Virtual Poster Session Abstracts

SAC 2021

April 7 & 8, 2021

72nd Meeting of the
MGH Scientific Advisory Committee

Contents

Agenda

Wednesday, April 7, 2021

Celebration of SCIENCE

11:00 am - 1:00 pm POSTER SESSION

2:00 - 5:00 pm CELEBRATION OF SCIENCE

Welcome and Opening Remarks
Peter L. Slavin, MD, President, MGH

ECOR Report
David E. Fisher, MD, PhD, Chair, Executive Committee on Research (ECOR)

Class of 2021 MGH Research Scholars
Susan A. Slaugenhaupt, PhD, Scientific Director, Mass General Research Institute

2021 Martin Prize for Fundamental Research
Large-Scale Topological Changes Restrain Malignant Progression in Colorectal Cancer
Martin Aryee, PhD, Assistant Professor, Pathology

2021 Martin Prize for Clinical Research
Trial of Sodium Phenylbutyrate-Taurursodiol for Amyotrophic Lateral Sclerosis
Sabrina Paganoni, MD, PhD, Assistant Professor, Physical Medicine and Rehabilitation

2021 Howard M. Goodman Fellowship
Development and Application of Therapeautic Genome Editing Technologies
Benjamin Kleinstiver, PhD, Assistant Professor, Pathology / Center for Genomic Medicine

2021 Howard M. Goodman Fellowship
Neuro-immune Circuits Controlling the Initiation of Allergic Immunity
Caroline Sokol, MD, PhD, Assistnat Professor, Medicine / Rheumatology, Allergy and Immunology

Agenda

Thursday, April 8, 2021

COVID Lessons Learned: Scientific and Community Impact

10:00 am - 12:00 pm **COVID-19, Health Equity, and Developing the Next Generation of Population and Health Care Delivery Team Scientists**

Welcome
Peter L. Slavin, MD, President, MGH

Part I: Developing the next generation

Completing the Translational Research Arc at MGH from Discovery to Delivery Science and Health Equity
Steve Bartels MD, MS, Director, The Mongan Institute

Impact of COVID-19 on Vulnerable Research Faculty- Challenges and Opportunities
Katrina A. Armstrong, MD, Physician-in-Chief, Department of Medicine

Advancing Equity and Diversity and Growing the Pipeline of the Next Generation of Population and Health Care Delivery Scientists: the MGH COVID-Corps Research Internship Program
Long Nguyen MD, MS & Aswita Tan-McGrory, MBA, MSPH

Moderated Q & A Session
Dr. Bartels

Part II: Examples of COVID-19 Population and Health Care Delivery Team Science

Data Science: Rapid Implementation of Realtime COVID-19 Clinical Epidemiology:

- **Mobile Epidemiology of COVID-19 and the Coronavirus Pandemic Epidemiology (COPE) Consortium**
  Andrew Chan MD, MPH & David Drew, PhD
- **Rapid Observational Data Collection for COVID-19 Research at Mass General Brigham**
  Ingrid Bassett, MD, MPH & Virginia Triant, MD, MPH

Data Science: Simulation Modeling of the Clinical and Economic Outcomes of COVID-19 Prevention and Treatment: From Homeless Adults in Boston to WHO Low and Middle Income Country Vaccine Distribution
Krishna Reddy, MD

Agenda

Thursday, April 8, 2021

Delivery Science: COVID-19, Health Disparities, and Interventions for Vulnerable Populations, a Panel Discussion
Margarita Alegría, PhD, Julie Levison, MD, MPH, Efren Flores, MD & Ali Raja, MD, MBA

COVID-19: Global Health Equity in Pandemic Response
Louise Ivers, MD, MPH

Research on Interventions for COVID Related Stress
Luana Marques, PhD

Moderated Q & A
Dr. Bartels

12:00 - 1:00 pm **Lunch**

1:00 - 2:15 pm **COVID-19 Innovation**
Moderator: Galit Alter, PhD, Ragon Institute of MGH, MIT and Harvard

Keynote: COVID-19 From Bench to Globe
Dr. Alter

Technology
Gary Tearney, MD, PhD

Diagnostics
A. John Iafrate, MD, PhD

Therapeutics
Keith Flaherty, MD

Immunology
Richelle Charles, MD

Vaccine Trials
Ricky Mofsen, DO

Q & A
Dr. Alter

2:15 - 2:45 pm **Executive Session (SAC members only)**

2:45 - 3:15 pm **Debrief: Research Institute Steering Committee & SAC Members (closed)**

