascular cells. To identify the molecular mechanism, we compared the nucleotide-exchange activity between the wild-type and mutant ARHGEF26 and found no significant difference. However, evaluation of ARHGEF26 protein stability by cycloheximide chase showed the Leu29 mutant displayed longer half-life than wild-type, suggesting the gain-of-function phenotypes of Leu29 may be secondary to its resistance to degradation. Quantitative proteomics revealed differential protein interaction between the wild-type and Leu29 ARHGEF26 in cells, highlighting critical inflammatory pathways. Together, our work identified ARHGEF26 is a novel genetic risk factor for CAD.

Poster Number 55

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Glioma reprograms microglial cells through extracellular transfer of miR-21

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Gliomas are primary brain tumors derived from neuroglia stem cells. Microglia are innate immune cells in the central nervous system and make up a large part of glioma tissue. Glioma cells shape their micro-environment communicating with and reprogramming surrounding non-neoplastic cells resulting in enhanced angiogenesis, immune suppression and remodeling of the extracellular matrix. Glioma cells communicate with microglia, in part by releasing extracellular vesicles (EVs) that include microvesicles and exosomes. By intracranial implantation of mouse glioma cells, stably expressing a palmitoylated fluorescent protein to label EVs, in syngeneic miR21 null mice we monitored the uptake of EVs by surrounding cells in the brain. Here, we demonstrate that microglia take up EVs derived from the tumor following functional delivery of miR-21 that regulates specific downstream mRNA targets. These findings show an EV dependent miRNA delivery and provide insight into the reprogramming of microglial cells by tumor cells creating a favorable micro-environment.

Poster Number 56

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Growth factor-induced macropinocytosis in tumorigenesis

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The architectural and biochemical features of the plasma membrane are governed by its intimate association with the underlying cortical cytoskeleton. The neurofibromatosis type 2 (NF2) tumor suppressor, merlin, and closely related membrane:cytoskeleton linking protein ezrin, organize the membrane:cytoskeleton interface, a critical cellular compartment that both regulates and is regulated by growth factor receptors. An example of this poorly understood interrelationship is macropinocytosis, an ancient process of nutrient uptake and membrane remodeling that can both be triggered by growth factors and manage receptor availability.

We show that merlin-deficiency primes the membrane:cytoskeleton interface for Epidermal Growth Factor (EGF)-induced macropinocytosis via a mechanism involving increased cortical ezrin, altered actomyosin and stabilized cholesterol-rich membranes. These changes profoundly alter EGFR trafficking in merlin-deficient cells, favoring increased membrane levels of its heterodimerization partner ErbB2, clathrin-independent internalization and recycling. Our work suggests that unlike Ras-transformed cells, merlin-deficient cells do not depend on macropinocytic protein scavenging, and instead, exploit macropinocytosis for receptor recycling. Finally, we provide evidence that the macropinocytic proficiency of NF2-deficient cells can be employed for therapeutic uptake. This work provides new insight into fundamental mechanisms of macropinocytotic uptake and processing and suggests new ways to interfere with or exploit macropinocytosis in NF2-mutant and other tumors.

Poster Number 57

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Human iPSC-derived neuronal cell-based assays for evaluating the therapeutic potential of small-molecule probes targeting the WNT/GSK3 pathway as regenerative medicines

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Glycogen Synthase Kinase-3 (GSK3) regulates diverse cellular functions via its interaction with a myriad of protein substrates. Recent human genetic studies, in combination with evidence from pre-clinical molecular, cellular, pharmacological and behavioral studies, increasingly recognize the importance of a balance in GSK activity and its regulation of WNT signaling and other critical neural substrates on neuroplasticity in the context of health and disease. Consequently, pharmacological control of GSK3 is being actively pursued as a therapeutic target for CNS disorders ranging from dementia to neurodevelopmental disorders such as Fragile X syndrome and Pitt-Hopkins syndrome. However, development of GSK3-targeted therapeutics for clinical use have faced hurdles of poor selectivity, low blood-brain barrier (BBB) penetration, and toxicity. To address these challenges for GSK3, and simultaneously identify other potential therapeutic targets relevant to WNT signaling and regenerative medicine, we developed three quantitative, high-throughput, cell-based assays that enable quantification of: 1) WNT pathway activity under basal and stimulated conditions; 2) neurogenesis and viability of NPCs; and 3) GSK3-dependent kinase activity toward CRMP2, a critical cytoskeletal regulator, in differentiated neurons. We report here the application of these assays to screen for novel GSK3 inhibitors and to also characterize novel compounds being synthesized for use as positron emission tomography (PET) imaging probes that will allow assessment of in vivo target engagement. Taken as a whole, our data indicate these assays are robust and suitable for high-throughput screening and optimizing potential novel disease-modifying therapeutic and diagnostic agents in the context of human disease biology.

Poster Number 58

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MITF specifies melanocytes by repressing neural crest genes

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Melanocytes arise from neural crest cells, which produce multiple cell types including neurons, glial cells, craniofacial bone and cartilage cells and smooth muscle cells, and produce melanin pigments, which decide our skin, hair, and eye colors and protect our skin from ultra violet.

Microphthalmia-associated transcription factor (MITF) is a master transcriptional regulator of the melanocyte development and differentiation. MITF positively regulates transcription of not only melanocyte specific (e.g., TYR, TYRP1, DCT, PMEL) but also ubiquitously expressed/fundamental (e.g., CDK2, BCL2, BCL2A1, PPARGCA) genes. MITF specifies melanocytes, but the precise mechanism of it remains unclear.

We performed RNA sequencing to analyze transcriptome of primary human melanocytes with MITF knockdown and unexpectedly found that MITF knockdown induced neuronal and glial genes. By analyzing genome-wide MITF-binding sites of primary human melanocytes by chromatin immunoprecipitation followed by sequencing, we found that MITF repressed neuronal and glial—two alternative lineages of neural crest cells—genes. Furthermore, we found that desmoplastic melanomas that express low level of MITF—but not nevi and non-desmoplastic melanomas—expressed a neuronal/glial gene repressed by MITF.

These findings suggest that MITF specifies melanocytes in two ways: (1) promote melanocyte differentiation by activating melanocytic genes and (2) protect melanocyte precursors from differentiating into other lineages by actively repressing neuronal and glial genes.

Poster Number 59

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Quantitative bone-marrow imaging reveals niche signatures of distinct types of adult and juvenile hematopoietic stem cells

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The bone marrow (BM) microenvironment consists of multiple cell populations supporting hematopoietic stem cells (HSCs). Despite extensive research, the cellular composition of the BM HSC niche remains controversial. Previous studies used different strategies to stain HSCs in situ and focused on only few niche components in bones with anatomical differences, thus leading to contradict results.

To systematically dissect the complex HSC microenvironment, we employed a new quantitative multicolor imaging pipeline enabling simultaneous visualization of HSCs and up to 4 distinct BM components. We imaged 9 putative niche types in combination with different HSC reporter systems using 121 full-bone sections from different bones. We reveal that both femoral and sternal HSCs are adjacent to sinusoids, megakaryocytes and Cxcl12 stroma, but not bone, adipocytes, arteries/arterioles, peri-arteriolar and non-myelinated Schwann cells. Multi-dimensional analysis revealed that HSCs occupy multiple spatially distinct niches with discrete cellular composition. To investigate whether those niches support different HSC sub-types, we identified dormant label-retaining (LR) and proliferating non-LR HSCs in situ. Surprisingly, LRs, non-LRs HSCs all have similar niche signatures, challenging previous studies. Finally, we quantify how HSC localization changes with age. Our data provide a detailed view of the BM HSC niche and the underlying cellular players involved in supporting stem-cell properties under homeostasis. We revise and extend previously contradictory findings by quantitatively image a large number of niche components and their combinations in different bones of reporter mice marking HSCs with distinct properties.

Poster Number 60

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Cell polarity and RTK signaling in biliary morphogenesis and tumorigenesis

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The NF2 tumor suppressor, Merlin, is required for the architecture and functional specification of cells and tissues. We previously found that liver-specific deletion of Nf2 in mice yields progenitor expansion that leads to both hepatocellular carcinoma and cholangiocarcinoma. Here we show that these neoductular lesions originate during intrahepatic bile duct (IHBD) formation in the embryonic liver via the over-conversion of progenitors to a biliary fate without proliferation. An investigation of this phenotype yielded fundamental insight into IHBD development; we found that in the normal developing liver IHBDs form via a self-organizing mechanism featuring the coordination of polarity, cell-cell communication, de novo lumen formation/expansion and apical constriction. In livers lacking Merlin, deregulated luminal expansion during this process recruits excess hepatoblasts to a biliary fate. Ectopic IHBDs that form in the Nf2^{-/-} embryonic liver later proliferate uncontrollably after birth, leading to tumorigenesis. This suggests that changes in the architecture of the cell cortex can influence cell fate during development and establishes a new paradigm for IHBD formation. This work also provides new links between IHBD formation and cholangiocarcinoma, which often features neoductular pathology. Indeed, the self-organizing, inductive nature of IHBD formation could be a driver of intratumoral heterogeneity in cholangiocarcinoma; moreover, the role of FGF/FGFR in similar self-organizing developmental processes in lower organisms suggests a model for the established role of FGFR in human cholangiocarcinoma. I have devised an in vitro 3D system, using cells derived from embryonic mouse livers, to investigate the role of unrestricted FGF signaling in livers lacking NF2.

Poster Number 61

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Microtubule acetylation promotes myofibroblast mechanoactivation and lung fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a severe lung disease characterized by the deposition, remodeling, and stiffening of extracellular matrix buildup. This matrix stiffening promotes lung fibrosis by inducing mechanoactivation of fibroblasts via mechanotransduction pathways. Although the role of the actin cytoskeleton in integrin-mediated mechanotransduction has been well documented, the role of the microtubule cytoskeleton in lung fibroblast mechanoactivation remains poorly understood. Here we investigate how mechanical signaling modulates microtubule acetylation to promote myofibroblast activation and lung fibrosis.

Mechanoactivation of human lung fibroblasts (HLFs) was induced by culturing these cells on soft (1kPa) or stiff (100kPa) polyacrylamide hydrogels. Mass spectrometry analysis revealed that HLFs cultured on stiff matrices showed hyper-acetylated α -tubulin at residue lysine 40 compared to cells cultured on soft matrices. α -tubulin K40 acetylation is regulated by the enzyme α TAT1, which showed upregulated in fibrotic lung fibroblasts compared to controls. Forced overexpression of WT α TAT1, but not catalytically dead α TAT1, induced increased microtubule acetylation and myofibroblast activation. In vivo, global α TAT1 knock out mice were significantly protected from bleomycin-induced lung fibrosis. Mechanistically, our studies demonstrated that leukocyte recruitment and vascular permeability were preserved in α TAT1-deficient mice following bleomycin injury. However, the number of α SMA+ lung myofibroblasts determined by immunofluorescence was significantly reduced in α TAT1-deficient mice compared to WT, indicating that the protective phenotype of α TAT1 knock out mice could be ascribed to impaired mechanoactivation of myofibroblasts following bleomycin injury.

Our studies highlight that α TAT1 inhibition has the potential to be a new therapeutic strategy for the treatment of IPF

Poster Number 62

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Unraveling the mechanism of neutropenia in patients with Barth Syndrome

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Barth syndrome (BTHS) is an X-linked recessive disorder characterized by neutropenia and cardiomyopathy. BTHS is caused by loss-of-function mutations in the tafazzin (TAZ) gene. TAZ is an enzyme that processes cardiolipin, a phospholipid found within the inner mitochondrial membrane, and the lack of TAZ activity results in a deficiency of unsaturated cardiolipins.

Patients with BTHS have a high early childhood mortality rate due to neutropenia and the risk of life-threatening infections. However, the cellular and molecular mechanisms for neutropenia in BTHS is unknown. In this study, we examined the metabolism, function, and survival of neutrophils lacking TAZ to understand the link between TAZ-deficiency and neutropenia.

We utilized an established ER-HOXB8 system to develop conditionally immortalized granulocyte-monocyte progenitors (GMPs) from TAZ-KO and WT mice. These progenitors can be expanded and differentiated ex vivo into functional neutrophils. The TAZ-KO neutrophils showed no significant defects in phagocytosis, migration, and cytokine secretion. However, TAZ deficiency in neutrophils significantly decreased mitochondrial oxygen consumption and intracellular ATP production rate. Moreover, a lipidomics analysis indicated that TAZ deficiency led to the expected reduction in unsaturated cardiolipins as well as a dysregulation of cholesterol, fatty acids, and other phospholipids.

Consistent with these findings, gene expression analysis indicated that transcripts regulating lipid metabolism, steroid biosynthesis, and AMPK signaling pathways were upregulated in TAZ-KO cells. Finally, we observed that TAZ-KO GMPs were more sensitive to apoptotic stress. These data suggest that neutropenia in BTHS may be attributed to abnormal mitochondrial energy metabolism, aberrant lipid regulation, and accelerated apoptosis of GMPs.

Poster Number 63

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A novel in vivo model of colonic aganglionosis using diphtheria toxin-mediated ablation of the enteric nervous system

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Hirschsprung disease (HSCR) is a congenital disorder characterized by the failure of neural crest cells to colonize the entire intestine during development, leaving the distal colon aganglionic. The current treatment involves surgical removal of the aganglionic gut but leads to significant intestinal morbidity in 50% children. Thus, innovative therapies are needed to treat HSCR. Testing new therapies is hampered by the poor survival of existing models of HSCR, which die within the first few weeks of life.

We developed a novel model of colonic aganglionosis by crossing Wnt1-Cre mice with R26R-iDTR reporter mice, thus generating a Wnt1-iDTR line in which active Cre recombination renders Wnt1-expressing neural crest cells sensitive to diphtheria toxin (DT). To create segmental aganglionosis, we injected DT into the colon wall via laparotomy. This resulted in focal loss of enteric neurons and glia in the myenteric plexus, which was maintained up to 3 months, suggesting persistence of ENS ablation. No obvious loss of smooth muscle cells or interstitial cells of Cajal was observed, confirming specificity of ablation. Significant changes in muscle thickness and epithelial morphology were revealed following ENS ablation. Moreover, focal loss of ENS did not alter solid or liquid gastrointestinal transit time or colonic contractility and did not produce a megacolon phenotype, resulting in markedly improved survival in this model of focal aganglionosis as compared to HSCR.

We have generated a novel, non-lethal, and highly specific in vivo model of colonic aganglionosis that can be utilized to assess therapeutic strategies for treatment of neurointestinal disease.

Poster Number 64

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Thymus regeneration is dependent on distinct mesenchymal stromal cell populations

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The regenerative ability of the thymus is an important factor in determining the outcome of a bone marrow transplantation. Still, the cytoreductive regimens employed prior to the transplantation invariably damage the thymus stroma, thus impeding recovery of T lymphopoiesis. Overall, the thymus niche is poorly defined. Thymic epithelial cells have been extensively characterized, but our understanding of how other stromal cell types contribute to T lymphopoiesis is limited. We therefore set out to further define the thymic niche under homeostasis and regeneration.

Using single-cell RNA-sequencing we demonstrate changes in distinct thymic mesenchymal stromal cell (MSC) populations 3 days post-irradiation and transplantation. At this time point, when thymus seeding progenitors are entering the tissue, one MSC subset that is capable of producing T cell promoting factors is reduced whereas another class of pro-adipogenic MSCs is increased. Suggesting that the slow regeneration of the thymus after a transplantation could in part stem from this imbalance in MSC subtypes. In further support of this, adoptive transfer of MSCs into irradiated and transplanted hosts accelerates short-term regeneration of the thymic microenvironment and T cell progenitor seeding.

Poster Number 65

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Human induced pluripotent stem cell derived pro-epicardial cells enhance survival of and facilitate compaction of three-dimensional cardiac disc, spherical organoids, and tube constructs ex vivo

INVESTIGATORS: G. Kaur, J. R. Gershlak, J. Tan, J. P. Guyette, H. C. Ott

The application of induced pluripotent stem cell derived cardiomyocytes (CMs) in cell therapy and cardiac tissue engineering is limited by their morphological and functional immaturity. During in vivo human heart development, non-myocyte support cells derived primarily from the epicardium influence CM proliferation, maturation, and alignment. Nevertheless, no study has been conducted to recapitulate these developmental events in engineering cardiac tissue using human cells, despite several reports showing successful generation of human epicardial cells from human induced pluripotent stem cells. Therefore, my project is focused on developing three-dimensional cardiac discs, spherical organoids, and tube constructs to model human heart development in vitro. I comparatively evaluate the functional and structural maturation: survival and compaction of CMs in constructs engineered with the additional pro-epicardial cells (PECs). The objective of this project is to determine if our highly efficiently differentiated PECs recapitulate their in vivo structural and functional heart morphogenesis roles ex vivo. To assess survival, I perform terminal deoxynucleotidyl transferase dUTP nick end labeling analysis on constructs engineered with or without PECs and find discs, organoids, and tubes improve their survival at over the course of 3 weeks. A rudimentary week-long compaction assay also supports facilitation of compaction ex vivo.

Poster Number 66

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Single cell sequencing of neonatal uterus reveals a subluminal endometrial stromal progenitor indispensable for female fertility

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The Mullerian duct is the anlage of the fallopian tube, uterus, cervix, and upper third of the vagina, which regresses in the male fetus in response to Mullerian inhibiting substance (MIS). This sexual differentiation process is driven by an MIS receptor 2 (MIS2) expressing subluminal mesenchymal cell directing the regression of the Mullerian luminal epithelium. In the female, this MIS2+ mesenchyme is retained in the absence of the MIS inhibitory signal, yet its specific contribution to the postnatal development of the uterus remains unknown. Characterizing the fate of this multi-potent uterine progenitor cells is crucial to our understanding of how uterine differentiation contributes to female fertility. Here, we report that subluminal mesenchymal MIS2+ expression persists in the uterus after birth. Using single cell RNA sequencing we demonstrate that the inner and outer endometrial stromal layers are derived from MIS2+ stromal progenitors and that this cell fate is specified in the first week of life. Inhibiting these MIS2+ stromal progenitors with MIS during that critical postnatal developmental period dysregulates paracrine signals necessary for uterine development (WNTs, BMPs, IGFs), prevents specification of the endometrial stromal layers, and results in uterine hypoplasia, absence of glandular duct formation, and complete infertility in the adult. Together, our results identify a new endometrial stromal progenitor cell type responsible for the formation of the inner and outer endometrial stromal layers, and elucidates novel signaling pathways that are necessary for uterine layer specification and indispensable for female fertility.

Poster Number 67

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Decreased oral glucose tolerance and insulin response during biological evening versus morning among adults under free-living conditions

INVESTIGATORS: H. S. Dashti, C. Vetter, J. Florez, M. Garaulet, F. A. Scheer, R. Saxena

Introduction: Melatonin secretion during the biological night impairs glucose tolerance in vivo and suppresses insulin secretion from beta-cells in vitro, yet its implication in adults under free-living conditions and its relevance for time-of-day assessment of glucose tolerance remain undetermined.

Methods: In the Shift work, Heredity, Insulin, and Food Time (SHIFT) Study (#NCT02997319), we tested the hypothesis that compared to the biological morning, an oral glucose tolerance test (OGTT) performed during the biological evening will reveal relatively impaired glucose tolerance and reduced insulin secretion. In this randomized crossover study, participants completed two 2-hour 75-g OGTTs: morning and evening. Incremental 2-hour area under the curve (iAUC) for increases in glucose and insulin using the trapezoidal method, insulin sensitivity index (ISI), corrected insulin response (CIR), and oral disposition index (Dio) were calculated.

Results: In 83 healthy, non-night workers, we confirmed 10-fold higher endogenous melatonin concentrations at biological evening compared to biological morning assessments (morning, 2.35 ± 3.93 pg/ml vs. evening, 26.16 ± 24.72 pg/ml; $P < 0.001$). Compared to the morning OGTT, evening OGTT postprandial glucose was 91% higher (glucose iAUC: morning, $4,523 \pm 2587$ mg.min/dl vs. night, $8,652 \pm 2773$ mg.min/dl; $P < 2.2 \times 10^{-16}$), while no difference in postprandial insulin iAUC ($P = 0.82$) was noticeable. Whereas time-of-day had no influence on ISI ($P = 0.87$), the evening OGTT had significantly lower CIR ($P = 0.022$) and Dio ($P = 0.003$) compared to the morning OGTT.

Conclusion: These observations extend earlier findings from in vitro and controlled human experiments by confirming impaired glucose tolerance likely from reduced insulin response to glucose during the biological evening relative to the biological morning.

Poster Number 68

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Serum neuroactive steroid levels in postmenopausal women with treatment-resistant major depressive disorder

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Neuroactive steroids such as allopregnanolone and 3α -androstenediol are modulators of traditional neurotransmitter receptors implicated in the etiopathology of psychiatric disorders. We hypothesized that fasting allopregnanolone and 3α -androstenediol by GC/MS, and the ratio of these steroid levels to their precursors (progesterone and testosterone, respectively), would be lower in postmenopausal women with treatment-resistant major depressive disorder (MDD, $n=12$) than non-depressed controls (HC, $n=28$).

Mean age and BMI did not differ between groups. In MDD vs HC, the mean allopregnanolone/progesterone ratio was lower (0.20 ± 0.19 vs 0.47 ± 0.46 , $p=0.03$) and progesterone levels were higher (153 ± 177 vs 57 ± 50 pg/mL, $p=0.04$). There was no difference in mean allopregnanolone. MDD had lower serum free testosterone vs HC (0.21 ± 0.16 vs 0.38 ± 0.18 ng/dL, $p=0.006$). There was no difference in 3α -androstenediol levels or 3α -androstenediol/total testosterone ratio between groups. There was a trend toward a positive association between progesterone levels and depression severity ($r=0.34$, $p=0.06$) and an inverse association between the allopregnanolone/progesterone ratio and depression severity ($r=-0.36$, $p=0.07$). Lower free testosterone levels were associated with greater depression severity ($r=-0.45$, $p=0.03$).

In postmenopausal women with treatment-resistant MDD, the allopregnanolone/progesterone ratio was lower and progesterone levels higher than in non-depressed controls. Allopregnanolone levels did not differ between groups. This may be due to reduced metabolism of progesterone to allopregnanolone and could have treatment implications. Additionally, testosterone levels were lower in depressed women, but there was no difference in 3α -androstenediol levels or the 3α -androstenediol/total testosterone ratio between depressed and non-depressed women, suggesting that testosterone may play a greater role in depression symptomatology than its metabolite.

Poster Number 69

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Integrated metagenomic and metabolomic investigation of host-microbiota interactions reveals the beneficial effects of combination of elevated tissue Omega-3 fatty acids and Genistein supplementation on high-fat diet induced obesity and metabolic syndrome

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The combined effect of an elevated tissue omega-3 polyunsaturated fatty acids (PUFA) and genistein (Gen) on high-fat diet-induced obesity and metabolic syndrome (MS) is not known. Male 6 weeks old wild-type (WT) and FAT-1 transgenic mice, which can endogenously produce omega-3 fatty acids, were divided into four groups (1. WT. 2. FAT-1. 3. WT+ Gen. 4. FAT-1+Gen; n=5) and fed either HFD (60%Kcal fat) or HFD supplemented with Genistein (1g/kg diet) for 16 weeks. Markers of obesity, MS, metabolic endotoxemia (ME), low-grade chronic inflammation (LGCI) were investigated. Multi-omics technologies were applied to resolve the taxonomic and functional attributes of gastrointestinal microbiota at the metagenomic and metabolomic levels. Compared to individual effects, the combination of elevated tissue omega-3 PUFA and Gen dramatically prevented the obesity and MS, which was associated with reduced ME (serum lipopolysaccharides) and LGCI (serum TNF-alpha) in the FAT-1 +Gen group. Alterations in the gut microbiome (alpha and beta diversity and taxon-based analysis) and fecal and serum metabolites (global and individual differences) were dramatic as well as beneficial in the FAT-1+Gen group compared to other 3 groups. Integrated metagenomic-metabolomic analysis uncovered a major role of healthy host-microbiome interactions associated with observed preventive effects of combination of n-3 PUFA and Gen. For the first time, our multi-omics study of host-microbiota interactions therefore demonstrates that elevated tissue n-3 PUFA and Gen may exert dramatically beneficial effect in combination, and indicates the potential necessity of combination of n-3 PUFA with Gen in lowering the risk for obesity and associated MS.

Poster Number 70

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Oxytocin significantly attenuates the functional connectivity between food motivation brain areas in overweight and obese men exposed to high caloric food images

INVESTIGATORS: E. A. Lawson, F. Plessow, L. Holsen, N. Hadjikhani, L. Kerem

The neurohormone oxytocin (OXT), shown to decrease food intake, is a promising novel treatment for obesity. We previously showed that in overweight and obese men, intranasal (IN) OXT reduced fMRI activation in the ventral tegmental area (VTA), the origin of the mesolimbic dopaminergic reward system, in response to high-calorie food vs non-food visual stimuli. Here we employed a dynamic method of fMRI analysis, functional connectivity, which refers explicitly to the influence that one neural system exerts over another in a context-dependent manner. We hypothesized that OXT would modulate the functional connectivity of the VTA with key brain areas known to be involved with food processing.

In this randomized, double-blind, placebo-controlled crossover study of IN OXT, 10 overweight/obese (mean±SD BMI 28.9±0.8 kg/m²), men age 31.4±1.8 years presented after a 10-h overnight fast. Following administration, subjects started an fMRI food motivation paradigm that included images of high and low-calorie foods. The VTA was anatomically defined as the seed region to explore effects of OXT on functional connectivity. Following OXT administration, compared to placebo, participants showed significant attenuation of the functional connectivity between the VTA and insula, parietal operculum, amygdala, cingulate, and hippocampus in response to viewing high-calorie food stimuli vs. objects ($Z \geq 3.1$, cluster corrected, $P=0.05$).

Conclusions: In overweight/obese men OXT attenuates the functional connectivity between the VTA and brain regions associated with processing of food images. These findings could partially explain the anorexigenic effect of OXT, providing insight into the mechanism through which OXT ameliorates food-cue-induced, reward anticipation in obese patients.

Poster Number 71

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A randomized placebo-controlled trial of low-dose testosterone therapy in women with anorexia nervosa

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Anorexia nervosa (AN) is a psychiatric illness with significant morbidity and no approved medical therapies. We have shown that relative androgen deficiency in AN is associated with depression and anxiety symptom severity. We hypothesized that physiologic testosterone therapy would improve weight, depressive and anxiety symptoms, and eating disorder symptoms/behavior.

Methods: 90 women 18-45 yo with AN (mean BMI 18.3 ± 1.6 kg/m²) and free testosterone levels below the median for healthy women were randomized to testosterone 300 mcg daily or placebo patch for 6 months. Primary outcome was BMI. Secondary outcomes were depression symptom severity [by Hamilton Depression Rating Scale (HAM-D)], anxiety symptom severity [by Hamilton Anxiety Rating Scale (HAM-A)] and eating disorder psychopathology/behaviors [by Eating Disorder Examination (EDE) and Eating Disorder Inventory-2 (EDI-2)].

Results: Mean BMI increased by 0.02 ± 1.01 kg/m² in the active, and 0.5 ± 1.1 kg/m² in the placebo, group ($p=0.03$) over 6 months. At 4 weeks, there was a trend toward a greater decrease in HAM-D score ($p=0.09$) in the active vs placebo group. At 6 months, mean HAM-D and HAM-A scores decreased similarly in both groups [HAM-D -2.9 ± 4.9 (active) vs -3.0 ± 5 (placebo), $p=0.72$; HAM-A -4.5 ± 5.3 (active) vs -4.3 ± 4.4 (placebo), $p=0.25$]. There were no significant differences in EDE or EDI-2 scores between groups.

Conclusions: Low-dose testosterone therapy for 6 months resulted in less weight gain, and did not lead to sustained improvements in depression, anxiety, or disordered eating symptoms, relative to placebo in women with AN.

Poster Number 72

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Tibial and radial bone structure as assessed by HRpQCT may explain differences in peripheral skeletal integrity and fracture risk across the weight spectrum that cannot be explained by areal BMD alone

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Context: Women with anorexia nervosa and obesity have elevated fracture risk, but bone structure and strength by high-resolution peripheral quantitative CT (HRpQCT) have not been reported. We investigated tibial and radial structure, strength, and their determinants in women across the weight spectrum. Design: Cross-sectional. Setting: Clinical research center. Participants: 139 women, 17-46yo: 1) anorexia nervosa (AN)(n=74), 2) eumenorrheic lean controls (HC)(n=33) and 3) eumenorrheic obese (OB)(n=32). Measures: Areal BMD (aBMD) and appendicular lean mass (ALM) by DXA; tibial and radial cortical and trabecular bone structure and strength by HRpQCT. Results: Mean aBMD Z-scores, tibial and radial trabecular vBMD, and failure load were lowest in AN, higher in HC, and highest in OB. Mean trabecular plate variables were lower in AN($p<0.05$), but not higher in OB, than HC. Mean cortical thickness, but not vBMD or porosity, was lower in AN than OB. At higher BMIs, increases in trabecular vBMD, failure load, and plate variables were minimal. ALM and IGF-1 levels were positively, and amenorrhea negatively, associated with skeletal variables. Despite similar aBMD, women with a fracture history had lower mean trabecular vBMD and plate variables than those without($p<0.05$). Conclusions: Tibial and radial structure and strength were impaired in women with anorexia nervosa, and only slightly higher in women with obesity, compared to controls, suggesting the skeleton may not adapt to higher weight loads in obesity. Higher muscle mass and IGF-1 levels were associated with greater, and amenorrhea more impaired, skeletal variables. Bone structure may explain differences in fracture risk not explained by aBMD.

Poster Number 73

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Night eating among night shift workers: Preliminary results from the SHIFT Study

INVESTIGATORS: C. R. Tucker, H. S. Dashti, C. Vetter, J. Florez, M. Garaulet, F. Scheer, R. Saxena

With over 15 million adults working night shifts in the US, it has become increasingly important to identify modifiable factors that mediate the link between night shift work (NSW) and type 2 diabetes. Food intake during the biological night has been shown to disrupt the synchrony across biological rhythms, however changes in the diurnal distribution of food intake among NSW has not been extensively explored. We test the hypothesis that compared to day shift workers (DSW), NSW have shifted caloric intakes towards later times in the Shift work, Heredity, Insulin, and Food Time (SHIFT) Study (NCT02997319), a multicenter, large, observational study. Here, we describe results from the first 21 NSW and 21 sex-/age-matched DSW. Participants recorded 24-hour food intake noting food type, time, and quantity for 2 weeks. Total energy intake and macronutrient composition were similar between NSW and DSW ($P > 0.05$). On work days, compared to DSW, NSW consumed meals later in the day, and most notably dinner, the most energy dense meal (DSW, 7:13pm vs. NSW, 11:12pm). The last eating episode was also later (DSW, 8:36pm vs. NSW, 3:23am). On non-work days, food intake times for all meals were similar. These observations support the hypothesis that NSW consume food coinciding with the biological night, which may mediate the link between night shift and type 2 diabetes. When completed, the SHIFT Study will be able to delineate the influence of different night shift schedules on mistimed food intake exposure.

Poster Number 74

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Ultra-rare genetic variation in the epilepsies: A whole-exome sequencing study of 17,606 individuals

INVESTIGATORS: Y. A. Feng, D. P. Howrigan, L. E. Abbott, K. Tashman, F. Cerrato, B. M. Neale, Epi25 Collaborative

Sequencing-based studies have identified novel risk genes for rare, severe epilepsies and revealed a role of rare deleterious variation in common epilepsies. To identify the shared and distinct ultra-rare genetic risk factors for rare and common epilepsies, we performed a whole-exome sequencing (WES) analysis of 9,170 epilepsy-affected individuals and 8,364 controls of European ancestry. We focused on three phenotypic groups; the rare but severe developmental and epileptic encephalopathies (DEE), and the commoner phenotypes of genetic generalized epilepsy (GGE) and non-acquired focal epilepsy (NAFE). We observed that compared to controls, individuals with any type of epilepsy carried an excess of ultra-rare, deleterious variants in constrained genes and in genes previously associated with epilepsy, with the strongest enrichment seen in DEE and the least in NAFE. Moreover, we found that inhibitory GABAA receptor genes were enriched for missense variants across all three classes of epilepsy, while no enrichment was seen in excitatory receptor genes. The larger gene groups for the GABAergic pathway or cation channels also showed a significant mutational burden in DEE and GGE. Although no single gene surpassed exome-wide significance among individuals with GGE or NAFE, highly constrained genes and genes encoding ion channels were among the top associations, including CACNA1G, EEF1A2, and GABRG2 for GGE and LGI1, TRIM3, and GABRG2 for NAFE. Our study confirms a convergence in the genetics of common and rare epilepsies associated with ultra-rare coding variation and highlights a ubiquitous role for GABAergic inhibition in epilepsy etiology in the largest epilepsy WES study to date.

Poster Number 75

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Repurposing large health insurance claims data to estimate genetic and environmental contributions in 560 phenotypes

INVESTIGATORS: C. M. Lakhani, B. R. Tierney, A. K. Manrai, J. Yang, P. M. Visscher, C. J. Patel

We analysed a large health insurance dataset to assess the genetic and environmental contributions of 560 disease-related phenotypes in 56,396 twin pairs and 724,513 sibling pairs out of 44,859,462 individuals that live in the United States. We estimated the contribution of environmental risk factors (socioeconomic status (SES), air pollution and climate) in each phenotype. Mean heritability ($h^2 = 0.311$) and shared environmental variance ($c^2 = 0.088$) were higher than variance attributed to specific environmental factors such as zip-code-level SES ($\text{varSES} = 0.002$), daily air quality ($\text{varAQI} = 0.0004$), and average temperature ($\text{vartemp} = 0.001$) overall, as well as for individual phenotypes. We found significant heritability and shared environment for a number of comorbidities ($h^2 = 0.433$, $c^2 = 0.241$) and average monthly cost ($h^2 = 0.290$, $c^2 = 0.302$). All results are available using our Claims Analysis of Twin Correlation and Heritability (CaTCH) web application.

Poster Number 76

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Genetic and molecular basis of circadian rhythm disorders: A patient-centered research study

INVESTIGATORS: J. M. Lane, F. A. Scheer, R. Saxena

Circadian rhythms regulate human behavior and physiology within the 24-hour, with dysregulation of those rhythms associated with sleep disorders, cognitive and physical performance, cancer, and chronic metabolic and neurologic disease. Despite the importance of circadian rhythms to human health, little is known about the biological connection between human circadian rhythms and our health. We propose to identify novel genetic factors involved in circadian rhythms by recruiting, phenotyping, and sequencing the genome of extreme circadian rhythm disorder patients. To achieve this goal, we are launching a new patient-driven study of circadian rhythm disorders. We will recruit participants from online advocacy groups, through fliers in circadian disorder clinics, and advertisement on our study website. In order to reach a diverse set of patients our study will forgo traditional laboratory-based tests of circadian rhythms and instead develop a novel home-based circadian phenotyping kit which can also be used by clinicians to diagnose circadian rhythm disorder patients at MGH and beyond. We will perform exome sequencing on DNA extracted from participant saliva samples and analyze our cases using reverse regression (revreg). Although the short-term goal of the study is a genetic study of circadian rhythm disorders, we hope by making it a lot easier to participate, more people will participate, our study will represent the population better, and lead to generation of data and insights that do not exist today significantly impacting people with circadian rhythm disorders.

Poster Number 77

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Systematic evaluation of prenatal and pediatric diagnostic yields from whole-genome sequencing in 8,954 individuals

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Clinical genetic screening in prenatal and pediatric cohorts have traditionally required a decision to test targeted genes or mutational classes, as evaluation of all variant classes has been intractable. Whole genome sequencing (WGS) has the potential to transform diagnostic testing by capturing all classes variation with a single technology. Here, we compared diagnostic yields from WGS to those from karyotype, CMA, and whole exome sequencing (WES) in a pediatric cohort of 2,100 quartet families with a proband diagnosed with autism spectrum disorder (ASD; n=8,400) and a prenatal cohort of 202 cases with a structural defect detected on ultrasound. We first benchmarked our bioinformatic pipelines on 519 ASD quartets, discovering 3.4M SNVs, 0.3M indels, and 5,863 structural variants (SVs) per genome. WGS recapitulated 99.6% of all CMA-predicted CNVs and >97% of all de novo coding variants from WES. Molecular validation of 171 de novo SVs revealed a 97% confirmation rate. The yield from WGS exceeded all other technologies but provided only ~0.3% increased diagnostic yield over the combination of all conventional methods. We next evaluated WGS in the fetal structural anomaly samples and discovered a pathogenic variant in 11.1% of cases that had negative karyotype and CMA results. This study suggests a modest overall increased diagnostic yield of WGS compared to the combination of all conventional methods and should temper enthusiasm regarding substantial increases in interpretable pathogenic variants from WGS. Nonetheless, WGS was superior to any individual method thus warranting evaluation as a first-tier screen in prenatal and pediatric diagnostic testing.

Poster Number 78

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Multi-trait genome-wide association meta-analysis of dietary intake identifies new loci and functional links with metabolic traits

INVESTIGATORS: J. Merino, H. S. Dashti, C. Sarnowski, J. M. Lane, J. C. Florez, R. Saxena, CHARGE Consortium and the UK Biobank

Dietary intake, a major contributor to the global obesity epidemics, is a complex behavior influenced by innate variability in biological processes. Recent genome-wide association studies for dietary intake have only identified a handful of genetic loci suggesting that new approaches are needed to depict the genetic architecture of dietary intake. Here, we present a large-scale multi-trait genome-wide association meta-analysis of dietary intake in 283,119 European-descent participants from the UK Biobank and the CHARGE Consortium that led to the identification of 96 independent genome-wide significant loci. Dietary intake signals mapped to multiple brain tissues and were enriched for genes predominantly expressed in serotonergic and GABAergic neurons and b1-tanocytes. Furthermore, we found enrichment of biological pathways related to neurogenesis and nuclear translocation. Fine-mapping through integration of multiple cell-lines and tissue-specific epigenomic information extended the number of variants with high-confidence of association by ~15% and refined likely causal variants. Clustering of dietary intake associated variants identified four primary variant subgroups (ketogenic diet, hyper caloric diet, low protein diet, and plant-based diet) with defined patterns of metabolic risk. Among other findings, a composite polygenic score of the "low protein diet" cluster showed associations with lower body mass index (BMI) in the UK Biobank and an independent clinical biobank (Partners Biobank). Accordingly, Mendelian randomization supported the causal link between protein intake and BMI. This study provides insights into plausible gene targets and underlying biological mechanisms for dietary intake and may provide new avenues for the primary prevention of prevalent common complex metabolic diseases.

Poster Number 79

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Identification of genetic modifiers of somatic CAG repeat instability by in vivo CRISPR-Cas9 genome editing

INVESTIGATORS: R. Murtha, A. Azevedo, M. Kovalenko, M. Andrew, F. Zhang, J. Lee, 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 expansion of the CAG repeat occurs in a time-dependent and tissue/cell-type specific manner, with high levels in striatum and liver, and is critically dependent on components of the DNA mismatch repair. In patients, somatic CAG expansions in the brain are inversely correlated with age of motor onset. Further, DNA repair genes have been identified as modifiers of HD onset or progression in genome-wide association studies (GWAS). 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 we report the development of a CRISPR/Cas9-based platform to identify novel instability modifiers in vivo. To this end, we generated HttQ111 knock-in mice that endogenously express Cas9 and validated single guide RNAs (sgRNAs) targeting known modifier genes that influence somatic CAG expansion. Following tail vein injection of AAV8 or PHP.B-based viruses carrying sgRNAs, we confirmed strong liver and brain transduction using an mCherry reporter, detected a high frequency of frameshift mutations at target sites, and successfully suppressed or hastened somatic CAG expansions. We will 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. Together, this promises to provide significant insight into mechanisms of somatic HTT CAG instability and novel targets for therapeutic intervention.

Poster Number 80

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Xist deletional analysis reveals a co-dependency between Xist RNA and Polycomb complexes for spreading along the inactive X

INVESTIGATORS: H. Sunwoo, D. Colognori, A. Kriz, C. Wang, J. T. Lee

Mammalian X-chromosome inactivation (XCI) compensates sex-chromosome dosage imbalance by silencing one of the two X chromosomes in female cells. This gene silencing on a chromosome-wide scale makes XCI one of the best model systems to study epigenetic gene regulation. In addition, a normal but silent allele on the inactive X chromosome (Xi) in patients often provides a therapeutic opportunity in that its reactivation may alleviate symptoms in numerous X-linked genetic diseases including Rett Syndrome. Xist RNA, the master regulator of XCI, spreads along an entire chromosome to establish silencing. However, the mechanism and functional RNA elements involved in spreading remain undefined. Here, we perform a comprehensive endogenous Xist deletion screen using CRISPR genome engineering and identify Repeat B motif as crucial for spreading Xist and maintaining Polycomb repressive complexes 1 and 2 (PRC1/PRC2) along the Xi. Unexpectedly, spreading of these three factors is inextricably linked. Deleting Repeat B or its direct binding partner, HNRNPK, compromises recruitment of PRC1 and PRC2. In turn, ablating PRC1 or PRC2 impairs Xist spreading. Therefore, Xist and Polycomb complexes require each other to propagate along the Xi, suggesting a feedforward mechanism between RNA initiator and protein effectors. Perturbing Xist/Polycomb spreading causes failure of de novo Xi silencing, with compensatory downregulation of the active X, and also disrupts topological Xi reconfiguration. Thus, Repeat B is a multifunctional element that integrates codependent Xist/Polycomb spreading, silencing, and chromosome architecture.

Poster Number 81

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CRISPR-mediated molecular dissection of Prader-Willi syndrome

INVESTIGATORS: D. Tai, X. Nuttle, K. Mohajeri, N. Burt, S. Erdin, M. Ryan, A. Mil-homens, C. Zhang, C. Lee, J. F. Gusella, M. E. Talkowski

Paternal deletions of chromosome 15q11-q13 are the most common cause of Prader-Willi syndrome (PWS), yet how these rearrangements translate into disease remains largely unclear. To explore this question, we generated PWS cellular models by leveraging CRISPR/Cas9 to engineer human induced pluripotent stem cell (iPSC) lines harboring both Type I (~6 Mbp) and Type II (~5.3 Mbp) PWS deletions against an isogenic background. Our study integrates genome editing with stem-cell and omics technologies to interrogate molecular mechanisms of PWS pathogenesis and establish a compendium of PWS cellular models. We are differentiating our Type I and Type II deletion models together with unedited isogenic controls into hypothalamic neurons (HNs) and 3D cerebral organoids. A battery of morphological and electrophysiological measurements will be performed on these HNs and organoids to define cellular consequences of PWS deletions. In parallel, we will perform transcriptomic profiling on these HNs and our existing neural stem cell (NSC) PWS deletion models, seeking to identify differentially expressed genes and dysregulated biological pathways linked to PWS deletions. To complement these studies, we are developing a piggyBac transposon system to deliver CRISPR components to create an allelic series of iPSC models. This work will yield iPSCs harboring deletions of candidate PWS drivers and critical noncoding regions including MAGEL2, SNURF-SNRPN, SNORD109A, SNORD116, IPW, and the PWS imprinting center. Ultimately, characterizing these models as described above and comparing them to our Type I and Type II deletion models will reveal genes and pathways underlying major components of PWS, suggesting potential novel therapeutic targets.

Poster Number 82

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High-throughput development of CRISPR genome editing technologies with enhanced properties

INVESTIGATORS: R.T. Walton, A.A. Sousa, M.M. Welch, Y.E. Tak, J.Y. Hsu, K. Clement, J.E. Horng, L. Pinello, J.K. Joung, B.P. Kleinstiver

CRISPR technologies have advanced genome editing by providing tools capable of permanently modifying DNA sequences in a variety of organisms. The ability to precisely target genomic sequences with CRISPR nucleases is restricted only by the requirement for a protospacer adjacent motif (PAM), a short sequence required to initiate target site recognition. While the Cas9 nuclease from *Streptococcus pyogenes* (SpCas9) prefers an NGG PAM, enabling targeting of approximately 1 in 8 base pairs of randomly distributed DNA, the Cas12a nuclease from *Acidaminococcus* species (AsCas12a) is restricted to targeting roughly 1 in 43 base pairs because it requires a TTTV PAM. Though the targeting ranges of Cas9 and Cas12a have been expanded by altering PAM recognition, many sites remain inaccessible. To address the need for CRISPR nucleases that robustly modify sites with non-canonical PAMs, we developed high-throughput selection and characterization methods to engineer variants with novel PAM preferences. We evolved several variants of SpCas9 capable of potentially targeting sites with NG PAMs, as well as variants to selectively modify NGA, NGC, and NGT PAMs. Furthermore, we engineered an enhanced AsCas12a variant (enAsCas12a) with roughly 7-fold increased targeting range and roughly 2-fold improved on-target activity. We demonstrate that in addition to potent activity as nucleases, our variants function as effective synthetic transcriptional activators and as base editors capable of mediating single nucleotide edits. In sum, we have developed high-throughput methods to engineer CRISPR nucleases with altered PAM preferences and enhanced activities, strategies that should be amenable to modify other properties of CRISPR nucleases.

Poster Number 83

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Role of RAB3GAP2 mutations in syndromic and non-syndromic forms of Isolated GnRH deficiency revealed by Whole exome sequencing

INVESTIGATORS: W. Crowley, R. Balasubramanian, W. Xu

Background: Human GnRH deficiency can occur either alone [Isolated GnRH deficiency (IGD)] or in the context of syndromic presentations. We have previously demonstrated that CHD7 mutations can cause IGD with/without features of CHARGE syndrome. We hypothesized that other genes causing syndromic IGD can also contribute to non-syndromic IGD.

Methods: Genes linked to syndromic-IGD were identified by querying the Online Mendelian Inheritance in Man (OMIM) database with the search term " ". Genes that were genetically constrained for loss of function (LOF) mutations (defined by pLI from ExAC population) and not linked to IGD alone were prioritized and examined in whole exome sequencing data of 1,309 IGD subjects.

Results: RAB3GAP2, a gene encoding RAB3 GTPase-Activating Protein 2, previously associated with Warburg Micro syndrome (WARBM) fulfilled our criteria. WES data showed 4 RAB3GAP2 LOF mutations in 3 IGD subjects (gene burden test vs. gnomAD, $p < 0.05$). One IGD subject harbored a compound heterozygous RAB3GAP2 LOF mutations. This normosmic IGD subject also displayed phenotypic features consistent with WARBM (congenital cataracts and intellectual disability). Two anosmic IGD subjects harbored heterozygous RAB3GAP2 LOF mutations without WARBM features.

Conclusions: We report the first compound heterozygous mutation in RAB3GAP2 causing WARBM. The WARBM diagnosis was clinically unsuspected prior to WES, highlighting the role of unbiased WES in unraveling cryptic syndromic diseases. We also demonstrate a putative contributory role for RAB3GAP2 heterozygous LOF mutations in anosmic IGD patients with anosmia but without WARBM features, suggesting a hitherto unrecognized role for RAB3 GTPases in embryonic GnRH development.