Posters

Bioengineering & Devices

Biomedical Imaging

Posters

Cancer

Posters

Posters

Immunology/Inflammation

Posters

Infectious Diseases

Musculoskeletal

Neurosciences

Posters

Posters

Posters

Posters

Bioengineering & Devices

Poster Number 1

Aylin Acun, PhD
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Development of Decellularized Whole Human Livers for Increasing Donor Pool

INVESTIGATORS: A. Acun, B. E. Uygun

Background: Eight patients die daily awaiting liver transplant. Although there is donor liver scarcity, thousands of livers are discarded due to conditions such as advanced age. To ameliorate donor scarcity, we aim repurposing discarded human donor livers through whole liver decellularization and subsequent recellularization with patient-specific cells. The purpose of this study is to assess the effect of advanced donor age on ECM composition and structural organization as the variation in quality and age will affect translating this approach to clinical settings.

Methods: We decellularized 3 young (<44 years old) and 5 aged (>44 years old) whole human livers through a 4 day-long protocol of perfusion with sodium dodecyl sulfate (SDS) through portal vein and hepatic artery. Total DNA, collagen, and glycosaminoglycan (GAG) contents were assessed and mass spectrometry and histology were used to examine structural composition and organization.

Results: More than 90% of DNA was removed from all livers. We observed that aged livers had a less homogenous decellularization. We determined that aged livers had higher collagen and GAG content compared to young livers. Histology analysis showed that the structural integrity was preserved in livers regardless of age while aged livers had higher remaining fat content.

Conclusions: Our results indicate that there are age-related alterations in liver ECM composition and structure. Through further characterization of livers from different aged donors, the widely varying discarded donor livers can be classified, thus be used effectively as decellularized scaffolds to develop human liver substitutes with high functionality and regenerative capacity.

Poster Number 2

Dara Ahmadi Azar, PhD
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Biomechanical Assessment of Inflammatory Bowel Disease in a Murine Model

INVESTIGATORS: D. Ahmadi Azar, Z. Zhou, N. Saeidi

Background - Inflammatory Bowel Disease (IBD) affects approximately 3 million people in the US. Diagnosis of IBD using current clinical methods is associated with challenges and confusion. Dextran sodium sulfate (DSS)-induces colitis in mice is established as a useful model of IBD. Our goal is to improve current clinical methods in diagnosis of IBD by introducing a novel approach which focuses on gastrointestinal mechanical response which is manifested via inflammatory disease.

Methods – An acute model of DSS was established in 10-week old wild type mice (n=5). The premise of our study is to investigate if mechanical response can help detecting disease. To that end, a LabVIEW-based benchtop test setup was developed and was equipped with a digital image correlation outer diameter measurement system. A series of semi-automated intraluminal inflation-deflation tests were performed using a balloon catheter at a rate of 0.5 ml/min to maintain steady state conditions. Sections of murine small intestine (duodenum, jejunum, ileum) and colon were examined ex vivo.

Results and Discussion - Analysis of unloaded configuration of intestine shows a decrease in outer diameter for ileum samples ($p<0.05$) and an increase in colon ($p<0.001$) compared to control. Our results indicate changes in mechanical properties induced by DSS are predominantly present in ileum and colon. Geometric measurements and mechanical properties show two distinct schemes of mechanical remodeling in ileum and colon in response to pathology. Our device prototype and experimental methods detect systematic changes which can be related to presence and possibly the severity of pathology.

Bioengineering & Devices

Poster Number 3

Elizabeth Flynn, BA
Cancer Center, Research Technician | eflynn6@mgh.harvard.edu
HyPE-seq: Hydrogel Protein Encapsulation and Sequencing

INVESTIGATORS: R. Thakur, S. Stott

The relationship between DNA, RNA, and protein is fundamental to the study of biology. A clearer picture of cell function can be achieved through the simultaneous analysis of its transcriptome and protein expression. Capturing the transcriptome and the proteome at a single cell level would broaden the scope of cellular research by allowing us to see how individual cells are functioning in larger systems. We want to use nucleic acid cytometry to enrich rare cell populations, and be able to simultaneously capture DNA, RNA, and proteins from those rare cells by encapsulating them in a functionalized hydrogel.

Rare cell populations can be difficult to profile without enrichment. With single cell sequencing, sequencing depth is spread among all cells of the sample, and low level transcripts may be lost. When a rare cell population is enriched by sorting, there will be more sequencing reads per rare cell, and analysis will be more in depth for that rare cell population.