Poster Number 84

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Interrogating the genetic determinants of TS and other tic disorders through genome wide association studies

INVESTIGATORS: J. M. Scharf, C. A. Mathews, G. Coppola, P. Paschou, D. Yu, Tourette Association of America International Consortium for Genetics

Tourette's syndrome is a polygenic and highly heritable neuropsychiatric disorder, characterized by both motor and vocal tics lasting over 1 year. Genome-wide association study approaches are useful for interrogating the genetic architecture of Tourette's syndrome and other tic disorders. We conducted a genome-wide association study in 4,819 Tourette's syndrome cases and 9,488 controls and identified one genome-wide significant locus within FLT3 on chromosome 13, rs2504235, although this association was not replicated in an independent population-based sample (706 cases, 6068 controls). We found genetic variants spanning evolutionarily conserved regions significantly explained 92.4% of Tourette's syndrome heritability. Tourette's associated genes significantly preferentially expressed in dorsolateral prefrontal cortex, implicating the modulation of gene expression through noncoding variants, particularly within cortico-striatal circuits, as a fundamental mechanism in Tourette's syndrome pathogenesis. Tourette's polygenic risk score significantly predicted both Tourette's syndrome and tic spectrum disorders status in the population-based sample. Significant graded increases were found in mean Tourette's polygenic risk score from population controls to cases with other tic disorder, to Tourette's syndrome cases, demonstrating that Tourette's syndrome and other tic disorders exist as a continuous spectrum of disease, and supporting the unification of Tourette's syndrome and other tic disorders in future diagnostic schemata. Tourette's polygenic risk score is also significantly correlated with worst-ever tic, demonstrating the possibility of using Tourette's polygenic risk score for predicting conversion of transient tics to chronic tic disorders, as well as tic persistence and lifetime tic severity.

Poster Number 85

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Visualization of multivariate stroke recovery data using radar plots

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. The World Health Organization's International Classification of Function, Disability, and Health (WHO ICF) provides a framework for understanding how stroke comprehensively affects people. Outcome measures spanning the WHO ICF framework can be used to build a multivariate visualization of stroke recovery. We investigate the use of radar plots for this visualization. Participants were enrolled in an ongoing single-center, prospective, observational cohort study (Stroke Motor Rehabilitation and Recovery Study). Participants ranged from 21 to 88 years of age, could follow simple commands, and presented with arm weakness due to ischemic stroke. Assessments spanning the WHO ICF domains were performed during hospitalization, at 6 weeks, 3 months, 6 months, and one-year post stroke. A radar plot was constructed for each individual participant. Each ray projecting from the central point of the plot represents one clinical outcome scale. Data from each assessment was normalized and plotted on a scale from zero to one, with one representing full recovery. We show that radar plots provide a method to represent longitudinal and multivariate data in a single graphical display. Each of the webs within the radar plot are equivalently spaced suggesting that there exists no single path to recovery, removing any presentation bias. Next steps include further analysis and thorough assessment of the interaction between presenting participants with their data and the resulting effect on patient engagement as well as stroke recovery.

Poster Number 86

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Assessment of mental health and stigma associations with HIV serostatus disclosure in a refugee settlement in rural Uganda: a cross-sectional study

INVESTIGATORS: K. N. O'Laughlin, A. Xu, S. Ashaba, M. Ravicz, J. Kasozi, K. Greenwald, R. A. Parker, Z. M. Faustin, T. Muwonge, A. Tsai, I. V. Bassett

Background: Disclosure of HIV status enables people to access social support, facilitating engagement in HIV care. People diagnosed with HIV in refugee settlements may be unlikely to disclose their status because of mental health problems and stigma.

Methods: In 2018 we offered HIV testing at 3 health centers in Nakivale Refugee Settlement. Prior to returning test results, we measured symptoms of post-traumatic stress disorder (Post-traumatic Stress Disorder Checklist [PCL-C] ≥ 14), depression (Patient Health Questionnaire [PHQ-9] ≥ 10), anxiety (Generalized Anxiety Disorder scale [GAD-7] ≥ 10), stigma (7 items adapted from the Demographic and Health Surveys, threshold score >3), and social support (Brief Social Support Scale [BS6] ≤ 11). One week later, people with HIV were asked about serostatus disclosure.

Results: Of 3,023 participants, 88 (2.9%) were diagnosed with HIV. Of these, 59/88 (67%) were re-surveyed and 52/59 (88%) reported they had disclosed their HIV status to at least one other individual. HIV status disclosure had no statistically significant correlates: age ($p=0.692$), sex ($p=0.428$), refugee status ($p=0.099$), PTSD ($p=0.385$), depression ($p=0.664$), anxiety ($p=1.000$), anticipated stigma ($p=1.000$), or lack of social support ($p=0.238$).

Conclusion: Mental health problems and anticipated stigma were not associated with disclosure of HIV status among refugees and Ugandan nationals. Most participants reported disclosure; however, individuals who did not disclose HIV status may not have been reached for follow-up. Disclosure may be high in this humanitarian setting where reliance on social support is important to help meet daily survival needs such as obtaining water and food.

Poster Number 87

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Transplant center perceptions of engagement in organization health literacy

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Introduction: Organizational health literacy (OHL) is an institution's ability to assist individuals with inadequate health literacy to navigate the healthcare system. However, it is unclear if transplant centers have the necessary infrastructure in place to address issues of OHL pertaining to the complex transplant process. Therefore, we assessed the perceptions of engagement in OHL among transplant staff at a tertiary academic hospital.

Methods: A 51-question survey from the Agency for Healthcare Research & Quality was administered to staff at Massachusetts General Hospital Transplant Center from 2/28/2018 to 4/6/2018. Respondents selected "Doing well", "Needs improvement", "Not doing", and "Not sure/NA" to a wide range of questions. 6 questions regarding infrastructure in place to address issues of OHL were included for analysis. A performance ratio was calculated by dividing the number of "Doing well" by the sum of "Needs improvement", "Not doing", and "Not sure or N/A". A ratio < 1.0 was considered as not performing well.

Results: 68 staff (20% response rate), including nurses (28.3% of total respondents), physicians (20.0%), and coordinators (12.8%). Respondents rated the institution as not performing well in all areas. The lowest performance ratios were regarding: having a written health literacy improvement plan (0.09), educating staff in health literacy (0.05), and regularly assessing the health literacy environment of the institution (0.04).

Conclusion: Healthcare providers play a key role in assisting patients navigate the transplant process. However, there is low confidence in institutional engagement in key components of maintaining universal

Poster Number 88

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Clinical impact and cost-effectiveness of genotype testing at HIV diagnosis in the United States

INVESTIGATORS: E. Hyle, J. A. Scott, P. E. Sax, L. R. Milham, C. M. Dugdale, M. C. Weinstein, K. A. Freedberg, R. P. Walensky, CEPAC (Cost-effectiveness of Preventing AIDS Complications)

US guidelines recommend standard genotype at HIV diagnosis ("baseline genotype") to detect transmitted drug resistance (TDR) to three major drug classes (i.e., NNRTIs, NRTIs, and PIs). However, INSTI-based regimens are now first-line antiretroviral therapy (ART), so the clinical and economic value of baseline genotypes is uncertain. We used the Cost-effectiveness of Preventing AIDS Complications (CEPAC) model to examine the clinical impact and cost-effectiveness of Baseline Genotype compared to No Baseline Genotype for people starting INSTI-based regimens. For people with no TDR (83.8%), baseline genotype does not alter regimen selection. With transmitted NRTI resistance (NRTI-R, 5.8%), baseline genotype guides NRTI pair selection and informs subsequent ART after adverse events (AE, 14%). With transmitted NNRTI resistance (NNRTI-R, 7.2%), baseline genotype influences care only for people with AE who move to an NNRTI-based regimen. We varied 48-week virologic suppression depending on TDR (40%-92%). Compared to No Baseline Genotype, Baseline Genotype would result in <0.03 additional undiscounted quality-adjusted life months (QALMs), cost \$600 more per person, and would not be cost-effective (ICER, \$620,000/quality-adjusted life year). In univariate sensitivity analysis, the clinical benefits of Baseline Genotype never exceeded 0.15 QALMs for all newly diagnosed people with HIV. Baseline Genotype was cost-effective at current TDR prevalence only under extreme conditions, such as $\leq 60\%$ suppression of transmitted NRTI-R with INSTI-based regimens and ≥ 6 months observed on a failing regimen before switch. With INSTI-based first-line regimens in the US, Baseline Genotype offers minimal clinical benefit, is not cost-effective, and should no longer be recommended by the US guidelines.

Poster Number 89

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Persistent treatment gaps and disparities in US diabetes mellitus care from 2005-2016

INVESTIGATORS: F. M. Shebl, N. McCann, R. P. Walensky, D. J. Wexler, P. Kazemian

We sought to evaluate whether diabetes mellitus diagnosis, linkage to care, and meeting individual and combined treatment targets improved over time and to investigate potential disparities in US diabetes care.

To do so, we derived percentages of diagnoses, linkage, and treatment goal achievement from the National Health and Nutrition Examination Survey (NHANES) in 2005-2008, 2009-2012, and 2013-2016, and developed a logistic regression model adjusting for time period, age, gender, race/ethnicity, education, and health insurance.

In 2013-2016, 74% of US adults with diabetes were aware of their diagnoses; 70% were linked to diabetes care, 70% met HbA1c, 70% met blood pressure, 51% met cholesterol target, and 84% did not smoke. Twenty-one percent of patients achieved all treatment goals simultaneously. Results were similar in the other two study periods.

None of the individual and combined outcomes improved over the 3 study periods ($p=0.08-0.98$). Compared to middle-aged adults with diabetes (45-64y), older adults ($\geq 65y$) were more likely (Odds Ratio [OR] 1.64, 95% Confidence Interval [CI] 1.20-2.24) and younger adults (18-44y) were less likely (OR 0.50, 95% CI 0.28-0.90) to meet the combined goals. Women achieved the combined goals less often (OR 0.59, 95% CI 0.46-0.76). Reporting non-Hispanic black race (vs. non-Hispanic white race) was associated with reduced likelihood of achieving the combined goals (OR 0.59, 95% CI 0.42-0.82).

Despite major advances in diabetes medications and new care models, US diabetes care has not significantly improved between 2005 and 2016.

Poster Number 90

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Assessing the burden of mental illness among refugees in Uganda

INVESTIGATORS: K. N. O'Laughlin, A. Xu, S. Ashaba, H. H. Khidir, R. A. Parker, Z. M. Faustin, T. Muwonge, A. C. Tsai, I. V. Bassett

Stressors in humanitarian settings such as poverty, violence, and disrupted social relations, may worsen mental health. We estimated the prevalence of mental illnesses among refugees accessing health services in Nakivale Refugee Settlement and compared the burden of illness in this population with Ugandan nationals in the surrounding community. From March-November 2018, we enrolled participants from 3 outpatient health centers in Nakivale. We collected demographic information including country of origin and years living in the settlement and administered validated scales adapted for use in the local context to measure symptoms of post-traumatic stress disorder (PTSD Checklist-Civilian Version 6-item scale), depression (Patient Health Questionnaire 9-item scale), and anxiety (Generalized Anxiety Disorder 7-item scale). Of 2,842 participants, 1,904 (67%) were refugees and 938 (33%) were Ugandan nationals. Compared to Ugandan nationals, refugees were younger (median age 30 vs 31, $p<0.001$) and less often female (52% vs 57%, $p=0.017$). A significantly higher percentage of refugees screened positive for PTSD (45% vs 36%, $p<0.001$), depression (27% vs 22%, $p=0.002$), and anxiety (23% vs 18%, $p<0.001$). Among refugees, we observed differences by country of origin in the percentage screening positive for PTSD (50% Rwanda, 44% Burundi, 42% Democratic Republic of Congo; $p=0.030$), but not depression ($p=0.58$) or anxiety ($p=0.68$). Duration of time in the settlement did not result in differences in percentage screening positive for PTSD ($p=0.16$), depression ($p=0.34$), or anxiety ($p=0.19$). Mental health screening paired with appropriate treatment may be an effective intervention to reduce the burden of disease among refugees.

Poster Number 91

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Cost-effectiveness of frequent HIV screening for self-identified high-risk young men who have sex with men in the United States

INVESTIGATORS: A. M. Neilan, A. J. Bulteel, K. A. Freedberg, S. G. Hosek, P. Kazemian, R. P. Landovitz, A. D. Paltiel, S. C. Resch, R. P. Walensky, M. C. Weinstein, C. M. Wilson, A. L. Ciaranello

Of new HIV infections among US youth, 92% occur among young men who have sex with men (YMSM), yet more than half are unaware of their HIV status. We examined the clinical benefit, cost, and cost-effectiveness of frequent HIV screening for HIV-negative high-risk YMSM.

Methods: Using a Monte Carlo model we simulated self-identified, high-risk 15-year-old MSM to examine 4 HIV screening strategies for 15-30-year-olds, each in addition to the status quo (SQ): 3-yearly, yearly, 6-monthly, and 3-monthly. Age-specific HIV incidences ranged from 0.91-6.41/100PY. We used published care continuum data (YMSM-specific when available) including: screen acceptance (80%), linkage-to-care/antiretroviral therapy (ART) initiation (76%), monthly ART costs (\$2,290-3,780) and HIV per-test costs (\$38). Model outcomes include CD4 count, one generation of new HIV transmissions through age 30, life expectancy, lifetime costs, and incremental cost-effectiveness ratios (ICERs, in \$/year-of-life saved [YLS]; threshold $\leq 100,000$ \$/YLS.) In sensitivity analyses, we varied key input parameters.

Results: Mean CD4 at detection for all screening strategies increases (403-515) compared to SQ (296) as does discounted HIV-infected life-expectancy (306.4-313.7 vs. 296.5 months). Discounted population lifetime costs increase from \$150,600 (SQ) to \$165,100 (yearly). The ICER for 3-monthly vs SQ is \$6,900/YLS; other strategies are strongly dominated. 3-monthly also reduced by 50% mean cumulative primary transmissions from each person with HIV by age 30. These results are most sensitive to HIV transmission rates; excluding transmission, screening yearly is cost-effective (\$71,900/YLS).

Conclusions: For HIV-uninfected, self-identified high-risk US YMSM, HIV screening 3-monthly compared to less frequent screening would improve clinical outcomes and be cost-effective.

Poster Number 92

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Cost-effectiveness of urine-based tuberculosis screening in hospitalized patients with HIV in Africa

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Background: In conjunction with the STAMP trial, we used a microsimulation model to examine the clinical impact and cost-effectiveness of urine-based tuberculosis screening in hospitalized patients with HIV (PWH).

Methods: We compared two tuberculosis screening strategies among unselected hospitalized PWH in Malawi and South Africa: (1) Standard of Care: sputum Xpert; and (2) Intervention: sputum Xpert, urine lipoarabinomannan (TB-LAM), and urine Xpert. We calibrated two-month model output to trial results and projected longer-term clinical and economic outcomes. We considered the Intervention cost-effective if its incremental cost-effectiveness ratio (ICER) was less than US\$750/year-of-life saved (YLS) in Malawi and \$940/YLS in South Africa. A Modified Intervention of adding only urine TB-LAM to Standard of Care was also evaluated. We performed a budget impact analysis of countrywide implementation of the Intervention.

Results: The Intervention increased life expectancy by 0.5-1.2 years and was cost-effective, with an ICER of \$450/YLS in Malawi and \$840/YLS in South Africa. The Intervention remained cost-effective under nearly all modeled assumptions. The Modified Intervention was at least as cost-effective as the Intervention. Over five years, the Intervention saved 51,000/171,000 years of life in Malawi/South Africa and increased healthcare expenditures for screened individuals by \$37 million (10.8%)/\$261 million (2.8%).

Conclusions: Urine-based tuberculosis screening among hospitalized PWH increases life expectancy and is cost-effective in two different resource-limited settings. Urine TB-LAM is especially attractive with high incremental diagnostic yield and low additional cost compared to sputum Xpert, making a compelling case for expanding its usage to all hospitalized PWH in high-burden HIV/TB areas.

Poster Number 93

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Experience with a hospital policy on not offering cardiopulmonary resuscitation when believed more harmful than beneficial

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Ethics consultant researchers sought to determine the utilization and impact of a hospital policy based on empirical research aimed to protect patients at end of life from the harms of cardiopulmonary resuscitation. This was a retrospective cohort study of all ethics committee consultations between 2007 and 2013 at MGH, a large academic hospital with a diverse inpatient population. We evaluated whether age, race, functional status prior to admission, or severity of illness at the time of consultation was associated with a recommendation not to offer CPR. This study demonstrated that there was no significant relationship between age, race, or functional status and the recommendation not to offer CPR. Patients who were not offered CPR were more likely to be critically ill (61.2% vs. 18.2%, $p < 0.001$). Seventy two percent of patients for whom ethics consultants recommended not offering CPR and for whom a DNR order was written died during their hospitalization. None of these patients received CPR. The overall 90-day mortality rate among the patients for whom ethics consultants recommended not offering CPR and for whom a DNR order was written was 90.2%. Despite wide spread concerns that policies empowering physicians to write these orders may be biased in their application, we found no association between age, race, or functional status and the decision not to offer CPR in our ethics consultation practice.

Poster Number 94

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Five-year Massachusetts General Hospital demographic trends in clinical trial enrollment

INVESTIGATORS: J. D. Jackson, A. Sanchez

Historically, recruiting participants into clinical trials has been a challenge for researchers and physicians. Despite federal regulations encouraging representative research populations, additional barriers to research remain for minority populations. We analyzed reported study enrollment data from the last five fiscal years (2014-2018) at Massachusetts General Hospital (MGH) to identify local demographic trends in clinical trials enrollment. MGH's enrollment data indicates that participants more than doubled in the five-year period, from 440,317 in 2014 to 980,227 in 2018. White participants, reflecting the racial trends of MGH's clinical population, were consistently the largest study population (82.23% in 2014 and 79.08% in 2018). Despite being Boston's fastest-growing population since 2000, Latino populations remain poorly represented in clinical trials in 2018 (2.39%). Further data revealed increasing participation from females over the five-year period (69.63%, 77.91%, 76.12%, 74.30%, 72.54%, respectively), which is higher than national trends for women (55%). The results also demonstrate, somewhat surprisingly, that unreported demographic information was pervasive at MGH across all years (12.47% in 2014 and 12.63% in 2018). In conclusion, MGH has significantly increased its research recruitment; however, despite changing demographics in Boston, this growth in research participation continues to underserve racial and ethnic minorities.

Poster Number 95

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Divergent immunomodulatory capacity of the healthy versus IBD human enteric virome

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Although the microbiome has been established as a critical regulator of health and disease, the role of commensal viruses that inhabit human intestine (collectively, the virome) is largely unknown. The fecal virome is altered in inflammatory bowel disease (IBD) patients suggesting a role for dysbiosis in disease susceptibility. Furthermore, a collective reduction in commensal viruses or loss of host virus receptors enhances intestinal inflammation in mice. How the resident virome contributes to host homeostasis or how changes in virome composition impacts gut inflammation is unknown. To advance our knowledge of the virome-intestine relationship, we compared the immunomodulatory capacity of healthy versus IBD human intestinal viromes. Viruses isolated from fresh ileostomies or colon resections taken from ulcerative colitis (UC) or Crohn's disease (CD) patients triggered enhanced pro-inflammatory responses as measured by exacerbated interferon (IFN), tumor necrosis factor (TNF) and interleukin (IL)-6 production from primary human macrophages. These viruses also significantly compromised intestinal epithelial barrier integrity in an endotoxin-independent fashion. Conversely, viruses isolated from healthy/non-IBD controls triggered more anti-inflammatory IL-10 and transforming growth factor (TGF β) responses. Importantly, these enteric viruses were capable of inciting intestinal protection in vivo since mice with a "humanized virome" derived from healthy/non-IBD patients were rescued from intestinal inflammation in vivo. Our data reveal that viruses commensal to the intestine are not ignored by the host but instead elicit homeostatic, protective immune responses in health that switch to pro-inflammatory responses in disease. Manipulation of the virome, or the host immune response to it, may be beneficial in IBD.

Poster Number 96

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Melanocyte-specific regulation of PD-L1 by MITF confers immune tolerance to UV mutagenesis

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Genomic sequencing of human cancers has revealed that melanoma exhibits a strikingly high mutational density, the vast majority being recognizably derived from UV irradiation. This high mutational burden is associated with greater numbers of tumor-specific neoantigens that contribute to effective immunotherapy responses. However, the same high mutational density raises the question of how precursor melanocytes could accrue UV-induced mutations without undergoing immune recognition and destruction. Here we identify MITF, the master transcriptional regulator of melanocyte development and survival, as a lineage-specific constitutive inducer of PD-L1 expression in melanocytes. MITF directly promotes transcription of PD-L1 in melanocytes upon binding a conserved E-box-containing upstream enhancer. In addition to determining baseline cutaneous PD-L1 expression, MITF helps drive PD-L1 upregulation in response to UV radiation. This PD-L1 regulatory pathway is independent of interferon (IFN) signaling, in contrast to PD-L1 on melanoma cells, which is regulated primarily by IFN signaling and is associated with inflammation in the tumor microenvironment. We further observed that UV irradiation of PD-L1 $^{-/-}$ mice produced an exaggerated inflammatory response together with vitiligo-like epidermal melanocyte destruction. These results suggest that within normal skin, melanocytes harbor a distinct MITF-mediated mechanism driving PD-L1 production. This PD-L1 in turn may confer local immune privilege, permitting survival of UV-mutated (neoantigen-containing) melanocytes and protecting against immune destruction of melanocytes. These findings raise the possibility that similar lineage-specific mechanisms may induce immune tolerance towards other cell types subjected to mutagenic exposures and eventual carcinogenic risk.

Poster Number 97

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Crohn's disease-associated epigenetic reader SP140 orchestrates immune cell identity through repression of chromatin accessibility

INVESTIGATORS: H. Amatullah, S. Digumarthi, S. Mehta, K. L. Jeffrey

Introduction: Speckled Protein 140 (SP140) is an epigenetic "reader" enzyme restricted to the immune system. Single nucleotide polymorphisms (SNPs) within SP140 associate with Crohn's Disease (CD), Multiple Sclerosis and Chronic Lymphocytic Leukemia. SP140 mutations result in a loss of SP140 in CD patients. We identified SP140 as an orchestrator of macrophage identity and function through occupancy and repression of lineage-inappropriate genes such as HOX bearing repressive H3K27me3. Sp140 depletion in mice exacerbated DSS-colitis, establishing it critical for intestinal health. The specific mechanism by which SP140 modulates chromatin and transcription to maintain identity of immune cells is unknown. **Methods:** We determined protein interactors of SP140 using Mass Spectrometry and confirmed with co-immunoprecipitation. We examined Topoisomerase activity, double stranded breaks (marked by gamma-H2AX) in THP-1, primary human macrophages, and in EBV-B cells from patients with and without SP140 SNPs.

Results: The top SP140 interacting proteins are involved in DNA unwinding (Topoisomerase I (TOP1), II alpha (TOP2A), DNA-PK and SUPT16H (FACT 160 subunit), which were all confirmed by co-IP. Depletion of SP140 increased activity of topoisomerases and overexpression of SP140 repressed topoisomerase activity. Moreover, cells bearing SP140 SNPs lost interaction of SP140 with topoisomerases and displayed increased topoisomerase activity and increased gamma-H2AX.

Conclusion: SP140 exists in protein complexes containing topoisomerases and other proteins involved in chromatin remodeling. SP140 suppresses these DNA unwinding mechanisms at loci of lineage inappropriate genes to maintain cell identity. Furthermore, this function of SP140 is lost in patients harboring SP140 SNPs, suggesting compromised chromatin dynamics contributes to immunological disease

Poster Number 98

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Platelet Thrombus Formation in eHUS is Prevented by Anti-MBL2

INVESTIGATORS: R. I. Kushak, D. C. Boyle, R. L. Silva, I. A. Rosales, J. R. Ingelfinger, G. L. Stahl, M. Ozaki, R. B. Colvin, E. F. Grabowski

Introduction: Epidemic Hemolytic Uremic Syndrome (eHUS) caused by Shiga toxin-producing bacteria is characterized by thrombocytopenia, microangiopathic hemolytic anemia, and acute kidney injury that cause acute renal failure in up to 65% of affected patients. We hypothesized that the mannose-binding lectin (MBL) pathway of complement activation plays an important role in human eHUS, as we previously demonstrated that injection of Shiga Toxin-2 (Stx-2) led to fibrin deposition in mouse glomeruli that was blocked by co-injection of the anti-MBL-2 antibody 3F8; however, the markers of platelet thrombosis in affected mouse glomeruli were not delineated.

Material and Methods: To investigate the effect of 3F8 on markers of platelet thrombosis, we used kidney sections from our mouse model (MBL-2+/+ Mbl-A/C-/-; MBL2 KI mouse). Mice in the control group received PBS, while mice in a second group received Stx-2, and those in a third group received 3F8 and Stx-2. Using double immunofluorescence (IF) followed by digital image analysis, kidney sections were stained for fibrin(ogen) and CD41 (marker for platelets), von-Willebrand factor (marker for endothelial cells and platelets), and podocin (marker for podocytes). Electron microscopy (EM) was performed on ultrathin sections.

Results: Injection of Stx-2 resulted in an increase of both fibrin and platelets in glomeruli, while administration of 3F8 with Stx-2 reduced both platelet and fibrin to control levels. EM studies confirmed that CD41-positive objects observed by IF were platelets. The increases in platelet number and fibrin levels by injection of Stx-2 are consistent with the generation of platelet-fibrin thrombi that were prevented by 3F8.

Poster Number 99

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Dynamic changes in chromatin accessibility and nucleosome positioning in the control of antigen receptor rearrangement

INVESTIGATORS: M. A. Oettinger, M. Lion

Antigen receptor genes are assembled through a series of site-specific DNA rearrangement events, called V(D)J recombination, that are crucial for the formation of diverse immunoglobulin and T cell receptor repertoires. V(D)J recombination is tightly controlled by many different layers of regulation.

Our recent work identified a previously unknown level of regulation for the process of V(D)J recombination. We showed that the physical distribution of nucleosomes at antigen receptor loci is subject to regulated cell-type and lineage-specific positioning at recombination signal sequences (RSSs) that correlates with the recombination potential at these loci. These findings suggest that developmentally-regulated changes in nucleosome location and occupancy, in addition to the known chromatin modifications, play a fundamental role in regulating V(D)J recombination.

We have now extended our findings to measure the extent of chromatin accessibility at antigen receptor loci. Using a novel assay developed by us, we were able to determine not only local and global regions of increased accessibility, but also regions that are repressed relative either to the surrounding chromatin or to the general level of accessibility genome-wide. In addition, we have extended our analysis at distinct stages of lymphoid development. Here we show how chromatin accessibility and nucleosome distribution at antigen receptor loci is regulated during the step-wise and lineage-specific process by which these loci become poised for recombination. Moreover, the influence of regulatory factors on this progression was investigated. Our observations revealed insights into distinct chromatin structures that define antigen receptors features during haematopoiesis and B-cell development.

Poster Number 100

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IL-4, a new player in CD8 resident memory T cell formation and persistence

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Resident memory CD8+ T cells (TRM) persist long-term in peripheral organs of mice and humans where they provide a first line of host defense from pathogens that cause local infections. Tissue cytokines drive the formation and persistence of CD8+ TRM. Epithelium-derived active TGF- β promotes CD8+ TRM by inducing expression of the adhesion receptors CD103 and CD49a, and down-regulating expression of the transcription factor, EOMES. However, whether the local microenvironment can inhibit TRM formation is unknown. Studies suggest that individuals suffering from Th2-dominated atopic dermatitis or asthma exhibit increased susceptibility to viral infection, but the reasons are unclear. We hypothesize that Th2 cytokines inhibit the formation or persistence of CD8+ TRM.

Our results demonstrate that Th2 cell supernatant downregulates CD103 and CD49a expression but induces EOMES expression by TGF- β -stimulated CD8+ T cells in vitro. IL-4 mediates these effects and requires CD8+ T cell-intrinsic STAT-6 signaling. In vivo, a greater fraction of lymph node CD8+ T cells express CD103 in IL-4RKO than WT mice. Additionally, more CD8+ CD103+ TRM are present in the skin of IL-4RKO compared to WT mice at homeostasis, and CD8+ CD103+ TRM accumulate more rapidly following DNFB-induced cutaneous inflammation. These data suggest IL-4 counters TGF- β -mediated signals to inhibit CD8+ TRM formation/persistence. We are now using mouse models of Th2-mediated disease to determine whether CD8+ TRM numbers and function are reduced within tissues undergoing Th2 inflammatory responses.

Poster Number 101

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Re-polarization of tumor associated macrophages for cancer immunotherapy

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Tumor-associated macrophages (TAMs) are highly abundant in many cancers, often displaying an M2-like (immune suppressive) phenotype that fosters tumor growth and promotes resistance to therapy. Yet, macrophages are highly plastic and can also acquire an M1-like (immune supportive) phenotype to prevent tumor growth. Here, we identify a potent pharmacological driver of the M1-like phenotype through morphometric-based screening. The identified drug, R848, is an agonist of the toll-like receptors TLR7/8 which induces robust expression of genes known to correlate with immunotherapeutic response, including IL12. To achieve efficient drug delivery to TAMs in vivo, we furthermore develop an R848-loaded β -cyclodextrin nanoparticles (CDNP-R848). The nanoparticle exhibited high drug loading (>10%wt), rapid uptake by TAMs in vivo, and ultimately increased the drug concentration in TAMs by greater than threefold relative to free drug controls. As a monotherapy, the administration of CDNP-R848 altered the functional orientation of the tumor immune microenvironment towards an M1 phenotype in mice, controlling tumor growth and protecting cured animals against tumor rechallenge. When used in combination with the immune checkpoint inhibitor anti-PD-1, immunotherapy response rates were improved, including in a tumor model resistant to anti-PD-1 therapy alone. Our findings demonstrate the ability of rationally engineered drug-nanoparticle combinations to efficiently modulate tumor-associated macrophages for cancer immunotherapy.

Poster Number 102

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CCR8 controls the stepwise migration of dendritic cells into the lymph node and the initiation and polarization of the allergic immune response

INVESTIGATORS: C. L. Sokol, R. B. Camire, M. C. Jones, A. D. Luster

Dendritic cells (DCs) play a central role in linking innate and adaptive immune responses by capturing antigens in peripheral tissues and bringing those antigens to the draining lymph node (dLN) where they can initiate adaptive immunity. CD301b⁺ DCs, which populate the dermis of the skin, migrate into the dLN after cutaneous allergen exposure and are required for T helper 2 (Th2) differentiation. The migration of mature DCs into the dLN is thought to depend solely on the chemokine receptor CCR7. However, we found that CD301b⁺ DCs poorly upregulated CCR7 expression after allergen exposure and required a second chemokine signal, mediated by CCR8 on CD301b⁺ DCs and its ligand CCL8, to exit the subcapsular sinus (SCS) that surrounds the lymph node (LN) and to enter the LN parenchyma. In response to allergen exposure, CCL8 was produced by CD169⁺SIGN-R1⁺ macrophages in interfollicular LN regions. This CCL8 synergized with CCL21 in a Src-kinase-dependent manner to promote CD301b⁺ DC migration across the SCS floor. In CCR8-deficient mice, CD301b⁺ DCs remained in the SCS and were unable to enter the LN parenchyma, resulting in defective Th2 differentiation. We have defined a CCR8-dependent stepwise mechanism of DC- subset-specific migration through which LN CD169⁺ SIGN-R1⁺ macrophages control the polarization of the adaptive immune response.

Poster Number 103

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Risks and benefits of dolutegravir-based ART for women with HIV of childbearing age in South Africa: A model-based analysis

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Introduction: Dolutegravir is superior to efavirenz for HIV antiretroviral therapy (ART) but may be associated with an increased risk of neural tube defects (NTDs) in newborns if used by women at conception. Our objective was to project clinical outcomes of ART policies for women of childbearing potential in South Africa.

Methods: Using the CEPAC-International and -Pediatric HIV models, we simulated 3.1M South African women with HIV (15-49y) starting or continuing first-line ART, and their children over five years. We compared three strategies: efavirenz (EFV) for all, dolutegravir (DTG) for all, or World Health Organization-recommended efavirenz without contraception or dolutegravir with contraception (WHO approach). Model inputs included published risks of NTDs (efavirenz: 0.05%, dolutegravir: 0.67%), 48-week ART efficacy (efavirenz: 60-91%, dolutegravir: 96%), and age-stratified fertility rates (2-139/1,000 women). We projected deaths among women and children, sexual and pediatric HIV transmissions, and NTDs.

Results: Compared to EFV, DTG averted 13,700 women's deaths and 57,700 sexual HIV transmissions; total pediatric deaths increased by 4,400 due to more NTDs. WHO approach offered some benefits compared to EFV, averting 4,900 women's deaths and 20,500 sexual transmissions, while adding 300 pediatric deaths. Overall combined deaths among women and children were lowest with DTG (358,000) compared to WHO approach (362,800) or EFV (367,300).

Conclusions: Though NTD risks may be higher with dolutegravir than efavirenz, dolutegravir will lead to many fewer deaths among women, and fewer overall HIV transmissions. These results argue against a uniform policy of avoiding dolutegravir in women of childbearing potential.

Poster Number 104

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Protective properties of anti-Vibrio cholerae antibodies: Implications for future vaccine design

INVESTIGATORS: A. S. Iyer, T. R. Bhuiyan, L. Lu, R. Becker, F. Qadri, G. Alter, J. B. Harris

Cholera, a rapidly dehydrating, diarrheal disease affects over one-quarter of the world's nations in either epidemic or endemic forms with an estimated annual 4 million morbidity and over 100,000 mortality cases. Unlike natural infection, oral cholera vaccines (OCV) are poorly protective in children ≤ 5 years. While vibriocidal antibodies are markers of response to V.cholerae infection and OCV, they are suboptimal predictors of protection.

Functional profiles of the Fc-region of antibodies have been found to influence disease outcomes, acting as potent disease biomarkers. However, such features of anti-V.cholerae specific antibodies remain unexplored. Using a systems serology approach, we have begun to delineate Fc-mediated effector functions like antibody-dependent (AD-) cellular phagocytosis (ADCP), neutrophil, dendritic cell mediated phagocytosis (ADNP, ADDCP), complement deposition (ADCD) during acute (day 2) and convalescent stages (day7/30) of infection from patients who had natural cholera infection (n=35).

In addition, we are evaluating these antibody features in household-members of naturally infected cholera patients who shared the same cooking pot for ≥ 3 days with the index case and turned out to be either V.cholerae rectal swab, culture positive and symptomatic (n=53) or culture positive but asymptomatic (n=86) or rectal swab negative, uninfected individuals (n=286) as a means of studying antibody features at baseline (day 2) that predict protection.

Preliminary data using convalescent sera from cholera-infected patients reveal protective antibodies have potent ADCP, ADNP, ADCD activities, functions that were previously uncharacterized. Ongoing studies will help in further characterizing these features in an age-specific manner, laying foundation for improved next generation OCV.

Poster Number 105

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TNFRSFs upregulation and survival pathway activation in productive and latent HIV-1 infection

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Background: HIV-infected patients rely on life-long antiretroviral therapy to suppress HIV activity, due to infected cells called reservoir cells carrying HIV genome that are able to resume viral production as soon as therapy discontinued. Rather than succumb to HIV-induced cytotoxicity, reservoir cells possess remarkable stability, and represent as major obstacle to HIV cure. There is an urgent need to identify reservoir cells and the molecular mechanisms supporting their durability.

Methods: CD4 T cells were infected with dual-reporter HIV-1 virus, allowing to distinguish productively and latently infected cells. Transcriptional and phenotypic profiles of cells with latent or productive infection were analyzed by single-cell RNA-Seq and flow cytometry, respectively.

Results: RNA-seq identified several members of TNF receptor superfamily (TNFRSF) that distinguished transcriptional signatures of HIV-infected cells from uninfected cells. Latently-infected and productively-infected cells contain higher proportions (35-60%) of cells that expressed high-level OX40, TNFR2, or GITR, while frequencies were substantially lower (18-24%) in uninfected cells. Correspond to transcriptomic data, HIV-infected CD4 T cells showed distinct increase in surface expression of OX40, TNFR2 and GITR. Union gating of OX40-TNFR2 allowed to distinguish cells enriched for productive ($p=0.003$) or latent ($p=0.01$) infection. Furthermore, computational analysis inferred activation of survival pathways in HIV-infected cells, which may be driven by TNFRSFs-expressing populations.

Conclusions: Multiple TNFRSFs are upregulated at both transcription and protein level in cells with productive and latent infection and are associated with transcriptional induction of cell survival signatures. Our data suggest the potential of reservoir identification and eradication by targeting TNFRSFs and TNFRSF-mediated signaling.

Poster Number 106

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Clinical and economic impact of ibalizumab for patients with multidrug-resistant HIV in the United States

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Ibalizumab, the first FDA-approved monoclonal antibody to treat multidrug-resistant HIV, improves virologic suppression when combined with an optimized background regimen (OBR) of antiretroviral therapy. We projected long-term clinical outcomes, cost-effectiveness, and budget impact of ibalizumab plus OBR for people with multidrug-resistant HIV. We used the Cost-Effectiveness of Preventing AIDS Complications (CEPAC) model to compare two treatment strategies for multidrug-resistant HIV: 1) IBA+OBR—ibalizumab plus OBR, and 2) OBR—OBR alone. Ibalizumab efficacy and patient characteristics were from phase 3 trial data.

Five-year survival increased from 38% with OBR to 47% with IBA+OBR. Life expectancy increased from 3.74 QALYs with OBR to 5.12 QALYs with IBA+OBR. Lifetime costs were \$299,600/person with OBR and \$660,700/person with IBA+OBR; the ICER for IBA+OBR compared to OBR was \$260,300/QALY. IBA+OBR became cost-effective if the cost of ibalizumab was reduced by more than 88%. There was no efficacy threshold at which IBA+OBR became cost-effective. Five-year transmission rates decreased from 4.81/100PY with OBR to 3.51/100PY with IBA+OBR. For the 5,000 people with multidrug-resistant HIV, IBA+OBR, compared to OBR, increased costs by \$708 million over 5 years, ~0.6% of US HIV treatment costs over that time.

We found that ibalizumab will substantially increase survival for patients with multidrug-resistant HIV, a group currently lacking other treatment options. While IBA+OBR is not cost-effective, the small number of eligible patients makes the budget impact of adding ibalizumab to OBR relatively small in the US.

Poster Number 107

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Tissue engineered reconstruction of large bicortical defects in minipigs

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

Use a bioreactor system to tissue engineer bone for the reconstruction of large bicortical mandibular defects using three-dimensionally (3D) printed β -Tricalcium Phosphate (β -TCP)/Polycaprolactone (PCL) scaffolds and autogenous cells in minipigs. 3D printed scaffolds templates (n=6) with defined channels (1 mm) sizes were fabricated using Poly Vinyl Alcohol (PVA) filaments. The scaffolds templates were filled with 50:50 ratio of β -TCP and PCL powder and sintered. The sacrificial PVA template was dissolved in water. Scaffolds were characterized by Scanning Electron Microscopy, Energy-dispersive X-ray Spectroscopy, and compression test. Bone marrow progenitor cells harvested from the iliac crest of 4 minipigs were seeded to the scaffold (constructs, n=4) and differentiated into bone cells in a rotational bioreactor system, prior to implantation into a 4x2 cm mandibular porcine defect (control unseeded scaffolds, n=2; empty defects, n=2). Cell viability and penetration prior to implantation and after harvest at 4 and 8 weeks were calculated. Formation of new bone, and collagen deposition were evaluated histologically as well as quantitative analysis using micro-computed tomography at both time points. The scaffold consisted a defined architecture, channels and pores and compressive modulus ranging from 58 MPa-150 MPa. Cell viability and penetration was evident throughout the scaffold. Mild inflammatory response with the activity of macrophages, and collagen deposition, consistent to early phase of bone remodeling were observed. An increase in bone volume/total volume ratio percentage was noted at 8 weeks for the construct group. 3D fabricated β -TCP/PCL scaffolds are a promising alternative bone graft and autogenous progenitor cells enhanced bone formation.

Poster Number 108

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Assessing somatic CAG repeat instability at the protein level

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Huntington's disease (HD) is due to a CAG repeat expansion resulting in an extended polyglutamine (polyQ) tract within huntingtin (HTT) protein. The inherited CAG repeat is unstable and undergoes progressive length increases over time in somatic cells. This instability has emerged as a major modifier of age of disease onset and a relevant therapeutic target. Sandwich ELISA-based methods use antibody that targets the expanded polyQ tract to quantify mutant HTT (mHTT). Using purified truncated HTT proteins and endogenous full length mHTT from brains of HD knock-in (KI) mice with different polyQ lengths, we observed that detected signal is strongly dependent on polyQ length: it increases with polyQ length. We have devised a method to predict CAG repeat instability at the protein level. With this approach, it is possible to quantify the average polyQ length in a mixed population of HTT proteins present in tissues prone to CAG repeat instability. We observed that average polyQ lengths exhibited a strong correlation with average CAG repeat length determined by PCR method in human post mortem brain from HD donors. We also found higher average polyQ lengths in human HD cortex than in cerebellum, in agreement with previous studies of CAG repeat instability. We speculate that applying our approach to mHTT protein detection in cerebrospinal fluid may be useful for predicting CAG repeat instability in brain of Huntington disease's patients. This method may be applied for pharmacodynamics in clinical studies of therapies that aim to reduce CAG repeat instability and in other polyQ disorders.

Poster Number 109

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Delayed post-traumatic neuronal death in the developing hippocampus

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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 first detectable event was loss of participation in network activity and mild, sustained elevation of cytoplasmic Ca²⁺. The second stage was marked by activation of caspases (evidenced by FLICA staining) and the loss of fluorescence of transgenic fluorophores. In the third stage, neurons admitted AM dyes including SBFI-AM. The fourth stage was marked by steady increases in cytoplasmic Na⁺ to concentrations approaching that of the extracellular solution. During this stage, cytoplasmic membrane damage (demonstrated by Annexin-V staining), retraction of dendrites and axons, and condensation of nuclear chromatin (visualized by NucBlue) became progressively evident. Throughout the fourth stage, TMRM staining, pharmacological antagonists, and ion-sensitive fluorophores confirmed ongoing glycolysis and mitochondrial respiration and ATP production, sodium transport via Na⁺/K⁺ ATPases, and secondary transport including cation-Cl⁻ cotransport and Na⁺/Ca²⁺ exchange. Key events in the fifth and final stage included microglial engulfment (indicated by isolectin staining), sharp rises in Na⁺ and Ca²⁺ concentrations, and terminal cell shrinkage. Overall, we describe here a new in vitro model of delayed neuronal cell death in the developing hippocampus.

Poster Number 110

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Early life immune activation induces sex-biased mitochondrial and behavioral alterations

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Autism Spectrum Disorder (ASD) is a collection of complex neurodevelopmental disorders characterized by alterations in social interaction and communication. ASD occurs in ~1 in 59 children in the US, with a strong sex bias in prevalence (~3-4 males diagnosed to every female). Postmortem analyses of brains from ASD patients revealed significant decreases in expression of mitochondrial electron transport chain (ETC) subunits, while other analyses have found evidence for immune activation in these brains. However, reports often fail to distinguish between the specific cell type affected or sex of the patient. Emerging evidence suggests that mitochondrial respiratory function is inhibited during activation of microglia, the innate immune cells of the brain. Using both RNA sequencing and PCR Array of isolated microglia from mice injected perinatally with the bacterial endotoxin lipopolysaccharide (LPS), we show that ~96% of ETC genes were significantly downregulated by LPS in male microglia. Only ~6% of ETC genes were comparably decreased in females, suggesting that reduced mitochondrial function may be preferentially implicated in male microglial activation. Importantly, this same perinatal LPS model resulted in ASD-relevant male-specific deficits in social exploration, sociability, and novelty preference behaviors, as well as increased anxiety-like behavior, consistent with the high comorbidity of anxiety and ASD. These data indicate sex-biased deficits in social interaction and anxiety related to clinical manifestations of ASD. We are currently examining whether this perinatal immune challenge results in cell type-specific sex differences in mitochondrial function, as well as examining factors that may impart this male susceptibility to immune challenge.

Poster Number 111

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Heterogeneity of tau seeding in the human brain: A step toward understanding the diversity of Alzheimer's disease

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Alzheimer's disease (AD) is characterized by the aggregation of the amyloid peptide and tau proteins. If both depositions follow sequential and regional patterns, only the "spread" of tau parallel neuronal death, synaptic loss and cognitive decline. The mechanisms of tau aggregation are unknown but recent studies pinpoint tau seeding as a potential pathway. Indeed, small tau aggregates -so-called seeds- can induce focal fibrillization of intracellular non-aggregated tau via direct protein-protein contact. Particularly, tau aggregation can be triggered by "seeds" isolated from human brains. However, the relevance of this phenomenon for human disease progression is yet to be proven. Here, we wondered if tau seeding correlates with the clinical progression of AD and if we can use it as a diagnostic or therapeutic target.

We selected a cohort of AD patients from our brain bank based on clinical and neuropathological records. We quantified tau seeding from these brains and systematically performed different histological, biochemical and cellular characterizations for each patient. Lastly, we tried to reduce seeding using diverse antibodies. We found a high degree of heterogeneity of tau seeding phenotypes among cases. This variability correlates with the speed of clinical progression and the age of onset in addition to histological and biochemical characteristics. Antibodies targeting total or post-translationally modified tau significantly reduced seeding, but each brain sample displayed unique sensitivity to reduction arguing for the existence of various tau "strains". These results provide a novel characterization of tau seeding in AD and add insights into the therapeutic potential of tau immunotherapy.

Poster Number 112

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Structural brain alterations and cognition in cerebral amyloid angiopathy: A longitudinal follow-up study

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Background: The aim of this study was to assess the longitudinal changes in cortical and white matter atrophy and to evaluate their relationship with longitudinal changes in cognitive scores in Cerebral Amyloid Angiopathy (CAA).

Methods: The study included 57 non-demented probable CAA patients, 57 Alzheimer's Disease (AD) and 57 Healthy Controls (HC), all age-matched. All had undergone 2 MRI scans 1 year apart. FreeSurfer was used to calculate cortical thickness (CT) and White Matter Volumes (WMV), expressed as percentage of intracranial volume. Thirty-six CAA patients had cognitive assessments with z scores during baseline and follow up. Percent differences of CT (pdCT), WMV (pdWMV) and cognitive z scores (pd-z) at two time-points were calculated.

Results: CAA had lower WMV and CT compared to HC and lower WMV compared to AD both at baseline and follow up. The pdWMV was worse in CAA (-1.54%) as compared to HC (-0.32%, $p=0.004$) and AD (-0.30%, $p=0.018$), whereas the pdCT was lower in AD patients compared to CAA and HC (both $p<0.05$). The reduction in WMV correlated strongly with the worsening z-scores in executive function ($r=0.42$, $p=0.011$), but not processing speed ($p=0.16$) or memory ($p=0.11$) in CAA. The pdCT in CAA did not correlate with any of the cognitive domains. All these associations remained significant in multivariable models.