Here we present a microfluidic workflow that would encapsulate individual cells into porous hydrogels, and through functionalization of that hydrogel, simultaneously capture DNA, RNA, and proteins from each individual cell. We are then sorting these encapsulated cells by a nucleic acid marker, before proceeding with sequencing analysis.

Sorting by nucleic acid allows us to enrich rare cell populations, and a multi-omic method of analysis can give us an in depth profile of rare populations that have yet to be extensively analyzed.

Poster Number 4

Hamid Ghaednia, PhD
Orthopaedics, Research Fellow | hghaednia@mgh.harvard.edu
Predicting joint replacement failure via smart materials, machine learning, and electrical impedance tomography

INVESTIGATORS: H. Ghaednia, C. E. Owens, L. E. Kiderling, A. J. Hart, J. H. Schwab, T. N. Tallman

At an estimated cost of $8 billion annually in the United States, revision surgeries to total joint replacements represent a substantial financial burden to the health care system. Fixation failures, such as implant loosening, wear, and mechanical instability of the poly(methyl methacrylate) (PMMA) cement, which bonds the implant to the bone, are the main causes of long-term implant failure. Early and accurate prediction and diagnosis of cement failure is critical for developing novel therapeutic strategies and reducing the high risk of a misjudged revision. Unfortunately, prevailing imaging modalities, notably plain radiographs, struggle to detect the precursors of implant failure and are often interpreted incorrectly. Motivated by this challenge, we present a novel approach for in vivo monitoring and failure prediction of cemented joint replacements. Poly(methyl methacrylate) (PMMA) bone cement is modified with low volume fractions of conductive nano fibers to impart piezoresistive-based self-sensing. Electrical Impedance Tomography (EIT) is then used to monitor load-induced deformation and fracture of CF/PMMA in a phantom tank. Finally, we implement a combination of theoretical analysis and machine learning to interpreted EIT signals and measured load magnitudes, detect failure of implant fixation, and even distinguish between cement cracking and cement de-bonding. Because EIT is a low-cost, physiologically benign, and potentially real-time imaging modality, the feasibility study herein presented has potential to transform the standard of care for post-operative monitoring of implant conditions. Beyond clinical relevance, this technique could positively impact orthopedic researchers by providing, via in vivo monitoring, insight into the factors that initiate replacement failure.

Poster Number 5

Elise Lupon, MD
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Decellularization of vascularized engineered scaffolds for facial reconstruction

INVESTIGATORS: E. Lupon, A. Acun, R. Oganesyan, M. P. Goutard, A. G. Lellouch, A. R. Andrews, L. A. Lantieri, M. A. Randoplh, C. L. Cetrulo, Jr, B. Uygun

Background: Since 2005, Facial Vascularized Composite Allotransplantation (FVCA) has proven to be a revolutionary reconstructive procedure for severe disfigurements. However, the inclusion criteria for FVCA remains limited due to the requirement for lifelong immunosuppression and the risk of chronic rejection. We have developed a complex engineered graft for facial reconstruction using a decellularized human face which has the potential to be subsequently repopulated with patient specific cells.

Method: The facial specimen was procured from human cadavers in a face allotransplant donor fashion. The specimen was placed in containers and immersed in 1% sodium dodecyl sulfate followed by 1% Triton X-100, without perfusion through the pedicle. Cell removal was assessed macroscopically, by DNA quantification and histology. Vascular patency was assessed by X-ray imaging, scanning electron microscopy, and microfil injection. Mechanical properties were assessed by a tensile test and the immunological response was assessed by in vivo implantation in mice. Hundred thousand human dermal fibroblasts were seeded on the dermal side of 0.5 square cm decellularized samples.

Results: Histologic analysis confirmed the removal of cellular materials through the lack of nuclei. Scanning electron microscopy showed a well-preserved morphologic surface and an intact microvasculature was observed with the microfil injection. No significant difference of mechanical resistance was found between the native and decellularized scaffold. The X-ray imaging pre-decellularization was comparable to X-ray post-decellularization.

Conclusion: We have successfully developed complex an immunocompatible acellular facial scaffolds through immersion in the solution. This technology may represent a novel alternative to live allografts and immunosuppression.