Conclusions: Progression of white matter atrophy is more prominent in CAA as compared to AD and HC and this progression correlates with worsening of executive function, confirming the importance of white matter changes in CAA-related cognitive impairment.

Poster Number 113

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Effect of therapeutic chronic hypoxia on cerebral arteriovenous differences in the Ndufs4 knockout mouse model of Leigh Syndrome

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Leigh syndrome (LS) is the most common pediatric presentation of a defined mitochondrial disease, characterized by progressive encephalomyelopathy. LS is caused by mitochondrial dysfunction and altered oxidative phosphorylation (OXPHOS), resulting from mutations in the NADH: ubiquinone oxidoreductase subunit S4 (Ndufs4) gene, which encodes a subunit of mitochondrial complex I. Recently, chronic hypoxia was shown to enhance survival and reverse encephalomyelopathy in the Ndufs4 knockout (KO) mouse model of LS, although the mechanism of this therapy remains unclear. Ndufs4 KO and wildtype (WT) mice were anesthetized and mechanically ventilated with either a fraction of inspired oxygen equal to 11% ($FiO_2=11\%$; hypoxia) or air ($FiO_2=21\%$); catheters were placed in their internal jugular vein (IJV) and femoral artery. Blood was drawn, simultaneously, from the IJV and femoral artery and arterial and venous partial pressure of O_2 (PaO_2 and $PijvO_2$) were measured. When WT and Ndufs4 KO mice breathed air, Ndufs4 KO mice had a markedly lower cerebral arterial-venous difference in the partial pressure of oxygen ($Pa-iJvO_2$) and higher brain-specific $PijvO_2$ with similar arterial PaO_2 . Chronic hypoxia decreased venous $PijvO_2$ in Ndufs4 KO mice to a comparable level observed in WT mice. These results suggest that the brains of Ndufs4 KO mice have an impaired ability to consume O_2 , leading to excessive exposure to potentially damaging levels of O_2 . Future studies will use metabolomic analysis of cerebral arterial and venous blood to investigate how lower cerebral $Pa-iJvO_2$ and higher $PijvO_2$ relate to the observed improved survival of Ndufs4 KO mice breathing 11% O_2 .

Poster Number 114

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Dentate granule cell recruitment of feedforward inhibition governs engram maintenance and remote memory generalization

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Memories generalize over time as memory traces re-organize in hippocampal-cortical networks. Although engram-bearing dentate granule cells (eDGCs) are thought to encode memories, how DGC connectivity governs engram properties and remote memory generalization is poorly understood. We show that learning increases connectivity of eDGCs with stratum lucidum inhibitory interneurons (SLINs). We identify a hippocampal mossy fiber terminal localized cytoskeletal factor, actin-binding LIM protein 3 (abLIM3) whose levels decrease upon learning. Viral downregulation of abLIM3 in DGCs increased DGC-SLIN connectivity, parvalbumin (PV)-SLIN activation, enhanced DGC recruitment of inhibition onto CA3 and maintained a fear memory engram in the dentate gyrus (DG) over time. Furthermore, abLIM3 downregulation in DGCs conferred conditioned context-specific reactivation of memory traces in hippocampal-cortical networks and the basolateral amygdala (BLA) and decreased fear memory generalization at remote timepoints. Consistent with age-related hyperactivity in CA3, learning failed to increase DGC-SLIN connectivity in aged mice. abLIM3 downregulation in DG of aged mice was sufficient to restore DGC-SLIN connectivity, increase PV-SLIN activation and improve remote memory precision. These studies exemplify a connectivity-based strategy targeting a molecular brake of feedforward inhibition in DG-CA3 that may be harnessed to decrease remote memory generalization in post-traumatic stress disorder and improve memory precision in aging.

Poster Number 115

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In vitro Parkinson's disease modeling reveals PARK7 mutation-associated mitochondrial dysfunction

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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, mitochondrial dysfunction and dopaminergic neuronal cell death. We have characterized a novel DJ1 mutation as the cause of a severe, early onset sporadic dystonia-parkinsonism syndrome and generated 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. Mitochondrial function assays using Seahorse technology showed a significant difference in basal oxygen consumption rate (OCR) and maximum OCR between control and patient mutation containing DA neurons, indicating a reduced capacity of PARK7^{-/-} DA neurons to deal with oxidative stress. Additional ongoing studies involve generation of fluorescent reporter lines for analyzing oxidative stress and mitochondrial function to further elucidate the contribution of DJ1 mutations to PD pathogenesis.

Poster Number 116

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Modelling interneuron dysfunction in schizophrenia using patient-derived neurons

INVESTIGATORS: A. Kathuria, K. L. Lopez, R. Karmacharya

The development of improved therapeutics for schizophrenia (SCZD) remains a significant unmet medical need. Drug development has been slowed by poor understanding of the pathophysiology of these illnesses. The discovery by Yamanaka and colleagues to generate induced pluripotent stem cells (iPSC) from human fibroblasts presents an unprecedented opportunity to generate neuronal cultures with the patients' genetic makeup. We have generated iPSCs from subjects with schizophrenia and healthy controls and differentiated them into cortical interneurons. Recent evidence points to dysfunction in the GABAergic transmission. It has been suggested that the symptoms of schizophrenia are related to both the concentration of cortical GABA and the activity of the rate-limiting synthetic enzyme glutamic-acid-decarboxylase (GAD) is reduced. In particular, a number of studies have consistently found reduced GAD67 levels in post-mortem brain tissue of patients with schizophrenia. These findings suggest that cortical interneurons are affected in schizophrenia. However, the essential question that remains unanswered is that how does deficits in cortical interneurons development lead to schizophrenia pathophysiology? In this study we attempt to answer this question, using iPSCs generated from patients with schizophrenia and healthy controls. We have derived cortical interneurons which are positive for parvalbumin, somatostatin, gephyrin, GAD 67 and calbindin. This was confirmed by immunocytochemistry and western blot analysis. Our data indicated that schizophrenia interneurons showed a decrease in gephyrin and GAD 65/67 when compared to control interneurons. This data not only corroborates with post-mortem brain tissue studies but also validates iPSCs as model to study neuropsychiatric disorders.

Poster Number 117

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Structural and physical aspects of the synaptic compartment are altered in the Q175/Q7 knock-in Huntington's disease mouse model

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Huntington's disease (HD) is a neurodegenerative disease caused by expansion of a normal CAG tract in the HTT gene which is translated to a polyglutamine tracts in Huntingtin, the protein product of HTT. Huntingtin is expressed in all tissues but enriched in brain, especially in neurons. Mutant Huntingtin causes degeneration and eventually death of neurons in neocortex and striatum. Mutant Huntingtin collects in nuclear and cytoplasmic aggregates, but it also distributes to the same sites as normal Huntingtin including on membranes and in synapses. Dysfunction at synapses is thought to be an early change contributing to both cognitive and motor disturbances. In this study we investigated the biochemical integrity of synapses in HD mouse striatum. We used density gradient centrifugation to achieve subcellular fractionation of striatal tissue from 6-month-old knock-in Q175/Q7 HD and wild-type (WT) mice. We found that both pre- and post-synaptic proteins and plasma membrane (PM) proteins from Q175/Q7 HD striata have altered distribution compared to those from WT striata. To understand the causes underpinning the altered physical distribution of synaptosomes and PM in the density gradient, we analyzed affected fractions by Transmission Electron Microscopy to look at organelle size. Lipidomic analysis of the fractions that contain proteins shifted in the density gradient was also performed to determine if altered lipid composition could account for the change in density. This study uncovers a previously unknown change in the physical state of the synapse early in an HD mouse model that may contribute to early disease symptoms.

Poster Number 118

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The impact of genetic knockout of Hdac2 and Hdac3 in striatal medium spiny neurons of Huntington's disease knock-in mice

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The underlying cause of Huntington's disease (HD) is the inherited expansion >35 repeats of a CAG tract within the HTT gene. The repeat undergoes further tissue-specific expansion, most pronounced in medium spiny neurons (MSNs) of the striatum. Human and mouse genetic data provide compelling evidence that somatic CAG expansion drives disease and that intervention in this process will have therapeutic benefit. We are therefore interested in identifying factors that contribute to somatic CAG expansion and in understanding the impact of these factors on disease phenotypes.

Based on initial observations that histone deacetylase (HDAC) inhibitors modify CAG instability in a cell-based assay, we deleted either Hdac2 or Hdac3 in MSNs of HD mice (HttQ111) by crossing this line with mice carrying a conditional (floxed) Hdac2 or Hdac3 allele and DARPP-32-Cre transgenic mice expressing Cre recombinase specifically in MSNs. Homozygous knockout of either Hdac2 or Hdac3 in MSNs moderately decreased HTT CAG expansion in the striatum and reduced nuclear huntingtin accumulation, a hallmark of HD pathogenesis. Differential gene expression analyses of RNA-seq data revealed a striking effect on the striatal transcriptome of the Hdac2 knockout, with ~11% of genes dysregulated overall (adjusted $p < 0.05$), and with a subset of these changes representing "correction" of transcriptional dysregulation mediated by the HttQ111 allele.

Together, our findings that Hdac2 and Hdac3 enhance HTT CAG expansion, together with the global impact on the transcriptome of Hdac2 knockout in MSNs have significant implications with respect to therapeutic targeting of these genes in HD.

Poster Number 119

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Novel [11C]-labeled Radioligands for Imaging Sigma-1 Receptor in the Brain Using Positron Emission Tomography (PET)

INVESTIGATORS: Y. Lan, P. Bai, Z. Chen, C. Wang

The sigma-1 receptor is recognized as a unique class of non-G protein coupled, non-ionicotropic intracellular chaperone protein within the mitochondria-associated endoplasmic reticulum (ER) membranes. The sigma-1 receptor plays a major role in various pathological conditions in the periphery and central nervous system (CNS), where they are implicated in several neuropsychiatric and neurodegenerative disorders including depression, epilepsy, Parkinson's disease (PD) and Alzheimer's disease (AD). Imaging of sigma-1 receptor in brain using positron emission tomography (PET) could serve as a noninvasively tool for elucidate the distribution and functional roles in vivo. Herein, we describe the radiosynthesis, in vivo PET/CT imaging and ex vivo autoradiography of two novel radioligands, [11C]HCC900424 (1) and [11C]HCC900329 (2). The results showed that these two probes have high specificity, good selectivity and appropriate kinetics and distribution: the brain uptake and wash-out kinetics of [11C]-1 and [11C]-2 in transgenic AD model mouse and health wild-type mice showed significant differences, the concentration of radioligands in AD model mouse is reduced compared to health wild-type mice. Besides, the specific interaction of [11C]-1 and [11C]-2 with sigma-1 receptor was reduced by administration of unlabeled 1 and 2 (self-blocking), confirming the selective labeling of sigma-1 receptor. In ex vivo radiography of a rat brain reveals high concentration of the radioligand in those regions, which are reported to be in rich of sigma-1 receptor. These studies performed with [11C]-1 and [11C]-2 demonstrated that both tracers possess ideal imaging properties as valuable tools for selective non-invasive visualization and quantification of sigma-1 receptor in brain.

Poster Number 120

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Dissecting ictogenesis in a model of post-traumatic epilepsy

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Acquired epilepsies are characterized by spontaneous, recurrent seizures that emerge following injury. Brain injuries account for 20-60% of all epilepsy and one third of patients with post-traumatic epilepsy are refractory to current treatment options. In order to guide the development of new treatment options, we need to know more about the process of seizure initiation, or "ictogenesis", in chronically epileptic networks. In this study, we investigated the patterns of preictal activity in the hippocampal organotypic slice culture model of post-traumatic epilepsy in mice. These slice cultures become spontaneously epileptic following the widespread axotomy that occurs during slicing. Immediately after preparation, slice cultures were placed on membrane inserts in a glass-bottomed 6-well plate. Organotypic slice cultures were then transferred to the Incuscope: a CO2 incubator customized to include optics for inverted fluorescence microscopy, a motorized stage for positioning the samples, and a computer-controlled multi-channel perfusion pump, which allowed for the longitudinal study of network activity, with single cell resolution. Calcium dynamics were serially imaged in both principal cells and interneurons using the green calcium sensitive transgenic fluorophore GCaMP7, over several weeks, with ictal activity emerging over the first week post-injury. We found that slice cultures generate different types of seizure onset, including low amplitude fast, high amplitude fast, and hypersynchronous onset patterns. Interestingly, the proportion of onset type evolved over time. Future studies will aim to parse the contribution of principal cell and interneurons in the preictal buildup to seizure initiation.

Poster Number 121

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A conserved role for the N-glycosylation pathway in sleep and seizures

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Protein N-glycosylation is important for protein folding, stability and secretion. Precursor glycan assembly is carried out by ALG enzymes, mutations in which cause a family of rare metabolic disorders called congenital disorders of glycosylation (CDGs). No CDG has yet been reported for mutations in either ALG10 or ALG10B (alpha-1,2-glucosyltransferases), two paralogous ALG enzyme coding genes created by intrachromosomal duplication in Hominoidea 25 million years ago. GWAS of human sleep disturbance (UK Biobank data), revealed variants near both ALG10 and ALG10B affecting multiple sleep and chronotype traits. Further, a patient with a quadruple mutant genotype for ALG10 and ALG10B exhibits epilepsy, among other symptoms. *Drosophila* has a single Alg10 gene making it ideal to study its role and the N-glycosylation pathway in seizure and sleep. Neuron-specific Alg10 RNAi knockdown leads to sleep defects and mechanically-induced seizures. Independent genetic evidence supporting a role for Alg10 in seizures and sleep makes use of CRISPR/Cas9 gene editing. Transgenic flies expressing human ALG10 and ALG10B bearing patient missense and truncating mutations are being used to address their pathogenicity. Target protein(s) with disrupted glycoregulation in Alg10 mutant flies are being identified using glycoproteomic approaches. Supporting our hypothesis, we found other N-glycosylation pathway enzymes are also important for neurological function, showing sleep and seizure phenotypes. Capitalizing on the lack of genetic redundancy in *Drosophila*, we have not only identified a gene (ALG10) and a pathway (N-glycosylation) that play a role in human and *Drosophila* sleep and seizures, but also gaining insights into an ultra-rare human neurological disorder.

Poster Number 122

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Modeling schizophrenia in a dish: A stem cell approach

INVESTIGATORS: K. Lopez - Lengowski, A. Kathuria, R. Karmacharya

Schizophrenia (SCZD) is a crippling neurological disorder with a world-wide prevalence of 1%. Cognitive impairments are the most important predictor of functional outcomes in patients with schizophrenia. However, efficacious treatment of cognitive deficits in psychotic disorders remains a significant challenge in clinical practice. Although antipsychotic medications provide symptomatic relief by reducing hallucinations, they do not improve the cognitive deficits that are a core feature in schizophrenia. There is an urgent need for new therapeutic approaches that target the neurobiology of these cognitive impairments. Our research focuses on developing stem cell-based models to study the molecular and cellular basis of schizophrenia using iPSCs (Induced pluripotent stem cells) generated from patients. We have derived nine control and nine schizophrenia lines from eighteen donors. To better understand the development of the telencephalon, we made cerebral organoids from these lines. Six-month-old in-vitro cerebral organoids were compared using immunohistochemistry and electrophysiology. Preliminary data revealed that the schizophrenia cerebral organoids showed no electrical activity when stimulated while the control organoids showed electrical activity. These findings suggest that these mini brains developed from patients with schizophrenia exhibit dysfunctions in connectivity.

Poster Number 123

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Histone deacetylase 3 localization is altered in ALS post-mortem motor cortex

INVESTIGATORS: A. N. Mills, K. A. Mueller, J. D. Berry, G. Sadri-Vakili

Previous studies have shown epigenetic dysregulation in ALS. Alterations in gene expression in post-mortem human tissue has suggested that correcting epigenetic abnormalities may alter pathogenesis. Moreover, class I and II HDACs were reported to be altered in post-mortem motor cortex and spinal cord and in in vitro and in vivo models of ALS. We demonstrated no significant difference in class I and II HDACs in ALS motor cortex and spinal cord tissue compared to control. These findings were confirmed in people living with ALS (PALS) using a novel PET tracer, [11C] Martinostat. The PET results indicate no visually or statistically detectable differences in patterns of HDAC expression in the motor cortices of PALS compared to controls as measured by [11C] Martinostat-PET uptake. However, our recent findings suggest that although HDAC protein levels are not altered in ALS, HDACs are mis-localized in the ALS motor cortex, which would alter their binding partners and cellular function.

Here, we aimed to determine whether HDACs are mis-localized in ALS and whether this alters binding partners and function. HDAC3 localization was assessed in post-mortem motor cortex using immunofluorescence and microscopy. Alteration in HDAC3 binding partners was assessed using co-immunoprecipitation in the same post-mortem samples. Our results indicate that while HDAC3 is depleted in ALS, there is an increase in nuclear HDAC3. In addition, there is a decrease in HDAC3 binding partner interactions, such as HDAC4, that may lead to alterations in protein acetylation downstream. These findings demonstrate HDAC mis-localization may cause epigenetic alteration in ALS.

Poster Number 124

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Targeting FUS mislocalization to mitigate disruption of nuclear integrity in ALS

INVESTIGATORS: N. Mishra, F. Freyermuth, N. Li

Fused in Sarcoma (FUS) is a DNA/RNA binding protein that normally shuttles between nucleus and cytoplasm, although its mutation and/or cytoplasmic mislocalization causes amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Disruption of the nucleocytoplasmic transport has recently emerged as a central disease mechanism in several neurodegenerative diseases. However, the association between FUS mislocalization and alterations of the nuclear pores remained elusive. By analyzing patient fibroblasts, CRISPR-edited isogenic human neurons and brains from mice carrying FUS mutations, we found that cytoplasmic accumulation of FUS triggers disruption of the nuclear pores and distortion of the nuclear membrane. In particular, confocal imaging and STORM super-resolution microscopy revealed that lamin B and several nucleoporins were mislocalized in the cytoplasm, including within FUS aggregates. We then tested whether restoring FUS nuclear localization would impact nuclear membrane abnormalities. We used both a genomewide CRISPR/Cas9 genetic screen and small molecule screens with FUS nucleo-cytoplasmic ratio as primary readout to identify molecular determinants of FUS cellular localization. Several compounds, including different PKC inhibitors, were shown to increase nuclear localization of FUS. Interestingly, PKC α knock-out was also found, among other hits, in the CRISPR/Cas9 genetic screen. Using imaging flow cytometry, we demonstrated that compounds restoring nuclear FUS, as well as siRNA reducing PKC α levels, were efficient in alleviating distortion of the nuclear membrane. This study provides the first evidence that mutant FUS sequesters central components of the nuclear membrane and establishes genetic or pharmacological restoration of FUS nuclear localization as a potential therapeutic approach in ALS and FTD.

Poster Number 125

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Simulations of artificial vision showing the effects of training, and the effects of gaze contingency

INVESTIGATORS: K. K. Rassia, J. S. Pezaris

How easy is it to adapt to the sensory experience of artificial vision? We performed an experiment with simulations of artificial sight to investigate the effects of daily training on a simple reading task. Six normally-sighted subjects performed a MNREAD-based reading task where they read simple, three-line sentences out loud under various viewing conditions and were assessed on reading accuracy (percentage of words read correctly) and reading speed (number of correctly read words per minute). Subjects came to the laboratory on a daily basis to perform the task. The number of phosphenes in the simulation (roughly equivalent to pixels of resolution) and the size of the fonts were varied as subjects read 40 sentences, two from each condition, per daily session. At the start, performance was consistent with earlier reports (Vurro et al. 2014); the population mean of reading accuracy decreased with decreasing font size or decreasing phosphene counts. By 40 sessions, accuracy had saturated at 100% in many cases, although speed was continuing to improve. Ending speed for both the two hardest phosphene conditions was as good or better than that for the next-easiest condition at the start of training, equivalent to a doubling of the number of phosphenes. We conclude that post-implantation rehabilitation will be of critical importance to optimize device utility.

Poster Number 126

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Characterization of neuronal chloride microdomains

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Cytoplasmic chloride plays an essential role in the regulation of the polarity of ligand-gated anion channels. Differences in the intracellular chloride concentration ($[Cl^-]_i$) of neurons lead to oppositely directed chloride flow, GABA signaling, and anticonvulsant effects. Therefore, understanding the mechanisms underlying $[Cl^-]_i$ regulation is critical for unraveling the variability in GABA reversal potential (EGABA) and the effects of anticonvulsants. Utilizing multiphoton microscopy and chloride-sensitive fluorophores, we demonstrate differences in $[Cl^-]_i$ in cell bodies, dendrites and different segments of the same dendrites both in vitro and in vivo.

If $[Cl^-]_i$ differs in these microdomains, and this underlies the variance in electrophysiological estimates of the reversal potential for GABAA receptor-mediated Cl^- currents (EGABA), then the local EGABA should be predictable from the fluorophore data. To test this hypothesis, we have embarked on characterizing the Cl^- microdomains using simultaneous microscopic analysis of the high-sensitivity Cl^- fluorophore SuperClomeleon, Fluorescent Lifetime Imaging (FLIM) of Cl^- sensitive, pH-insensitive dye MEQ, as well as electrophysiological measures of the local EGABA in organotypic slice cultures and dissociated cultured neurons of murine hippocampus. Our data demonstrate that $[Cl^-]_i$ varies in different segments of dendrites, and that the borders of these microdomains are highly stable over a course of an hour. In addition, we show that there is a highly significant correlation between the dendritic $[Cl^-]_i$ calculated by SuperClomeleon and the local EGABA measured by gramicidin-perforated patch-clamp recordings. These studies will shed light on neuronal chloride homeostasis and the existence of functionally significant neuronal Cl^- microdomains.

Poster Number 127

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Hypertension severity and stroke recurrence risk among oldest intracerebral hemorrhage survivors

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Introduction: Oldest old (age ≥ 80 years) survivors of Intracerebral Hemorrhage (ICH) may be at higher risk for stroke recurrence, reflecting increasing hypertension severity with aging. Dedicated longitudinal studies of ICH in the oldest old are urgently needed as they represent an ever-increasing proportion of survivors. We sought to determine whether: 1) oldest old ICH survivors are at higher risk for stroke recurrence; 2) hypertension severity accounts for disparities in stroke recurrence risk across age categories.

Methods: We analyzed data from survivors of primary ICH. Exposures of interest included patients' demographics, medical history, ICH characteristics, Blood Pressure (BP) measurements and medications exposures during follow-up. Outcomes of interest included ICH and/or ischemic stroke events during follow-up.

Results: We enrolled 1464 ICH survivors (441 ≥ 80 years and 1023 < 80 years). In multivariable analyses, lobar ICH location, white non-Hispanic background and female gender were independently associated with ICH at age ≥ 80 years (all $p < 0.05$). ICH survivors age ≥ 80 years had higher systolic BP during follow-up (≥ 80 years: median 148 mmHg, IQR 139-156 vs. < 80 years: median 138, IQR 132-145, $p < 0.001$), but they were less likely to receive anti-hypertensive treatment (≥ 80 years: 233/442, 53% vs < 80 years: 603/1023, 59%, $p = 0.003$). Hypertension severity interacted with advancing age (interaction $p < 0.001$) to further increase stroke recurrence risk among oldest old ICH survivors with frank hypertension (Figure 1).

Conclusion: Oldest old ICH survivors are more likely to demonstrate inadequate blood pressure control. They are at higher risk for stroke recurrence, with hypertension severity playing a pivotal role.