Poster Number 6

Jason Lynch, PhD
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Development of designer probiotics for targeted delivery of immunomodulatory payloads

INVESTIGATORS: J. P. Lynch, C. González-Prieto, A. Z. Reeves, N. Godbole, N. Sahli, L. Lemos, C. B. Shoemaker, W. S. Garrett, C. F. Lesser

Drug delivery platforms that target the deposition of therapeutics to sites of disease are needed to increase efficacy and decrease off-target effects of current interventions. To address these limitations, we developed a probiotic-based strategy capable of directly secreting therapeutic proteins into the gut lumen. Our chosen chassis E. coli Nissle 1917 (EcN) is a human probiotic with a proven safety record in human subjects. To enable EcN to secrete therapeutic proteins into the gut lumen, we engineered it to encode a type III secretion system modified to secrete proteins into its surroundings rather than into host cells. Next, we developed variants of this strain, referred to as T3EcN, that secrete functional nanobodies, variable domains of heavy chain only antibodies, isolated from immunized alpacas and identified to sequester and neutralize target proteins of interest. This was done by appending the N-termini of the nanobody with a type III secretion sequence. To enable T3EcN to stably colonize and constitutively secrete nanobodies within the mammalian gut, nanobody-coding genes were placed under the control of constitutive synthetic promoters and introduced into a plasmid that can be maintained in the absence of antibiotic selection. We observe that nanobody-secreting T3EcN variants stably colonize and maintain secretory activity within the gastrointestinal tract of mice. Using a bioluminescent reporter for type III secretion activity, we have demonstrated that T3EcN's type III secretion system is active in the mammalian gut. Ongoing work in preclinical murine models is focused on establishing proof-of-concept for this novel targeted drug delivery platform.

Poster Number 7

Daniel Rabe, PhD
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Microfluidic Detection of SARS-CoV-2 and Virus-Related Extracellular Vesicles

INVESTIGATORS: S. A. Rabi, U. K. Ho, W. A. Michaud, E. A. Flynn, A. N. Hoang, P. S. Lai, M. B. Goldberg, D. Kwon, R. C. Charles, X. Yu, G. M. Boland, S. L. Stott

Robust, efficient, and reliable testing for SARS-CoV-2 is extraordinarily challenging due to our lack of ultra-sensitive assays and ever evolving knowledge of the virus. Additionally, standard PCR based assays do not provide information on the infectivity or potential outcome of patients that test positive for SARS-CoV-2. Alternatively, microfluidic processing of clinical samples can provide low-cost assays with great promise for clinical translation. Our laboratory was one of the first to apply microfluidic technologies for the isolation of extracellular vesicles (EVs) in the blood of patients with cancer. Thus, for this project, we have optimized our EV capture technology, the EVHB-Chip, to isolate intact SARS-CoV-2 virus, as well as epithelial and immune EVs. Through the use of COVID-19 infection related EVs in addition to intact SARS-CoV-2 virion detection, our assay has the potential to provide further insight into the infectivity and outcome for COVID-19.

Poster Number 8

Haya Raef, MS
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Testing of a light dosimeter for use in erythropoietic protoporphyria

INVESTIGATORS: L. Rebeiz, R. Karp-Leaf, I. Kochevar, A. K. Dickey, S. B. Elmariah

Background: Erythopoietic protoporphyria (EPP) is a rare disease, caused by decreased activity of ferrochelatase, the final enzyme of heme biosynthesis. Reduced ferrochelatase activity results in the accumulation of protporphyrin IX, leading to painful skin photosensitivity that can decrease quality of life. Sunscreens are ineffective for EPP, as they do not shield against visible light, to which EPP patients are sensitive. While afamelanotide reduces phototoxicity to some extent, there are no effective therapies that relieve the pain of phototoxic reactions once begun. Therefore, reliable tools that accurately monitor photosensitivity in EPP are needed to predict and prevent symptoms in these patients. A more effective method to measure light exposure may also benefit future clinical trials in EPP by providing quantitative clinical trial endpoints.

Aim: To conduct a prospective observational study to assess the utility of the SunSense wearable light sensor for monitoring cumulative light exposure in EPP.

Methods: Light exposure was quantified using a light dosimeter in 25 EPP patients. The wearable light sensor was compared to patient self- reporting of light exposure. Cutaneous reactions to light were simultaneously measured with text-based surveys. Associations between light exposure and symptoms were assessed over a period of two months.

Results: Results suggest that light exposure correlates more closely with symptoms in EPP when light exposure is quantified with a light dosimeter versus by self- report.