Poster Number 128

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Endogenous estrogen exposure and delay of gratification in females with anorexia nervosa

INVESTIGATORS: M. S. Rogers, A. M. Izquierdo, C. J. Mancuso, M. Slattery, K. T. Eddy, M. Misra, E. A. Lawson, F. Plessow

Background: Anorexia nervosa (AN) is characterized by restrictive eating despite self-starvation and frequently accompanied by (oligo-)amenorrhea. Additionally, individuals with AN show an increased delay of gratification by means of resisting smaller immediate monetary rewards for larger delayed gains. While increased delay of gratification to both food and non-food stimuli has been repeatedly discussed as being at the core of the psychopathology of AN, the potential underlying neurobiological mechanisms are unknown. We hypothesized that hypoestrogenemia, known to affect dopamine availability and thereby potentially altering activation in neurocircuits regulating reward sensitivity and self-control, would be associated with delay of gratification in a monetary intertemporal choice task.

Methods: Forty-one post-menarchal females with AN (mean age \pm SD: 19.5 \pm 2.4 years) completed an intertemporal choice task. From their choices between smaller immediate and larger delayed monetary rewards, the extent to which they discounted the value of rewards with increasing delay to delivery (discounting rate) was calculated. Endogenous estrogen exposure over the past nine months was determined from menstrual history. Body mass index (BMI) Z-scores were calculated as a measure of disease severity.

Results: Lower estrogen exposure was associated with a lower discounting rate ($p=0.42$, $p=0.006$), translating into an increased delay of gratification. This association remained significant after controlling for BMI Z-scores ($p=0.04$).

Conclusion: In females with AN, low endogenous estrogen exposure is associated with increased reward delay. This suggests a potential involvement of estrogen pathways in the regulation of these behaviors. Future studies are required to further investigate their relevance for cognitive-behavioral aspects of AN psychopathology.

Poster Number 129

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Genetic variants and alterations in Hippo/YAP signaling in post-mortem ALS motor cortex

INVESTIGATORS: T. Petrozziello, A. N. Mills, S. M. Farhan, J. Abramovich, K. A. Mueller, E. J. Granucci, K. Vakili, G. Sadri-Vakili

Understanding the underlying pathogenic mechanisms and identifying disease-modifiers capable of altering the course of Amyotrophic Lateral Sclerosis (ALS) are critical for the development of new therapies. One such candidate is the Hippo signaling pathway, a tumor suppressor pathway, which exerts its nuclear activity through its terminal effector component, the transcriptional activator Yes-Associated Protein (YAP). YAP nuclear activity is required for neuronal survival and organ size maintenance. Two proteins that regulate YAP are the receptor tyrosine kinase, ErbB4, mutations of which have been associated with ALS, and WWOX, the WW domain-containing oxidoreductase. While ErbB4 interactions with YAP increase its nuclear function, WWOX suppresses YAP activity. In this study, we sought to determine whether alterations in Hippo/YAP signaling contribute to motor neuron death in ALS. We also screened for genetic variants in key Hippo pathway genes in an ALS cohort. Our results demonstrate that Hippo signaling is dysregulated in ALS and may contribute to neuronal death. Specifically, there is a significant decrease in neuronal nuclear YAP levels in the motor cortex of ALS patients compared to healthy controls. In addition, there is a concomitant decrease in WWOX and ErbB4 expression. Importantly our genetic analysis revealed several rare, genetic variants in YAP1, WWOX, and ErbB4 in 4,366 ALS samples from Project MinE and completely absent in gnomAD. Together, these findings demonstrate that the genetic variation in Hippo pathway genes may translate to deleterious alterations in Hippo signaling in ALS and can provide mechanistic insight into the causes of motor neuron death in ALS.

Poster Number 130

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Selective partial decrease of GSK-3 beta reduces tau hyperphosphorylation, aggregation and propagation

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Glycogen synthase kinase 3 beta (GSK-3 β) is a pivotal kinase responsible for tau hyperphosphorylation in Alzheimer's disease (AD). It is aberrantly activated in human AD brains and may trigger abnormal accrual of hyperphosphorylated tau in synapses and subsequent aggregation and propagation, ultimately leading to synaptic and neuronal anatomical collapse and cognitive impairment. To examine the efficacy of selective partial inhibition of GSK-3 β to decrease tau pathology, we injected a viral construct (AAV) encoding for the fluorescent reporter protein eGFP and 4-repeat wild type human tau (4R-wt hTau) into the entorhinal cortex of wild-type (WT) and GSK-3 β hemi-knockout (HK) mice. After 12-week survival, brains were collected for histopathological and biochemical analyses. While levels of total hTau in synaptic and cytosolic compartments were comparable between WT and HK mice, we detected a significantly higher accumulation of hyperphosphorylated tau at synapses in WT mice. Quantification of AAV-transduced neurons expressing hTau ("donor neurons") and neurons receiving hTau through protein uptake ("recipient neurons") showed a significant decrease of tau propagation in HK compared to WT mice. Parallel in vitro experiments using AAV (eGFP-4R-wt htau) in primary neurons derived from WT and HK embryos further confirmed that selective partial decrease of GSK-3 β significantly reduced tau propagation and the formation of tau aggregates. Our results suggest that selective partial decrease of GSK-3 β represents an intervention that significantly modifies, in in vitro and in vivo settings, a tau initiated neurodegenerative cascade by significantly reducing aberrant accrual of hyperphosphorylated tau in synapses, and therefore tau aggregation and propagation.

Poster Number 131

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Vasomotion as a driving force for paravascular clearance in the awake mouse brain

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Paravascular drainage of solutes including amyloid beta appears to be an important process in brain health and diseases such as Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA). Previous studies have suggested that arterial pulsations are a major driving force for clearance, but an important alternative motive force are much slower oscillations (~0.1 Hz) such as vasomotion. We used in vivo two-photon microscopy in awake head-fixed 8-month-old mice to record spontaneous vasomotion in surface arterioles after intravenous injection of fluorescent dextrans. Spontaneous vasomotion correlated with paravascular clearance of extravasated dextran after focal laser irradiations of nearby vessels. Moreover, when the amplitude of vasomotion was increased by means of functional hyperemia (using a visual stimulation paradigm) clearance was observed to be increased in the visual cortex of awake mice. Spontaneous vasomotion was preserved in 8-month-old transgenic mice with CAA, as were clearance rates. However, evoked vascular reactivity was impaired in mice with CAA at the same age, which corresponded to slower clearance rates under the same conditions, compared to wild-type mice. Functional neuronal imaging with the calcium indicator GCaMP suggested that the observed impaired evoked vascular reactivity was not due to differences in neuronal activation between transgenic and wild-type mice. Our findings suggest that slow rhythmic arteriolar fluctuations may be responsible for drainage of solutes. Targeting naturally occurring vasomotion in patients with CAA or AD may be a promising early therapeutic option for prevention of amyloid beta accumulation in the brain.

Poster Number 132

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Assessing urate effects on mutant LRRK2 toxicity in human iPSC-derived dopaminergic neurons

INVESTIGATORS: N. Xia, E. A. Macklin, M. A. Schwarzschild, R. Bakshi

Mutations in leucine-rich repeat kinase 2 gene (LRRK2) are the most common cause of autosomal dominant Parkinson's disease (PD). The incomplete penetrance of LRRK2 mutations indicates a protective role of other genetic or environmental factors in the pathogenesis in LRRK2 PD. Urate, an endogenous antioxidant as well as the end product of purine metabolism has emerged as a major inverse (reduced) risk factor not only for PD onset but also for its clinical progression. Our clinical biomarker findings show that non-manifesting LRRK2+ carriers had significantly higher levels of urate than those who developed PD in three large independent cohorts. In a cellular model, urate conferred neuroprotection of dopaminergic cells expressing LRRK2 G2019S mutation against oxidative toxin-induced cell death in an astrocyte-dependent manner.

Here, we aim to explore whether urate protects human induced pluripotent stem cell (hiPSC)-derived dopaminergic cells against LRRK2 mutation-mediated toxicity. We utilized the hiPSCs derived from 4 participant groups: idiopathic PD, LRRK2+ PD, and unaffected controls (healthy volunteers) with or without a LRRK2 mutation. Twelve cell lines (n=3 per group) with 4 independent replicates for each treatment group were used to achieve 80% power with type I error rate at $p = 0.05$. So far, we have successfully differentiated the hiPSCs into dopaminergic cells, which provide a useful PD model for the further investigations. We are currently assessing the neuroprotective potential of urate treatment and other therapeutic candidates in this hiPSC-derived LRRK2 model of PD to further advance our understanding of PD neurobiology and treatment.

Poster Number 133

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Characterization of plasma EVs following 5-ALA use in malignant gliomas

INVESTIGATORS: A. Yekula, E. Lansbury, S. Mordecai, L. Balaj, P. Jones, B. Carter

Malignant gliomas (GBM and anaplastic gliomas) are rapidly progressive brain tumors with very high morbidity and mortality. Gross total resection, when possible, is the first line of treatment. The recent FDA approval of 5-aminolevulinic acid (5-ALA, Gliolan) provides the neurosurgeon with real-time fluorescent delineation of malignant tissue which allows significantly higher rate of complete resections of malignant gliomas and longer progression-free survival compared to conventional white-light resections. Extracellular vesicles are nanosized membrane bound vesicles which are released by all the cells of the body including tumor cells and contain genetic and proteomic information that reflect the cell of origin. Liquid biopsy enables detection of tumor-specific information in EVs secreted by cancer cells. Here, we characterize EVs isolated from glioma cell lines treated with 5-ALA for 24 hours. We also evaluated plasma derived EVs from glioma patients following preoperative oral administration of 5-ALA. We used a highly sensitive fluorescence-based analysis known as Amnis ISX mkII imaging flow cytometer to measure fluorescent signals from individual nanoparticles with the added value of being able to individually visualize particles being measured. This is particularly useful when working with EVs in the range of ~100-500 nm in size. Here, we report a sensitive and robust assay to detect fluorescent EVs in the plasma of glioma patients upon administration of 5-ALA and undetectable upon tumor resection. This study is as a proof of concept to determine our ability to utilize fluorescent based tumor specific EV characterization to aid in the diagnostics and prognostics of malignant gliomas.

**Poster
Number
134**

Poster Number 135

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Exploring the experience of Partners Healthcare agency nurses

INVESTIGATORS: A. B. Coakley, D. A. Burke, J. M. Flanagan

Background: Cost of hiring agency nurses to fill vacancies for temporary employment is expensive. To combat these costs, Partners Healthcare developed an internal agency that hires new graduate nurses. These nurses trained on inpatient units within Partners hospitals for fourteen weeks. **Purpose:** This study used an open-ended qualitative design to understand the experience of new graduate nurses who had been hired to work for the Partners Health Care Agency (PHCA). This study employed purposive maximum variation sampling to meet study aims focused on new graduates' experience in the PHCA.

Methods: The new graduate nurses hired to work in the PHCA were sent a letter informing them about the study. The Principal Investigator called the participants who expressed interest in participating in the study. Consent was obtained, interviews conducted, recorded, transcribed and analyzed.

Results: Five themes emerged: 1. Uniqueness of this experience creates opportunities for informed choices and potential professional growth. 2. Institutional support and infrastructure results in employee satisfaction. 3. Clear communication contributes to sense of confidence - lack of clear communication leads to uncertainty and anxiety. 4. A welcoming culture towards new staff fosters teamwork and collaboration. 5. Preceptors willingness to share knowledge to new nurses leads to a positive learning experience.

Conclusions: New graduate RNs hired into the PHCA were satisfied with the experience and felt well prepared for employment within Partners Healthcare. This experience provided them with an opportunity for informed choices related to employment. **Implications:** This model represents a strategy to explore different clinical settings before choosing a place of employment.

Poster Number 136

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Retention of research participants in a longitudinal HIV clinical trial

INVESTIGATORS: M. N. Feldpausch, E. Kileel, M. V. Zanni, S. E. Looby, S. K. Grinspoon, K. V. Fitch

The large multi-center, randomized, placebo-controlled trial (Randomized Trial to Prevent Vascular Events in HIV, REPRIEVE) is evaluating statin therapy as a primary cardiovascular disease prevention strategy in people with HIV (PWH). Retention in this longitudinal study (up to 96 months) is essential for successful measurement of study outcomes. Thus, members of the REPRIEVE Clinical Coordinating Center (CCC) sought to improve understanding of retention strategies and barriers utilized at participating sites. A survey was administered to a site-identified "Retention Champion" by members of the CCC via phone, responses were keyed into REDCap and data analyzed with descriptive statistics. Fifty-one of 116 sites completed the survey. Retention strategies reported were: visit scheduling flexibility (78%), coordinating study visits with clinic visits (76%), team meetings to discuss participants at risk for lost to follow-up (59%) and team meetings to discuss retention practices (51%). Site-identified barriers to retention reported were study duration/fatigue (35%) and participant characteristics (27%). Among sites with less than 5% premature termination of participants, significantly more sites utilized monthly check-in calls ($P=0.03$), team meetings to discuss participants at risk of lost to follow-up ($P=0.01$), provision of transportation ($P=0.02$) and coordination of study and clinic visits ($P=0.02$) as retention strategies.

Here we show common retention strategies utilized at clinical sites participating in a large, multi-center trial of PWH. Data from this outreach will be useful to establish best practices for retention in clinical trials. Strategies identified in this project will inform the development of a nurse-initiated retention tool kit to help study sites enhance participant retention.

Poster Number 137

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Psychometric evaluation of the power influencing Professional Practice Changes (PIPPC Scale)

INVESTIGATORS: D. A. Jones, M. A. Ditomassi, J. Ives Erickson, M. E. Duffy

The purpose of this research was to examine the psychometric properties of the Power Influencing Professional Practice Changes (PIPPC Scale).

Background: The PIPPC scale is derived from Elizabeth Barrett's mid-range theory on power as knowing and active participation in change (Date 1983,1986, 2004) and informed by a qualitative investigation (Jones, 2013). The PIPPC scale is designed to measure two (2) components of power and its influence on professional practice changes used by nurses to evaluate their participation decision-making.

Methods: Psychometric evaluation was undertaken with nurses (n=899) who were engaged in providing ban on- line electronic survey developed using Qualtrics. There were no missing data on the 17-item PIPPC scale.

Results: Cronbach's Alpha(a) internal consistency reliability total score was .971 with 17 items loading more than .50, the factor cut off used to define component subscales. Principal Component Analysis with varimax rotation demonstrated two components, explaining 74.4 % of the variance. Cronbach's Alpha coefficients for Component 1 (Knowledge Influencing Change in Professional Practice) was .971 and for Component 2 (Power Promoting Professional Growth) was .782

Conclusion: The PIPPC scale is a psychometrically sound measure of 2 components of power grounded in Unitary Nursing Science and Barrett's conceptualization of Power as knowing and active participation in change and choice in an acute care setting. It is sufficiently reliable and valid for use as independent sub-scales in nursing and health care research.

Poster Number 138

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Nurse comfort and knowledge with assessing firearm access and providing patient education on safe firearm storage

INVESTIGATORS: K. S. Sheppard, E. Gettings, K. Hall, M. Rudolph, J. Carney, M. Zelinsky, C. Griffith, S. E. Looby

Nurses are well-positioned to lead patient-directed initiatives to increase awareness of safe gun storage (SGS). However, hospital-based policies to support nurses' ability to ask inpatients these questions are lacking, and barriers to providing patient education on SGS are poorly understood. Nurse comfort and knowledge with asking inpatients about firearm accessibility and providing education on SGS was assessed via an online survey among 42 nurses (21 from inpatient psychiatry; 21 from a general medical unit). Questions related to knowledge, practice, comfort and education on SGS. Descriptive statistics were computed to analyze survey responses within each unit. Here we show that >50% of nurses from both units were not familiar with the Massachusetts law on SGS, and 0% had never participated in a class on educating others on firearm safety and SGS. Nurses from the medical unit were less comfortable asking patients about firearm access/SGS and barriers included lack of knowledge and patient-education information, safety and many felt they didn't know what to do with the collected information. Nurses from this unit supported the need for a safety protocol, policy for documentation and nurse education on this topic. More than 50% of nurses from both units reported they would feel comfortable educating patients on SGS if information was available and a pamphlet was the endorsed best method for patient education. Collectively, participants supported the need for nurse education on this topic and >50% favored a 1-hour inter-professional class including security. Findings will inform policy and training strategies for educating nurses and inpatients.

Poster Number 139

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Does the day of the week predict a cesarean section? A statewide analysis

INVESTIGATORS: G. A. Del Carmen, S. Stapleton, M. Qadan, M. G. Del Carmen, D. Chang

Introduction: Although guidelines for clinical indications of Cesarean sections (CS) exist, non-clinical factors may affect CS practices. We hypothesize that CS rates vary by day of the week.

Methods: An analysis of the Office of Statewide Health Planning and Development database for California from 2006-2010 was performed. All patients admitted to a teaching or non-teaching hospital for attempted vaginal delivery were included. Patients who died within 24 hours of admission were excluded. Weekend days were defined as Saturday and Sunday and weekdays were defined as Monday through Friday. The primary outcome was CS versus vaginal delivery. Multivariable analysis was performed, adjusting for patient demographics, clinical factors, and system variables.

Results: 1,855,675 women were included. The overall CS rate was 9.02%. On unadjusted analysis, CS rates were significantly lower on weekends versus weekdays (6.65% vs. 9.58%, $p < 0.001$). On adjusted analysis, women were 27% less likely to have a CS on weekends than on weekdays (OR 0.73, 95% CI 0.71-0.75, $p < 0.001$). In addition, Hispanic ethnicity and delivery in teaching hospitals were associated with a decreased likelihood of CS (OR 0.91, 95% CI 0.86-0.96, $p = 0.01$; OR 0.80, 95% CI 0.69-0.93, $p < 0.001$, respectively).

Conclusion: Cesarean section rates are significantly decreased on weekends relative to weekdays, even when controlling for patient, hospital, and system factors.

Poster Number 140

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Adverse perinatal and long-term outcomes differ among physiologic subtypes of gestational glucose intolerance

INVESTIGATORS: P. K. Edelson, K. Corelli, K. E. James, M. F. Hivert, R. Thadhani, J. L. Ecker, C. E. Powe

Gestational diabetes mellitus (GDM) is associated with adverse perinatal and long-term outcomes; it is unclear if these outcomes are equally distributed among all affected women. We aimed to define physiologic subtypes of gestational glucose intolerance using beta-cell (BC) function and insulin sensitivity (IS) parameters and characterize adverse outcomes associated with each subtype.

We used homeostasis model assessment to estimate BC function and IS from fasting glucose and insulin levels at 16-20 weeks gestation. We defined BC and IS defects using the 50th percentile in 1369 women with normal subsequent 50g glucose load tests. We categorized 158 women whose GLTs were abnormal according to the predominant physiologic defect. We compared hyperglycemia-associated adverse outcomes across physiologic subtypes. We used logistic regression to adjust for potential confounders.

Among 158 women, 59 (37%) had BC defects, 83 (53%) had IS defects, 10 (6%) had both, 6 (4%) had neither. Women with IS defects (vs those with BC defects) were more likely develop GDM (13% vs 2%, $P = 0.03$), while women with BC defects were more likely to develop pregnancy-associated hypertension (20% vs 7%, $P = 0.02$). Women with IS defects were more likely to develop postpartum glucose intolerance (29% vs 3%, $P < 0.001$) during a mean follow up of 12 years. Differences persisted after adjustment for BMI, age, and GDM. There were no differences in macrosomia, cesarean delivery, or neonatal intensive care unit admission across subtypes.

Here we show that distinct physiologic mechanisms leading to GDM are associated with distinct adverse clinical outcomes.

Poster Number 141

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Substance use stigma, avoidance coping, and missed HIV appointments among MSM who use substances

INVESTIGATORS: C. O'Cleirigh, A. Batchelder

Substance use among men who have sex with men (MSM) living with HIV has been associated with sub-optimal engagement in HIV care. Stigma associated with substance use and HIV may account for this relationship. The revised stress and coping theory suggest that internalized stigma elicits avoidance, resulting in missed stigma-related health behaviors. Using logistic regression and bootstrapping techniques we investigated direct and indirect relationships between internalized, anticipated, and enacted substance use stigma (SUS); HIV-related internalized stigma; avoidance coping; and missing, without rescheduling, one or more HIV appointments in the past 6 months among 202 HIV+ MSM with problematic substance use. The sample was 22% Black, 69% White, 29% Hispanic, and over 50% reported $\leq \$20,000$ annual income. Internalized, anticipated, and enacted SUS were associated with missed appointments (OR=1.47, 95%CI: 1.15, 1.87; OR=10.44, 95%CI: 1.10, 1.88; and OR=2.08, 95%CI: 1.52, 2.84, respectively). Internalized, anticipated, and enacted SUS were also associated with avoidance coping ($\beta=0.38, p \leq 0.001$; $\beta=0.37, p \leq 0.001$; and $\beta=0.43, p \leq 0.001$, respectively). HIV-related internalized stigma was not associated with missed appointments but was associated with avoidance coping ($\beta=0.37, p \leq 0.001$). Additionally, we found a full indirect effect of avoidance coping on the association between anticipated SUS and missed appointments and a partial indirect effect between internalized SUS and missed appointments. We did not find an indirect effect of avoidance between enacted SUS and missed appointments. While longitudinal investigation of the pathway between SUS, avoidance, and missed HIV appointments is needed, our results indicate that avoidance coping related to anticipated and internalized SUS contributes to missed HIV appointments among MSM living with HIV and problematic substance use.

Poster Number 142

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Neural correlates of threat processing in women with PTSD following a traumatic childbirth

INVESTIGATORS: Z. Berman, A. Kaim, L. M. Shin, S. Dekel

Although childbirth is considered a uniformly happy event, accumulating evidence suggests that as many as one third of women experience their delivery as psychologically traumatic, and some also proceed to develop childbirth-related posttraumatic stress disorder (CB-PTSD). Nevertheless, research investigating the neural aberrations which may underlie this condition has been completely lacking. In particular, it is unclear whether the neural substrates of processing of threat signals, a central feature of PTSD, are altered in CB-PTSD. It is also unknown whether previously established risk factors for postpartum psychopathology and/or PTSD are linked with such neural abnormalities. Here we use fMRI to investigate neural responses to a validated emotional face-matching task in women who report a highly stressful childbirth, with and without CB-PTSD. Sixty postpartum women will be enrolled in this study. BOLD responses to angry and fearful faces will be compared between the groups and associations with obstetric and psychosocial factors will be assessed. Differences in BOLD responses to emotional faces in women with CB-PTSD compared with non-CB-PTSD controls are expected, including increased amygdala activations. Furthermore, CB-PTSD-related amygdala hyperactivity is expected to be associated with the obstetric and psychosocial factors.

Gaining insights into CB-PTSD's neural mechanisms is a critical step in the efforts to enhance our understanding of this overlooked condition and may help to map and distinguish among postpartum psychopathologies to inform better identification and care of women with this disorder, to the consequential benefit of both the mother and the infant.

Poster Number 143

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Physical activity and weight loss after bariatric surgery: Influences of self-efficacy and internalized weight bias

INVESTIGATORS: E. H. Feig, J. C. Huffman

Physical activity is critical for weight loss maintenance after weight loss surgery (WLS), but most patients are insufficiently active. Self-efficacy for exercise (SEE) and weight bias internalization (WBI) have been associated with physical activity in WLS candidates. However, these associations have not been tested post-WLS. Thus, the purpose of the present study was to investigate the relationship between SEE and WBI in a post-WLS sample, and to test the influence of these variables on physical activity and weight loss.

Participants were recruited from online support groups for this cross-sectional study. Validated self-report measures assessed SEE, WBI, and physical activity. Hierarchical linear regression analysis tested proposed associations controlling for age, gender, time since surgery, and body mass index (BMI).