Conclusion: Digital devices such as the SunSense wearable light sensor quantitatively measure light exposure and more effectively predict photosensitivity symptoms in EPP patients than self-reporting of sunlight exposure.

Poster Number 9

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Comparison of spread of cortical activation for electric and magnetic stimulation

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

Our goal is to develop a cortical visual prosthesis that can restore vision to the blind. Previous approaches have used arrays of electrodes, implanted into visual cortex, with the goal that each electrode elicits a discrete light percept (phosphene). In this manner, multiple electrodes can be activated simultaneously to create more complex spatial images. It is not clear however whether activation from implanted micro-electrodes is indeed focal previous studies suggest activation may spread well beyond the electrode site. Here, we compare the spread of activation arising from implanted micro-electrodes to that arising from a novel stimulation strategy in which implanted micro-coils are used to magnetically stimulate surrounding neurons.

A custom 128-channel μ-ECoG recording array was centered over V1 in anesthetized mice. A hole in the center of the recording array allowed micro-electrodes or micro-coils to be inserted into cortex; a micro-positioner controlled the depth of insertion and ECoG responses from stimulation of layers 2/3 and 5 were compared.

We found that electric stimulation resulted in widespread activation of the cortical surface; responses typically extended > 1 mm, even for relatively modest stimulation levels (10-20 μA). In contrast, the spread of activation with micro-coils was typically confined to within 0.3 mm and results were consistent for stimulation of both cortical layer 2/3 and layer 5 as well as across a range of stimulus strengths. Our results therefore suggest that magnetic stimulation from implantable micro-coils better confines activation in cortex and thus may have advantages over conventional electric stimulation.

Poster Number 10

Hojeong Yu, PhD
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Fast detection of SARS-CoV-2 RNA via the integration of plasmonic thermocycling and fluorescence detection in a portable device

INVESTIGATORS: H. Yu, H. Lee

The diagnosis of severe acute respiratory syndrome 2 (SARS-CoV-2) infection by quantitative PCR with reverse transcription (RT–qPCR) typically involves bulky instrumentation in centralized laboratories and an assay time of 1–2 h. Here, we show that SARS-CoV-2 RNA can be detected in 17 min via a portable device integrating reverse transcription, fast thermocycling (via plasmonic heating through magneto-plasmonic nanoparticles) and in situ fluorescence detection following magnetic clearance of the nanoparticles. The device, termed nanoPCR, correctly classified all nasopharyngeal, oropharyngeal and sputum samples from 75 patients with COVID-19 and 75 healthy controls, with good concordance in fluorescence intensity with standard RT–qPCR (Pearson coefficients > 0.7 for the N1, N2 and RPP30 genes). nanoPCR has the potential to decentralize COVID-19 molecular diagnosis. It has practical advantages: (1) the assay is based on well-established RT–PCR to produce more reliable and accurate (>99%) results than isothermal amplification tests; and (2) nanoPCR considerably shortens the PCR reaction time to enable on-site diagnostics. With these advantages, nanoPCR could contribute to the decentralization of COVID-19 tests into mobile, ambulatory clinics, mitigating the logistic burden of sample transports.

Poster Number 11

Theodore Zwang, PhD
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Implantable mesh electronics for long term recording of place cell stability and remapping

INVESTIGATORS: T. J. Zwang, C. D. Harvey, B. T. Hyman

Aging changes the adult brain both structurally and functionally. The long time period over which these changes occur can complicate studies attempting to understand how aging might affect cognitive processes at the level of single neurons. Here we utilize a novel biotechnology, mesh electronics, to overcome these challenges and measure electrophysiology of individual neurons and circuits over multiple months. Combined with behavioral tasks in virtual reality, these experiments show the ability to monitor individual place cell stability and remapping over at least two months.

Poster Number 12

Taylor Cannon, BS
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Volumetric mapping of the optical properties of atherosclerotic plaque components using intravascular Optical Coherence Tomography