In 97 participants (M age 50, 90% female), SEE was negatively associated with WBI ($b = -0.01$, $p = 0.047$). It was positively associated with moderate-to-vigorous physical activity ($b = 0.05$, $p = 0.004$), but unrelated to walking ($b = 0.01$, $p = 0.170$) or weight loss ($b = 0.03$, $p = 0.384$). WBI was not associated with moderate-to-vigorous physical activity ($b = -0.43$, $p = 0.11$) or walking ($b = 0.04$, $p = 0.822$), and was negatively associated with weight loss ($b = -1.25$, $p = 0.005$).

We found that SEE predicted moderate-to-vigorous physical activity and WBI predicted weight loss after WLS, even after controlling for BMI. Future research should examine these relationships longitudinally to better understand causality. Interventions targeting self-efficacy and WBI could improve health and well-being in post-WLS patients.

Poster Number 144

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An innovative SMS intervention to improve adherence to stimulants in children with ADHD

INVESTIGATORS: R. Fried, R. Perlis, M. DiSalvo, I. Biederman, C. Kelberman, J. Biederman

Objective: ADHD is a prevalent neurobiological disorder associated with a wide range of adverse outcomes. Large datasets document that stimulants decrease the risk for many adverse outcomes, yet compliance with stimulants remains poor. This study examined the effectiveness of a novel text messaging intervention aimed to improve the poor rate of adherence to stimulant medications in children with ADHD.

Methods: Subjects were children ages 6-12, who were prescribed a stimulant medication by their primary care physician for ADHD treatment. For comparators, we identified at a 10:1 ratio (age and sex matched) pediatric patients from the Partners HealthCare electronic medical record, who had been prescribed stimulant medications over a 2-year period. Timely prescription refills were determined using prescriptions documented in the electronic medical record.

Results: Results showed that 51% of patients receiving treatment as usual refilled their prescriptions in a timely fashion promptly enough to be considered consistently medicated. In contrast, 91% of the SMS intervention group refilled their prescriptions in a timely manner.

Conclusion: These data indicate that a novel ADHD-centric text messaging intervention significantly improved patient engagement to treatment with stimulants in children with ADHD. Findings provide strong support for the utility of this readily accessible, inexpensive and widely available technology to improve the poor rate of adherence to stimulant treatment in children with ADHD. To the best of our knowledge, this study is the first digital health intervention aimed at improving adherence to stimulant medication for children with ADHD.

Poster Number 145

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A bridge too far? Happiness exercises in people with low happiness in recovery from substance use

INVESTIGATORS: S. S. Hoepfner, B. B. Hoepfner

Well-being and psychopathology have been established as moderately correlated yet independent constructs of mental health, and numerous positive psychology interventions have been established to enhance well-being in clinical and non-clinical populations. One concern is that persons with low happiness may be disinclined to engage in positive psychology exercises and may find them harder to complete. We conducted a secondary analysis of a randomized controlled survey study of five brief happiness exercises in a sample of people seeking or in recovery from substance use ($n=531$), where we compared participants with "low" (LOW) versus "high" (HIGH) initial happiness ratings and evaluated changes in happiness using a repeated measures analysis of happiness ratings from pre- to post-exercise. Results show that LOW participants were less likely to complete assigned exercises (87% vs. 94%, $p=0.035$), felt exercises were harder ($p<0.001$), and were less likely to endorse that happiness exercises could help them maintain or increase their happiness ($p=0.025$). In contrast to these perceptions, both groups reported increases in happiness following happiness exercises relative to neutral/negative exercises ($p=0.001$), an effect that was not moderated by initial happiness ($p=0.724$), and there were no differences on actual time spent to complete exercises ($p=0.385$) or willingness to complete them as part of the daily routine going forward ($p=0.943$). Our findings suggest that people with low levels of happiness may require additional coaching to see the value of happiness exercises and to complete them successfully but will benefit similarly from completing them as persons with high levels of happiness.

Poster Number 146

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Predictive utility of autistic traits in youth with ADHD: A controlled 10-year longitudinal follow-up study

INVESTIGATORS: G. Joshi, N. Dallenbach, M. DiSalvo, S. V. Faraone, J. Wozniak, R. Fried, M. Galdo, B. Hoskova, J. Biederman

Background: Both ADHD and ASD are highly heritable disorders. Although underlying etiologies have yet to be fully elucidated, twin and family studies indicate shared genetic susceptibility between ADHD and ASD, suggesting traits of both disorders may be observed in affected individuals with either disorder. These cross-sectional studies suggested that a substantial minority of ADHD children manifest autistic traits (AT), which are associated with greater morbidity and dysfunction. Questions remain about the longitudinal stability of this AT profile and its predictive utility in ADHD youth.

Method: Participants were referred youth with and without ADHD, without a diagnosis of ASD, and their siblings, derived from identically designed longitudinal case-control family studies of boys and girls with ADHD. Participants were assessed with structured diagnostic interviews and measures of social, cognitive, and educational functioning. Presence of ATs at baseline was operationalized using a unique profile of the Child Behavior Checklist (CBCL) consisting of an aggregate T-score ≥ 195 on the Withdrawn, Social, and Thought Problems subscales (CBCL-AT profile).

Results: At follow-up, 83% of ADHD youth with positive AT profiles at baseline had a positive CBCL-AT profile. Presence of positive CBCL-AT profiles at baseline in ADHD youth heralded a more compromised course characterized by higher levels of psychopathology and adverse interpersonal, educational, and neurocognitive outcomes when compared with other ADHD youth and Controls.

Conclusion: Findings indicate high levels of persisting ATs in ADHD youth over time, as indexed through the CBCL-AT profile. Their presence prognosticates a compromised course in adult life in multiple domains of functioning.

Poster Number 147

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Relationships of sleep with cortical thickness among trauma exposed individuals

INVESTIGATORS: K. I. Oliver, C. Daffre, E. F. Pace-Schott, J. Seo

Sleep difficulties may contribute to the functional abnormalities of neural circuitry that underlie the development and persistence of posttraumatic stress disorder (PTSD). We examined associations between sleep physiology, hyperarousal symptoms and regional cortical thickness in trauma-exposed individuals. Persons exposed to trauma within the past 2 years (N=77) completed hyperarousal measures derived from the Clinician Administered PTSD scale and PTSD Checklist-5, as well as 2 weeks of actigraphy and sleep diaries, an acclimation and baseline night of ambulatory polysomnography (PSG), and a 3T structural MRI scan. Slow wave sleep (SWS)% and REM% were computed from PSG and mean sleep efficiency (SE) from actigraphy. Cortical thickness analyses were performed with FreeSurfer V6. Among all subjects, correlation maps were generated between cortical thickness and hyperarousal and sleep measures. A Monte Carlo simulation with 10,000 repetitions ensured a family-wise error of <0.05. We observed that hyperarousal was negatively correlated with cortical thickness in the left paracentral/precuneus and SE was positively correlated with cortical thickness in the isthmus-cingulate/precuneus, medial orbitofrontal cortex and right middle temporal gyrus. Greater hyperarousal—a prominent symptom of PTSD—was associated with lesser thickness in parietal portions of the default mode network (DMN). In contrast, greater sleep quality (SE) was associated with greater thickness in frontal and parietal portions of the DMN. SE is often reduced in PTSD. Thus, better sleep may protect, but hyperarousal may degrade thickness and perhaps function of the DMN in trauma-exposed individuals.

Poster Number 148

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Cingulum bundle anomalies in children with emotional dysregulation: A diffusion tensor imaging study

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Background: Emotional dysregulation in youth has been frequently shown to predispose individuals to adverse health outcomes and increase risk for mood disorders. However, the underlying neurodevelopmental mechanism of emotional dysregulation remains unclear. The goal of this study is to determine whether pediatric emotional dysregulation is attributed to specific neural abnormalities using advanced neuroimaging techniques.

Methods: 43 healthy children and children with emotion regulation difficulties (mean age, 10.3 years; standard deviation, 2.3 years) underwent diffusion tensor imaging. The imaging yielded diffusivity measures (i.e., fractional anisotropy, mean diffusivity, radial and axial diffusivity), which characterized the strength of directionality in neural connectivity along the brain's connection pathways. Emotional dysregulation severity was measured by the empirically-derived Child Behavior Checklist Emotional Dysregulation Profile, including Attention, Aggression, and Anxiety/Depression subscales.

Results: Whole-brain tests demonstrated that mean diffusivity and axial diffusivity in the dorsal cingulum bundle and the anterior corpus callosum areas significantly increased with higher emotional dysregulation severity.

Conclusions: This is the first study to provide whole-brain structural connectivity evidence in youth. Our results suggest impaired microstructural connectivity in the cingulum bundle pathways may underlie neurodevelopmental susceptibility for mood disorders as implicated in pediatric emotional dysregulation. Moreover, altered dorsal cingulum and anterior corpus callosal connectivity may manifest as a susceptibility neural biomarker or a developmental precursor of a pathological course toward mood disorders. Our findings provide biologically-relevant neurodevelopmental targets as well as contribute to improving early identification and prevention efforts aimed to mitigate the compromised course of mood disorders for susceptible cohorts.

Poster Number 149

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Racial health disparities in the prevalence and persistence of substance use disorders

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Racial-ethnic health disparities have been observed in the course of illness and remission from substance use disorders. Racial-ethnic minorities typically demonstrate equivalent or lower prevalence rates of substance use disorder than Whites individuals, however, there is evidence to suggest that, upon disorder onset, they are at elevated risk for more persistent and chronic disorders for reasons related to education, income, and potentially nativity. This study harmonized four national datasets including one survey completed in six languages that administered diagnostic assessments to a sample of 21,024 Asian, Black, Latino, and White adults. Persistence was operationalized as individuals with a lifetime history of substance use disorder who also met diagnostic criteria within the previous 12 months. Prevalence rates were highest among Whites and persistence rates were equivalent across racial-ethnic groups. Further analysis revealed that among respondents without a high school degree, individuals who identified as Black were almost twice as likely than Whites to have persistent substance use disorders. Additionally, higher education was associated with lower odds of a persistent disorder for Black individuals but not for the other minority populations. These results suggest that education, rather than income, is a socio-economic marker that differentially affects racial disparities in persistent substance use disorders. Higher education was protective against persistent substance use disorders for Blacks but not other minority groups suggesting other factors may be protective for Latinos and Asians. Blacks with low education may encounter barriers to access information, knowledge, and resources for remission and recovery from substance use disorders.

Poster Number 150

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Effects of clozapine and haloperidol on dendritic spine dynamics and function in iPSC-derived cortical neuron-like cells

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Dendritic spines are protrusions on the membranes of neuronal dendrites which accept neurotransmitter input and contribute to neuronal function. Abnormalities in dendritic spine dynamics in the upper layers of the prefrontal cortex have been implicated in psychotic disorders, including schizophrenia and bipolar disorder, because of consistently strong data from post-mortem imaging studies. Previous work in rodents has shown that clozapine and haloperidol, atypical and typical antipsychotic drugs respectively, modulate dendritic spine density; however, the effects of these drugs on spines from live human neurons has not been studied. We generated two iPSC lines from healthy patients to make human cortical neuron-like cells in vitro and found that both clozapine and haloperidol increased markers of dendritic spines including SiR actin, HOMER1, and PSD-95. Additionally, we found that neuron-like cells also expressing markers found in the upper layer of the prefrontal cortex were significantly more responsive to clozapine and haloperidol's effects upon spine markers. Finally, we showed that neuron-like cells treated with these drugs displayed a significant increase in spontaneous calcium ion firing after stimulation with KCl. These results show that human dendritic spines are sensitive to the effects of antipsychotic compounds and suggest modulation of spine dynamics as a future treatment target for psychotic disorders.

Poster
Number
151**Tae-Sung Yeum, MD**Psychiatry, Research Fellow | tyeum@mgh.harvard.edu***A novel method to improve the quality of clinical trials using the computer-administered Montgomery-Åsberg Depression Rating Scale data***

INVESTIGATORS: T. Yeum, G. Sachs, S. Edman, D. DeBonis, D. Vanderburg

Background: The high failure rate of randomized controlled trials (RCT) is a well-recognized obstacle to drug development but remains poorly understood. We report analyses utilizing data collected by interactive computer interviews to examine the impact of protocol specified eligibility criteria and rating reliability on signal detection in a bipolar depression RCT.

Methods: The data were derived from a double-blind placebo-controlled trial to test adjunctive ziprasidone as treatment for acute depression in 256 bipolar I subjects. In the analyses, we compared drug-placebo differences on change from baseline Montgomery-Åsberg Depression Rating Scale administered by site-based raters (MADRS.SBR) and by a computer (MADRS.COMP) for subgroups based on the computer assessments including protocol specified baseline severity criteria, presence of mixed episode, baseline inflation, and overzealous subject reporting. Rating reliability was defined as poor and attributed to baseline inflation by the SBR if $(\text{MADRS.COMP} - \text{MADRS.SBR}) \leq -10$ or attributed to overzealous reporting if $(\text{MADRS.COMP} - \text{MADRS.SBR}) \geq +10$.

Results: According to the signal detection analyses, the baseline inflation subgroup showed drug-placebo response differences of 5.9 favoring placebo by MADRS.SBR and 2.7 favoring placebo by MADRS.COMP. The overzealous subgroup showed drug-placebo differences of 6.8 favoring placebo by MADRS.SBR and 10.9 favoring placebo by MADRS.COMP. The subgroup with mixed episodes also showed the similar improvement to placebo than the actual medication.

Conclusions: These results suggest more stringent subject selection processes using computer algorithm may improve RCT signal detection.

Poster Number 152

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Occipital neuralgia and migraine: Intra-operative evidence for extracranial pathology

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Introduction: Recent clinical data supports a theory that aberrant anatomy and inflammation of structures surrounding peripheral/extra-cranial sensory nerves can provoke migraines through compression/irritation. The greater occipital nerve (GON) may be distorted with thickened fascia/muscle, dilated vessels adherent to nerves, and atypical nerve course. This study scientifically evaluated this hypothesis.

Materials and Methods: 92 subjects were prospectively enrolled. Intra-operative anatomy was systematically evaluated, the resulting data analyzed.

Results: Preoperatively, 67% of subjects reported bilateral pain which was associated with abnormal tissue anatomy bilaterally ($p=0.016$). Unilateral pain was not predictive of unilateral tissue aberration. 63% of subjects with unilateral pain had abnormal findings bilaterally. 94% of patients had abnormally thick trapezius fascia, and 30% demonstrated nerve encasement in fibrotic tissue. The occipital artery interacted with the GON in 88% of cases and 20% had dilated veins. The GON had an anomalous course in 42% of patients and appeared crushed/discolored in 32%.

Conclusions: This study is an ongoing effort to understand the extra-cranial pathophysiology of occipital neuralgia/migraine. Although nerve compression/irritation seems the common endpoint, it is currently unclear which tissues are involved in triggering migraine. Patients with unilateral pain had bilateral pathology, further highlighting the importance of bilateral nerve release surgery. Pathology varied between subjects with both anatomic abnormalities and pathological tissue changes. The majority of patients had thickened/fibrotic-appearing trapezial fascia (94%). Interaction of the occipital artery was seen in 88% of cases. This nerve/artery interaction is far more prevalent in migraine surgery patients than previously reported in cadaveric studies (0-54%).

Poster Number 153

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Patient mobility by race in New York City

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Background: Migration of whites for residential reasons peaked almost half a century ago. It is unknown whether this phenomenon persists in healthcare.

Methods: Analysis of the New York Statewide Planning and Research Cooperative System was performed for 2010-2016. All patients residing in one of two boroughs (Bronx, Manhattan) seeking elective surgical care were included. These boroughs were chosen given their sharp socioeconomic contrast despite geographic contiguity. Primary outcome was borough of surgical care.

Results: A total of 32,046 (76.9%) discharges from Manhattan and 9,641 (23.1%) from the Bronx were included, which correspond to 40,273 patients, 609 surgeons, and 29 hospitals. 9,186 (52.5%) Bronx residents underwent surgery locally vs. 23,730 (98.1%) Manhattan residents. Compared to blacks and Hispanics, whites from the Bronx were more likely to leave and obtain care in Manhattan regardless of primary insurance (Private: OR 2.3, 1.8; Medicaid: 2.8, 1.9; vs. black and Hispanic respectively; $p<0.03$ all). On the other hand, both blacks and Hispanics with private insurance from Manhattan were more likely to obtain care in Bronx hospitals when compared to whites (OR 13.5, 22.6 respectively; $p<0.001$).

Conclusion: Mobility among racial minorities within New York City's healthcare system appears to be in opposite directions to that of whites, raising concern regarding disparities in care. It is unclear whether this is due to patient preference or system-wide issues, but this divide only exacerbates existing struggles with cultural competence within healthcare. Future studies should investigate contributors to this segregation including obstacles to access, referral biases, and system-wide issues.

Poster Number 154

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Increasing the value proposition in trauma: A case for an intermediate care unit

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Massachusetts General Hospital (MGH) is a Level I trauma center with approximately 2,000 trauma admissions per year. Prior to 2014, moderately injured patients were admitted to an intermediate care unit known as the Trauma Rapid Assessment and Care Unit (TRACU). Subsequent to 2014 the TRACU was not staffed, and these patients were admitted to a Surgical Intensive Care Unit (SICU). We hypothesized that care in an intermediate care unit has unchanged outcomes and lower costs than admission to a SICU.

Utilizing the MGH trauma registry we identified all patients admitted to the TRACU (2011-2014), and to the SICU in the post-TRACU phase (2015-2018). The phase-out period was excluded. Using 1:1 case control matching, 536 patients were matched based on demographic factors, physiologic parameters, and injury burden on variables such as age, systolic blood pressure, Glasgow Coma Scale, injury type (blunt vs penetrating), injury severity score, need for immediate operation, and transfusion requirement. The primary outcome studied was mortality, and the secondary outcomes were morbidity, length of stay (LOS), and hospital costs.

Here we show that patients admitted to the TRACU had unchanged morbidity and mortality compared to SICU patients, and shorter LOS (5.2 vs 9.3 days, $p < 0.05$). Hospital costs were 3 times higher for patients admitted to the SICU. There was no difference in underlying comorbidities in the matched groups. Our findings make a strong case for an intermediate care unit for trauma patients. These findings also have implications for huge cost-savings at level I trauma systems nationwide.

Poster Number 155

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High throughput kinome and transcriptome analyses reveal novel therapeutic targets in NF2-deficient meningioma

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Meningioma (MN), the most common adult primary intracranial tumor, arise from the arachnoid/meninges and are non-responsive to chemotherapies, with 50-60% showing loss of the NF2 tumor suppressor gene. Previously we showed that NF2 loss activates mechanistic target of rapamycin complex 1 (mTORC1) and mTORC2 signaling, leading to past NF2 clinical trials using rapalogs (RAD001/everolimus), and current meningioma trials with dual mTORC1/2 inhibitor (mTORi) AZD2014. To understand additional dysregulated, potentially druggable pathways, we undertook large-scale 'omics (kinome and transcriptome) screening employing CRISPR-modified human arachnoid cells (ACs), NF2-null versus NF2-expressing. In NF2-null cells, we identified several erythropoietin-producing hepatocellular (EPH)-receptor tyrosine kinases, Src family kinases, and c-KIT, all targets of dasatinib. In vitro treatment of NF2-null ACs and MN using mTORi+dasatinib showed synergistic effects versus single drugs. Treatment of an orthotopic mouse MN model using mTORi or mTORi+dasatinib showed greater tumor reduction than dasatinib alone. In addition, transcriptome data also revealed increase of several ligands, particularly NRG1/neuregulin. NF2-null ACs showed NRG1 secretion along with ERBB3 receptor activation. Furthermore, conditioned-media from NF2-null ACs as well as exogenous NRG1-stimulation activated ERBB3, EPH-RTK and mTOR signaling, suggesting pathway cross-talk. Treatment with lapatinib (multi-ERBB inhibitor) but not erlotinib (EGFR inhibitor) blocked NRG1-stimulated pathways, whereas ERBB3-specific monoclonal attenuated both NRG1-stimulated and basally activated pathways. Taken together, here we show potential existence of an autocrine loop where NF2 loss leads to NRG1 secretion that in turn activates downstream pathways that may be critical drivers of NF2-deficient meningioma, thus providing new opportunities to co-target these pathways with combination drug therapy.

Poster Number 156

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CIMIT's Guidance and Impact Tracking System (GAITS)

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

Background: While supporting >1000 projects over >20 years, CIMIT has studied the complex journey innovators must navigate to create impactful healthcare solutions. A primary conclusion is that innovation in healthcare is a learnable, teachable process that far too many early stage innovators only learn by doing, which is very inefficient. CIMIT calls the process the Healthcare Innovation Cycle. It establishes a sequence of 10 healthcare specific milestones with each milestone defined by a core set of required deliverables in four domains critical to success in healthcare: Clinical/Workflow, Market/Business, Regulatory/Approvals, as well as Technology.

Rationale: To scale the delivery of its experience and expertise to help more innovators maximize the probability of success, CIMIT created the Guidance and Impact Tracking System (GAITS). This is an online platform that provides teams with clear milestones, curated guidance resources to help complete them, the ability to plan and document their progression and the ability to measure the impact of the innovation from a patient, knowledge and commercial perspective. It streamlines the work of portfolio managers and creates a robust database to study and benchmark approaches used by different groups to establish and share best practices.

Results: GAITS is now being used by CIMIT and several collaborators. Early feedback has been very positive, but more time is needed for quantitative assessment.

Conclusions: GAITS has the potential to become an industry standard, helping teams and portfolio managers create more patient impact from available resources.

Poster Number 157

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Blood-based monitoring in hormone receptor positive metastatic breast cancer identifies acquired HER2 mutations conferring susceptibility to targeted inhibition

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Plasma genotyping identifies potentially actionable mutations at variable mutant allele frequencies, often admixed with multiple subclonal variants, highlighting the need for their clinical and functional validation. We prospectively monitored plasma genotypes in 143 women with endocrine-resistant metastatic breast cancer (MBC), identifying 12 (8.4%) with HER2 mutations. These mutations were not detectable in matched archival tumor specimens, and within plasma they frequently co-existed with acquired ESR1 mutations. A patient with HER2-mutant MBC treated with the irreversible HER2 inhibitor neratinib sustained a prolonged clinical response, with complete molecular resolution of two distinct clonal HER2 mutations, with persistence of other passenger subclones. Ex-vivo culture of HER2-mutant circulating tumor cells (CTCs) from another patient showed resistance to endocrine and CDK 4/6 inhibitors, but exquisite sensitivity to neratinib. In these CTCs, neratinib abrogated downstream signaling, consistent with oncogenic dependency. Thus, HER2 mutant alleles that emerge during blood-based monitoring of endocrine-resistant MBC may confer novel therapeutic vulnerability.

Poster Number 158

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Modulation of KCC2 activity for control of anticonvulsant resistant seizures

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Objective: Neuronal chloride concentration ($[Cl^-]_i$) is an important determinant of post-synaptic GABA_A-receptor mediated signaling and cell volume regulation. Altered Cl^- equilibrium and corresponding reversal potential (E_{Cl}) result in cell swelling, accumulation of $[Cl^-]_i$, and depolarizing GABA responses, which foster seizures, epileptogenesis and anticonvulsant resistance via failure of inhibition. Cl^- equilibrium is mediated by a Donnan forces in which the cation-chloride co-transporters (CCCs) KCC2 (a canonical Cl^- exporter) and NKCC1 (a canonical Cl^- importer) comprise the requisite cation and Cl^- membrane permeability. Under pathological conditions, this permeability may limit the rate at which Cl^- equilibrium and GABA signaling can be restored.