INVESTIGATORS: T. M. Cannon, N. Uribe-Patarroyo, M. Villiger, B. E. Bouma

Improved classification of atherosclerotic plaques prior to coronary events could reduce mortality in patients with lesions at high risk of rupture, but even with advanced ultrasonic or optical imaging techniques, identifying specific plaque components remains challenging. Obtaining high-resolution images of coronary artery walls is possible using intravascular optical coherence tomography (OCT), but this structural imaging technique is unable to directly differentiate fibrous from lipid-rich areas of atherosclerotic plaques, limiting its utility in assessing the likelihood of plaque rupture in patients. Recently, our lab has developed novel signal processing methods to generate 3D maps of the optical properties of tissue, which are intrinsically linked to tissue physical characteristics. In particular, lipid, fibrous, and calcified areas of plaques are expected to differ in their optical properties, both from each other and from healthy arterial tissue. Here, we show preliminary results correlating volumetric maps of our calculated optical properties in cadaveric coronary arteries to matched ground truth histological results, as well as volumetric maps of optical properties in a human subject in vivo. Our new methods stand to improve the accuracy of optical imaging in evaluating the composition of atherosclerotic plaques in coronary arteries in vivo. We hope that our techniques will ultimately provide a promising clinical tool for assessment of patients at risk for severe coronary events, as well as an informative research tool for better understanding the incidence and progression of atherosclerosis and coronary artery disease.

Poster Number 13

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MarkIt: A Medical Collaborative Annotation Platform

INVESTIGATORS: J. M. Choi, S. M. Jeon, D. Y. Kim, J. W. Jung, S. H. Do

Ensuring high-quality annotations is especially important in medical image supervised machine learning. Annotation is a tedious task, but there are no supporting tools to make a high-volume and qualified dataset. We developed MarkIt, a web-based tool for collaborative annotating medical images. The developed platform handles both DICOM and non-DICOM images with a user login system. Users can annotate images by clicking labels and setting their confidence levels or mark region of interest by using rectangular or free line tools. Artificial intelligence tools, like prediction position, gender, age, and feature detection, can be tested or be used to speed up the annotation process. And various A.I. tools can be easily implemented and scaled up by using Docker systems. Each annotator's activities are tracked and stored in an immutable form using a blockchain system. And as proof of concept, we are utilizing this activity tracking system to measure each annotation or images' value in combination with other information, like concordance of a label, A.I. prediction result, and its confidences. We have been testing MarkIt with several researchers and physician groups to produce qualified dataset from 2020 and upgrading the system to fit various user tasks. We hope the MarkIt and blockchain system can be used to measure the value of an image-annotation dataset and can easily and freely trade it between researchers or provide grants for annotators in the near future. This platform is publicly available for testing on markit.mgh.harvard.edu.

Poster Number 14

Alvin Das, MD
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A Risk Prediction Score for Intracerebral Hemorrhage in Patients Taking Direct Oral Anticoagulants

INVESTIGATORS: R. W. Regenhardt, E. Gokcal, M. J. Horn, A. D. Warren, U. Gurol, A. Biffi, J. N. Goldstein, W. T. Kimberly, C. D. Anderson, A. Viswanathan, L. H. Schwamm, S. M. Greenberg, J. Rosand, M. E. Gurol

Background and Aims: Current risk prediction bleeding scores fail to predict intracerebral hemorrhage (ICH) with high accuracy in direct oral anticoagulants (DOACs) users. Herein, we develop a risk prediction score based on clinical risk factors and MRI biomarkers.

Methods: Clinical/radiological data were collected from consecutive DOAC-ICH patients and ICH-free patients from 2017 to 2020. The frequency/topography of MRI biomarkers in both cohorts were assessed. Baseline demographics and neuroimaging markers were compared in univariate tests between both groups. Significant associations ($p < 0.05$) were entered into a multivariable regression model to determine factors that predict the development of ICH.

Results: 53 NOAC-ICH patients and 94 ICH-free DOAC users were included. Diabetes, any smoking, and antiplatelet usage were more common in DOAC-ICH than ICH-free patients. Severe white matter hyperintensities (WMHs), lacunes, deep lacunes, cortical superficial siderosis (cSS), and cerebral microbleeds (CMBs) were more common in the ICH cohort than the ICH free cohort. When entered into a multivariate regression model, deep lacunes and severe WMHs were not significant. Ultimately, diabetes (1), any smoking (1), antiplatelet usage (1), CMBs (2), and cSS (3) comprised the final hemorrhagic risk score. Using this system, a score of 2 provided a sensitivity and specificity of 88% for predicting the development of ICH.

Conclusions: In this study, we demonstrate a score that predicts the development of ICH by incorporating risk factors and neuroimaging markers. Although screening MRIs are not currently performed prior to initiating DOAC therapy, these data suggest that patients of high-hemorrhagic risk may be identified.

Poster Number 15

Felicitas Detmer, PhD
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Imaging of mitochondrial function for monitoring anthracycline-induced cardiotoxicity

INVESTIGATORS: F. J. Detmer, Y. Petibon, M. Sung-Hyun, M. Dhaynaut, J. L. Guerrero, P. Brugarolas, M. D. Normandin, M. Pelletier-Galarneau, G. El Fakhri, N. M. Alpert

Cardiotoxicity is a serious side effect of several chemotherapeutic agents, especially anthracyclines. Prompt detection of anthracycline-induced cardiotoxicity (AIC) is crucial to mitigate myocardial impairment, however, current approaches to monitor patients for signs of AIC are mostly insensitive to early stages of the disease. Therefore, new imaging approaches for early detection of AIC are direly needed. While the mechanisms of AIC are incompletely understood, a key role has been attributed to mitochondrial damage and impaired mitochondrial function. Our group has recently introduced an approach for non-invasive mapping of total cardiac membrane potential (ΔΨT), a proxy of mitochondrial membrane potential (ΔΨM), using PET imaging with the tracer 18F-triphenylphosphonium (18F-TPP+). ΔΨM represents a comprehensive index of mitochondrial function and ΔΨT mapping could therefore serve as an early biomarker of AIC. This study aimed to determine whether ΔΨT mapping could detect an acute cardiotoxic effect of doxorubicin, a common anthracycline, in a swine model. We tested this hypothesis by administering doxorubicin intracoronarily during 18F-TPP+ PET imaging in minipigs.

We found that in all three studied animals, an acute depolarization of ΔΨT could be observed in areas directly exposed to the doxorubicin infusions, whereas no changes were measured for a control saline infusion administered prior to the doxorubicin infusions nor for the unexposed control areas. These results indicate that an acute effect of doxorubicin on cardiac mitochondria can be detected using ΔΨT mapping. Future studies aim at assessing the potential of ΔΨT mapping as a biomarker for early detection of AIC in a chronic setting.

Poster Number 16

Itika Garg, MD
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Vascular Metrics and Non-Perfusion Area Measured by Wide-Field Swept-Source OCT-Angiography (WF-SS-OCTA) in Different Stages of Diabetic Retinopathy

INVESTIGATORS: I. Garg, C. Uwakwe, Y. Cui, E. Lu, R. Le, Y. Zhu, K. M. Wai, J. Y. Moon, Y. Lu, C. Y. Li, T. Elze, J. W. Miller, L. A. Kim, D. Husain, D. G. Vavvas, J. B. Miller

There has been increasing interest in WF fundus photography and fluorescein angiography in the management of diabetic retinopathy (DR). We explore the added value of WF-SS-OCTA in a prospective observational study of diabetics compared to controls. We imaged 469 eyes of 288 patients (69 eyes of 39 control patients and 400 eyes of 234 patients with diabetes) using WF-SS-OCTA between December 2018 to September 2020. 6x6mm and 12x12mm scans centered on the fovea were analyzed for vessel density (VD), vessel skeleton density (VSD), foveal avascular zone (FAZ) area, circularity and perimeter; and non-perfusion area (NPA) on 12x12mm scans only. Mixed-effects multivariate regression models were used to test for significance. Among diabetics: 45, 88, 64, 34 and 169 eyes had no DR, mild non-proliferative DR (NPDR), moderate NPDR, severe NPDR, and proliferative DR (PDR) respectively. Trend analysis revealed a progressive decline in VD and VSD with increasing DR severity. Severe NPDR and PDR patients had an increased FAZ area, perimeter and irregularity ($p<0.05$). Also, all NPDR and PDR groups had irregular FAZ on 12x12mm scans ($p<0.05$). NPDR and PDR had greater NPA versus controls ($p<0.001$). On receiver operating curve (ROC) analysis, wide-field NPA was the best predictor among all for the presence of DR, AUC 0.97. Herein, we present a comprehensive study of a large cohort of DR patients imaged on the WF-SS-OCTA. The enhanced resolution and larger area highlights the importance of NPA which can be more readily and repeatedly measured with WF-SS-OCTA for earlier and accurate diagnosis of DR.