Methods: We investigated whether altering CCC activity affects $[Cl^-]_i$ in injured neurons, and the downstream effects on GABAergic inhibition and seizures in the in vitro model of post-traumatic epileptogenesis.

Results: We found (i) synchronous $[Cl^-]_i$ transients during recurrent seizures and progressive baseline Cl^- accumulation and depolarizing shift of E_{Cl} underlying epileptogenesis; (ii) the NKCC1/KCC2 inhibitor Furosemide progressively suppressed Cl^- transients and recurrent seizures, and reduced E_{Cl} ; (iii) the VU0240551 and VU0463271 that selectively inhibit KCC2 over NKCC1 transiently increased the duration and/or power of epileptiform discharges and corresponding Cl^- transients; (iv) sodium channel blocker TTX abolished VU induced epileptiform discharges and corresponding Cl^- transients without affecting E_{Cl} ; (v) KCC2 enhancer CLP257 likely improved $[Cl^-]_i$ homeostasis and reduced seizure activity in a concentration-dependent manner.

Conclusions: Our data validate KCC2 mediated Cl^- extrusion during recurrent seizures and highlight the ongoing need to develop more specific activators of KCC2 co-transport for therapeutic benefit.

Poster Number 159

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Effects of respiratory-gated auricular Vagal Afferent Nerve Stimulation (RAVANS) in the modulation of blood pressure in hypertensive patients

INVESTIGATORS: R. G. Garcia, J. Stowell, R. Sclocco, H. Fisher, V. Napadow, R. Barbieri, J. Goldstein

A reduced cardiac vagal activity has been proposed as an etiological mechanism for hypertension. Non-invasive brain stimulation methods targeting the vagus nerve and brain regions regulating parasympathetic tone could have the potential to modulate cardiovascular function in subjects with hypertension. The objective of this study was to determine the frequency-dependent effects of a novel, non-invasive, respiratory-gated auricular vagal nerve stimulation (RAVANS) technique in blood pressure levels of hypertensive patients. Twelve hypertensive subjects (54.6 ± 6.8 years, 7 females) were enrolled in the study and underwent five stimulation sessions, during which they received exhalatory-gated RAVANS at frequencies 2, 10, 25, and 100 Hz or sham stimulation in a randomized order. Electrodes were placed over vagal-innervated auricular regions (cymba concha) in the left ear and a continuous blood pressure signal was collected during 10-minute baseline and recovery periods, as well as a 30-minute stimulation period using a Finometer device (Finapres Medical System, the Netherlands). Our study showed that RAVANS administration resulted in a significantly lower systolic blood pressure during the recovery period in the 100 Hz session compared to the sham group (142.3 ± 17.9 vs 156.1 ± 13.9 mmHg, $p=0.04$), and significantly lower pulse pressure values during stimulation (64.6 ± 11.1 vs 71.8 ± 13.2 mmHg, $p=0.01$) and recovery (65.9 ± 11.6 vs 73.1 ± 12.1 mmHg, $p=0.04$) in the 100 Hz session compared to the sham session. Our results reveal that RAVANS has a frequency-dependent effect on the modulation of systolic blood pressure and pulse pressure and could have a potential therapeutic effect in patients with hypertension.

Poster Number 160

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Regulation of BRAF inhibitor resistance by protein degradation pathway

INVESTIGATOR: Z. Ji

RAF inhibition by the small-molecule drugs achieves enormous success in the treatment of melanoma. However, melanoma patients lose their responsiveness after 6 months of treatment. Activation of receptor tyrosine kinase (RTK) was found in vemurafenib resistant melanomas and considered as a critical factor contributing to vemurafenib resistance. To investigate the cause of the BRAF inhibitor (BRAFi) resistance, we examined RTK activation in a set of induced vemurafenib resistant melanomas and identified EGFR activation as a frequent event during the resistance development process. ACK1, a protein kinase that regulates EGFR turnover, was then identified to be downregulated in vemurafenib-resistant melanoma cells. We report that ACK1 reduction decreases EGFR degradation speed and increases EGFR protein expression. ACK1 reduction induced resistance could be reversed by EGFR inhibitor gefitinib and ALK inhibitor crizotinib. Furthermore, the addition of ligands of EGFR leads to significant increase of vemurafenib resistance. Finally, we confirmed that vemurafenib directly binds ACK1 and inhibits its protein expression. Our data suggest that BRAFi treatment is intrinsically pro-resistance by inhibiting ACK1, which subsequently increases EGFR and induces drug resistance.

Poster Number 161

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Illuminating DA-9803's potential as a therapeutic candidate for Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by deposition of amyloid plaques, progressive memory loss, and cognitive decline currently without a cure. Our recent results demonstrated that treatment of young APP/PS1 mice, an animal model of amyloidosis, with DA-9803, a multimodal natural extract, prevented amyloid plaque deposition and maintained neuronal calcium homeostasis, which is disrupted in this animal model. However, the mechanism(s) of DA-9803 action remained unknown. Here we studied the effects of DA-9803 on calcium homeostasis in primary neuron and astrocyte co-cultures after application of amyloid β (A β) oligomers. A β oligomers are toxic and lead to intracellular calcium elevations, or calcium overload, in neurons and astrocytes in vitro. 10-18 days in vitro (DIV) neuron-astrocyte co-cultures were loaded with the ratiometric calcium reporter Indo-1 as well as the astrocytic marker SR-101 and were pretreated with either 300 μ g/ml DA-9803 or vehicle for 45 minutes. Imaging was performed with multiphoton microscopy before and 1 hour after application of A β oligomers. We used transgenic conditioned media (TgCM) collected from Tg2576 neurons in culture as a source of naturally secreted human A β -40 and A β -42 oligomers. Exposure of cortical neurons and astrocytes to TgCM led to calcium overload while pretreatment with DA-9803 protected cells from TgCM-dependent calcium elevations. In summary, DA-9803 has protective effects on cellular calcium homeostasis, a functional readout of treatment efficacy, in vitro and in vivo. Thus, DA-9803 should be considered a promising candidate for therapeutic development.

Poster Number 162

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A pathway to primary prevention in genetic prion disease using antisense oligonucleotides

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Genetic prion disease is a fatal, incurable neurodegenerative disease caused by misfolding of the prion protein (PrP). Its extremely rapid progression, variable age of onset, lack of an identifiable prodrome, and small patient population pose challenges for conventional clinical trials. Primary prevention may be critical, and well-understood biology makes genetic prion disease a strong test case for use of a surrogate biomarker endpoint in clinical trials. We are developing antisense oligonucleotides (ASOs) to lower PrP and are working with regulators to craft a strategy for testing them in presymptomatic genetic mutation carriers with a surrogate endpoint of cerebrospinal fluid (CSF) PrP lowering. Here we show that PrP-lowering ASOs extend survival in prion-infected mice, with a clear dose-response relationship, efficacy against diverse prion strains, and a time dependence whereby preventive treatment is more effective than late treatment. In an MGH clinical cohort of presymptomatic PrP mutation carriers, we show that CSF PrP exhibits excellent within-subject test-retest reliability (mean CV = 6%) over 8-16 weeks, suggesting that ASO-mediated PrP lowering should be readily detectable in a trial of just tens of individuals lasting a few months. Genetic prion disease is poised to become a model for primary prevention in neurodegeneration.

Poster Number 163

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Increasing skin functionality in patients with radiation damaged skin

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Background: Radiation injury to the skin is a major source of dysfunction and disfigurement for thousands of patients who undergo adjunctive treatment for internal cancers. Radiation therapy can result in permanent skin changes including fibrosis, telangiectasias and skin atrophy. This can negatively impact patients' quality of life, due to pain, limited mobility and reduced cosmesis. There are currently limited treatment options for radiation dermatitis and no gold standard of care. As fractional laser treatment (FLT) has been shown to treat fibrosis associated with hypertrophic scars and morphea, leading to tissue repair, we hypothesize that FLT can normalize the fibrotic process and induce normal scar remodeling in patients with chronic radiation dermatitis.

Study Design: A prospective study of patients with significant radiation induced fibrosis. Each study site is treated with a CO2 fractionated laser and has an internal control which does not receive any intervention. Evaluations are subjective and objective, including the SF-36 survey, clinical photographs, scar thickness measured by ultrasound, scar compliance measured by Derma Torque Meter and erythema measured by a DermaSpectrometer. Evaluations occur after 3 laser treatments and 3-12 months after the last treatment.

Results: Preliminary data shows that elasticity improves after the laser treatments, indicating reduction in fibrosis. Clinical photographs indicate that telangiectasias improves with laser therapy.

Conclusion: Initial analysis indicates that FLT can improve cosmesis and increase functionality in radiation damaged skin. Further research is needed to understand the mechanisms of chronic radiation injury and devise appropriate interventions.

Poster Number 164

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Assessing the incidence of skin and soft tissue infection in patients on biologics

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Background: Skin and soft tissue infections (SSTIs) often occur at a site of disruption in the epidermal layer, creating an entry point for infection. Ustekinumab have been established in the treatment of moderate-to-severe plaque psoriasis, psoriatic arthritis, and Crohn's disease. The immunomodulating effects of biologics have been thought to predispose patients to SSTIs. Several guidelines recommend discontinuing this agent for 4 half-lives before surgery; however, given the lack of understanding of the risk of infection associated with these agents, it is difficult to ascertain if these guidelines are necessary.

Objective: To assess the incidence of skin and soft tissue infections in patients on ustekinumab for all indications. A secondary aim is to assess those undergoing surgeries to determine if there is risk of post-operative cutaneous infections.

Methods: A retrospective medical record review was conducted in patients at MGH with ustekinumab use from 2016-2018.

Results: 74 patients were found to be on ustekinumab. 9.47% of these individuals experienced skin and soft tissue infections. Of these patients, mean time on ustekinumab prior to infection was 13 months (SD 9.14). 32 of 74 (43.2%) were concomitantly receiving corticosteroids with ustekinumab. Only 2 of 42 patients (4.8%) had SSTIs on ustekinumab alone, while 7 of 32 patients (21.9%) who received both steroids and ustekinumab experienced SSTIs. 27% (20/74) of patients receiving ustekinumab underwent elective surgery within a mean of 3.41 months of index date.

Conclusions: In this cohort, incidence of SSTIs was 4.8%. There was no increased risk in patients undergoing surgery.

Poster Number 165

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Epicardial application of bioadhesive hydrogel improves survival and preserves left ventricular function in a mouse model of myocardial infarction

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Background: Delivery of hydrogel scaffolds to the heart is a promising strategy for mitigating the detrimental impact of myocardial infarction (MI). Challenges associated with the in vivo delivery of currently available hydrogels may limit clinical implementation. The use of a biocompatible, biodegradable, and visible-light crosslinked hydrogel could address many of the limitations of available hydrogels.

Objectives: The goal of this study was to evaluate the cardioprotective potential of epicardial application of gelatin methacryloyl (GelMA) hydrogel in a mouse model of MI.

Conclusions: In vitro testing revealed that GelMA is fully biocompatible and biodegradable. GelMA was also shown to form a tight bond with myocardial tissue. Elasticity of GelMA is comparable to that of mouse myocardium. GelMA was successfully delivered to the epicardial surface of the heart in vivo. GelMA was delivered as a liquid precursor solution that was efficiently photo-crosslinked with visible spectrum light. GelMA was also effectively applied to the heart in the context of experimental MI. GelMA treatment improved post-MI survival: 89% of mice treated with GelMA during experimental MI survived three weeks after MI, as compared with 50% of mice that did not receive GelMA treatment ($p = 0.001$). After experimental MI, left ventricular contractile function (as measured with trans-thoracic echocardiography) was better in GelMA-treated mice than in untreated mice (fractional shortening 37% vs. 26%, respectively; $p < 0.001$). Average scar burden after MI was also lower in GelMA-treated mice than in untreated mice (6% vs. 22%, $p = 0.017$).

Poster Number 166

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Development of new strategies to target Peripheral T cell lymphomas with Chimeric Antigen Receptor T cells

INVESTIGATORS: I. Scarfo, S. Akula, F. Powers, R. Larson, M. J. Frigault, D. Weinstock, M. V. Maus

Patients with peripheral T-cell lymphoma (PTCL) have a poor prognosis and are underserved by current therapies, making this a high priority set of diseases for the development of new CAR T cell approaches. However, the paucity of antigens differentially expressed on malignant and non-malignant T cells made the development of CAR T cells for PTCL more challenging. CD37 is a tetraspanin found exclusively on leukocytes. It is abundant on B cells, but it is low on normal T cells and dendritic cells. On the malignant counterpart, it is highly expressed on B-cells non-Hodgkin lymphomas and chronic lymphocytic leukemia. We investigated CD37 expression on PTCL and developed a second-generation CAR against this surface molecule (CAR-37).

We identified PTCL cell lines and primary samples with surface CD37 expression. Notably, some PTCLs express high and uniform levels of CD37. We also found a CD37 negative PTCL line suggesting that CAR-37 can be used for a subset of patients. CD37-directed CAR T cells demonstrated antigen-specific activation, proliferation, cytokine production, and cytotoxic activity in vitro. Furthermore, we found that CAR-37 T cells induced a rapid response in a preliminary in vivo experiment with a PDX PTCL model.

Taken together these results show that T cells expressing anti-CD37 CAR have substantial activity against PTCL. CAR-37 cells are a potential strategy to target some PTCLs and could spare normal T cells. Eradication of PTCL would be a significant achievement.

Poster Number 167

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Prognosis of liver fibrosis via $\alpha v\beta 3$ -targeted PET imagining studies

INVESTIGATORS: T. Shao, Z. Chen, V. Belov, W. Lin, R. T. Chung, S. Liang

The key factors in the pathogenesis of liver fibrosis are the activation and proliferation of hepatic stellate cells (HSCs), which express integrin $\alpha v\beta 3$ after activation. This study aimed to explore the potential of ^{18}F -AL-NOTA-PRGD2, denoted as ^{18}F -Alfatidea, as a positron emission tomography (PET) radiotracer to image hepatic integrin $\alpha v\beta 3$ expression to reflect HSC activity in fibrotic livers. Mouse models of liver fibrosis caused by thioacetamide or carbon tetrachloride (CCl_4), thioacetamide (TAA) treatment were employed to examine the expression and distribution of integrin $\alpha v\beta 3$ during fibrotic progression. The binding activity of radiolabeled cRGD to integrin $\alpha v\beta 3$ was assessed in liver sections. PET was performed to determine hepatic integrin $\alpha v\beta 3$ expression in mice with different stages of liver fibrosis. Protein and messenger RNA (mRNA) levels of integrin αv and $\beta 3$ subunits were increased with the progression of liver fibrosis in mice and human liver sections. ^{18}F labeled PRGD bound to mice and human fibrotic liver sections and the binding activity was the highest in advanced fibrosis. A PET imaging study with ^{18}F -AL-NOTA-PRGD2 as a tracer demonstrated that the radioactivity ratio of liver to heart increased progressively along with severity of hepatic fibrosis in different animal model. Conclusion: Hepatic integrin $\alpha v\beta 3$ expression in fibrotic liver reflects HSC activity and its imaging using ^{18}F -AL-NOTA-PRGD2 as a PET radiotracer may distinguish different stages of liver fibrosis in mice and human.

Poster Number 168

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Using low dose of quinoline methanol derivatives as novel treatments for primary brain cancer and brain metastasis

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Both primary brain tumors and secondary brain metastases that start in another part of the body and spread to the brain, such as lung, breast, melanoma, kidney, nasopharynx, and colon cancers have limited or no response to chemotherapy. Through repurposing drug screen of panel of primary brain tumor cells and brain metastasis cells established from newly diagnosed and recurrent patients, we identified that FDA-approved off-patent compound mefloquine has low nanomolar efficacy. It crosses the blood-brain barrier and accumulates in the brain at a relatively high concentration, thus causing adverse neurological effects. We therefore thought to take advantage of this unique characteristic and use very low dose of similar compounds to achieve enough amount in the brain and target the metastasis cells while avoiding the neurological side effects by conducting a structural modification campaign by designing a panel of quinoline methanol derivatives with high levels of BBB penetration and low levels of calcium homeostasis disruption based on key stereo-electrostatic potential and lipophilic features. Our results showed that those compounds were highly effective in inhibiting cellular proliferation and migration, and induced apoptosis in primary and secondary brain tumors with IC_{50} all below 100 nM and had very low toxicity in normal brain cells. These effects were mediated by increased intracellular reactive oxygen species (ROS) levels and modulation of autophagy. In orthotopic xenograft mouse model low dose of those compounds reduced tumor growth and significantly extended animal survival. Our results pave the way for translation of those compounds to the clinic.

Poster Number 169

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Extracorporeal removal of carbon monoxide using phototherapy in rats with normal or injured lungs

INVESTIGATORS: L. Zazzeron, A. Fischbach, W. Franco, W. A. Farinelli, F. Ichinose, D. B. Bloch, R. R. Anderson, W. M. Zapol

Carbon monoxide (CO) competes with oxygen for binding to hemoglobin, reducing the blood oxygen content and impairing tissues oxygenation. CO poisoned patients are treated with normobaric or hyperbaric 100% oxygen. When CO poisoning is associated with pulmonary edema due to CO-induced cardiogenic shock and ARDS secondary to smoke inhalation, burns or trauma, breathing 100% oxygen may be ineffective and harmful to the lungs. Visible light is known to selectively dissociate CO from hemoglobin. We hypothesized that the photo-dissociation of CO from hemoglobin, in blood passing through an extracorporeal membrane oxygenator, could increase the rate of CO elimination.

We developed a membrane oxygenator with optimal characteristics for exposure of blood to light and tested the device in a rat model of CO poisoning with or without simultaneous lung injury. After poisoning, animals were treated with 100% oxygen with or without extracorporeal CO removal using phototherapy (ECCOR-P). In rats with healthy lungs, treatment with ECCOR-P doubled the CO elimination rate compared to rats breathing oxygen alone (COHb half-life: 8.6 ± 0.5 vs. 17.7 ± 1.2 min, $n=4$ per group, $P<0.0001$). When lung injury was present, all CO poisoned animals treated with oxygen alone died within 46 minutes. Treatment with ECCOR-P resulted in approximately three-fold increase in the CO elimination rate compared to rats breathing oxygen alone (COHb half-life: 11.6 ± 2.4 vs. 28.5 ± 3.5 min, $n=4$ per group, $P=0.002$), and all animals survived until the study endpoint (60 min). The ECCOR-P may represent a unique alternative treatment for CO poisoned patients, with and without concomitant lung injury.

Alphabetical Listing of all Poster Presenters

Abels, Erik	55	Greenwald, Kelsy	86
Adiliaghdam, Fatemeh	95	Guarino, Carla	34
Adlueu, Eeswar	11	Guastaldi, Fernando	107
Aganj, Iman	12	Guo, Nannan	114
Allouche, Jennifer	96	Gustafsson, Karin	64
Alt, Clemens	13	Hansdorfer, Marek	152
Amatullah, Hajera	97	He, Shun	51
Aviolat, Hubert	108	Hendriks, William	115
Balena, Trevor	109	Hoeppner, Susanne	145
Batchelder, Abigail	141	Hsu, Yu-Tien	87
Beauchamp, Roberta	155	Hyle, Emily	88
Berman, Zohar	142	Iyer, Anita	104
Bhave, Sukhada	63	Ji, Zhenyu	160
Bordt, Evan	110	Jo, Min Joo	2
Bukhari, Syed	29	Jones, Dorothy	137
Chen, Ivy	30	Joshi, Gagan	146
Chiasson-MacKenzie, Christine	56	Kaliannan, Kanakaraju	69
Chindavong, Peter	57	Kang, Homan	16
Cloutier, Alison	85	Katagiri, Wataru	17
Coakley, Amanda	135	Kathuria, Annie	116
Collins, John	156	Kaur, Gurbani	65
Dashti, Hassan	67	Kawakami, Akinori	58
del Carmen, Gabriel	139	Kazemian, Pooyan	89
Dichtel, Laura	68	Kegel-Gleason, Kimberly	117
Dubash, Taronish	157	Kerem, Liya	70
Dugdale, Caitlin	103	Khan, Amjad	35
Dujardin, Simon	111	Khidir, Hazar	90
Dzhala, Volodymyr	158	Kimball, Allison	71
Edelson, Paula	140	Kiyokawa, Juri	36
Feig, Emily	143	Ko, Jina	3
Feldpausch, Meghan	136	Kokkaliaris, Konstantinos	59
Feng, Yen-Chen Anne	74	Kontos, Filippas	37
Ferraro, Gino	31	Kovalenko, Marina	118
Ferrer, Christina	32	Kuo, Hsiaohsuan	105
Fried, Ronna	144	Kurokawa, Tomohiro	38
Fuenfer, Michael	1	Lakhani, Chirag	75
Garcia, Ronald	159	Lan, Yu	119
Girouard, Vincent	14	Lane, Jacqueline	76
Goenka, Chhavi	15	Lariviere, Amanda	161
Gokcal, Elif	112	Lau, Lauren	120
Goruppi, Sandro	33	Lee, Seungwoo	4
Grabowski, Eric	98	Leger, Brittany	121
Grange, Robert	113	Li, Tao	39

Alphabetical Listing of all Poster Presenters

Li, Yanjing	40	Sajjadi, Amir	8
Lion, Mattia	99	Sanchez, Angie	94
Lipkin, Lev	41	Sangji, Naveen	154
Lopez-Lengowski, Kara	122	Santos, Alba	61
Lowther, Chelsea	77	Scarfo, Irene	166
Lv, Xiangmin	42	Schorr, Melanie	72
Man, Cheuk-Him	43	Schweiger, Markus	46
McAlpine, Cameron	52	Seo, Jeehye	147
McCurley, Jessica	53	Sepulcre, Jorge	25
Merino, Jordi	78	Shao, Tuo	167
Millham, Lucia	106	Sheppard, Kimberly	138
Mills, Alexandra	123	Shi, Lei	47
Minikel, Eric	162	Silva Amaral, Ana Claudia	130
Mishra, Nibha	124	Sohn, Jihee	62
Mo, Dandan	18	Sokol, Caroline	102
Mohan, Raghav	44	Su, Mack	48
Montesi, Sydney	19	Sun, Haoqi	9
Mora Buch, Rut	100	Sun, Yao	49
Morehouse, Elizabeth	163	Sunwoo, Hongjae	80
Murtha, Ryan	79	Tai, Derek	81
Neilan, Anne	91	Teng, Jian	168
Nguyen, Emily	164	Tetrault, Marc-Andre	26
Nomura, Shinsuke	20	Tucker, Chandler	73
Oh, Juhyun	45	Uchida, Mai	148
O'Loughlin, Evan	60	van Veluw, Susanne	131
Park, Kate	21	Vilsaint, Corrie	149
Parrish, John	5	Walton, Russell	82
Perez, Numa	153	Watmuff, Bradley	150
Pezaris, John	125	Wu, Tong	10
Ptaszek, Leon	165	Xia, Ning	132
Rahmati, Negah	126	Xu, Wanxue	83
Ramos-Torres, Karla	22	Yekula, Anudeep	133
Reddy, Krishna	92	Yen, Yi-Fen	27
Robinson, Ellen	93	Yeum, Tae-Sung	151
Rodell, Christopher	101	Yin, Kanhua	50
Rodriguez-Torres, Axana	127	Yu, Dongmei	84
Rogers, Madeline	128	Zazzeron, Luca	169
Ryu, Sang Baek	6	Zhang, Yiyi	134
Ryu, Jiheun	23	Zhou, Yu-Peng	28
Saatcioglu, H. Duygu	66	Zhu, Qiuyu Martin	54
Sabuhi, Wali	7		
Sadri-Vakili, Ghazaleh	129		
Sajedi, Salar	24		