Poster Number 17

Lindsay Hanford, PhD
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Exploration of highly-accelerated multi-echo MPRAGE using compressed sensing for brain morphometry applications

INVESTIGATORS: E. M. Iannazzi, T. Hilbert, T. Kober, R. L. Buckner, R. W. Mair

Compressed-sensing (cs) has the potential to shorten scan acquisition time. These time savings may help to reduce patient burden, artifacts in MR due to motion, and cost for repeat acquisitions. A key open question is whether time-saving cs T1w structural images will yield quantitative morphometric estimates comparable to traditional longer sequences. To test this, images were acquired for numerous cs T1w image variations across 2 independent datasets. Structural estimates were compared within- and between-subjects to assess scan stability and the effect of acceleration. x6 cs acceleration (~86s scan) was sufficient to yield comparable morphometrics for most measures in typical applications.

Poster Number 18

Katharina Hoebel, MD
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Radiomics Repeatability Pitfalls in a Scan-Rescan MRI Study of Glioblastoma

INVESTIGATORS: K. V. Hoebel, J. B. Patel, A. L. Beers, K. Chang, P. Singh, J. M. Brown, M. C. Pinho, E. R. Gerstner, B. R. Rosen, J. Kalpathy-Cramer

To determine the influence of preprocessing on the repeatability and redundancy of radiomics features extracted using a popular open-source radiomics software package in a scan-rescan glioblastoma MRI study. In this study, a secondary analysis of T2-weighted fluid-attenuated inversion recovery (FLAIR) and T1-weighted postcontrast images from 48 patients diagnosed with glioblastoma were included from two prospective studies (NCT00662506 and NCT00756106). All patients underwent two baseline scans 2–6 days apart using identical imaging protocols on 3-T MRI systems. No treatment occurred between scan and rescan, and tumors were essentially unchanged visually. Radiomic features were extracted by using PyRadiomics under varying conditions, including normalization strategies and intensity quantization. Subsequently, intraclass correlation coefficients were determined between feature values of the scan and rescan. Shape features show a higher repeatability than intensity (adjusted P,.001) and texture features (adjusted P,.001) for FLAIR and T1-weighted postcontrast images. Normalization improved the overlap between the region of interest intensity histograms of scan and rescan (adjusted P,.001 for both FLAIR and T1-weighted postcontrast images), except in scans where brain extraction fails. As such, normalization significantly improves the repeatability of intensity features from FLAIR scans (adjusted P=.003 [z-score normalization] and adjusted P=.002 [histogram-matching]). The use of a relative intensity binning strategy as opposed to default absolute intensity binning reduces correlation between gray-level co-occurrence matrix features after normalization. Both normalization and intensity quantization have an effect on the level of repeatability and redundancy of features, emphasizing the importance of both accurate reporting of methodology and understanding the limitations of pipeline design choices.

Poster Number 19

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Explainable artificial intelligence applying the stream of human consciousness

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Developing artificial intelligence is the process of developing stable algorithms that can mimic real intelligence. In particular, to make AI smarter and practically applicable, it must be possible to explain why this algorithm infers the result, as does a human with real intelligence. Once developed, the user should be able to clearly observe which part's performance is limited, which functions interfere with each other in the algorithm, and furthermore, which performance is reliable and which part is not. Most of the currently developed algorithms are black-box methods with limitations that do not have these functions.

On the other hand, in order to create an especially explainable artificial intelligence, artificial intelligence, like the flow of human consciousness, must be able to represent the reasons for making predictions in real-time. For humans to understand and evaluate the processes of artificial intelligence's internal reasoning, artificial intelligence algorithms must express this process. The explanation method of artificial intelligence will be most effective when it can imitate the flow of thinking that humans think.

Our newly developed artificial intelligence model adopts a method of evaluating chest x-ray images in the same way as human consciousness flow. Therefore, this model is similar to how a human radiologist evaluates X-ray images. In particular, using this flow of consciousness, AI algorithms automatically annotate new X-ray images, allowing them to become smarter and smarter.

Poster Number 20

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Mapping the subcortical functional connectome of the default mode network for targeted neuromodulation

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For patients with disorders of consciousness, connectome mapping can be used to identify widely-connected network hubs that could be therapeutic targets for stimulation. Evidence shows that the default mode network (DMN) plays a crucial role in modulation of consciousness in humans. However, the subcortical connectome of the DMN, which includes many regions of interest (ROI) targeted by neurostimulation, has not been well characterized. In this work, our goal is to identify the subcortical functional connectome of the DMN and explore which regions are strongly connected to the cortical DMN nodes. To achieve that, we applied a Nesterov-Accelerated SCAlable and Robust (NASCAR) tensor decomposition method, in conjunction with the BrainSync algorithm, to a group of 84 subjects' 7T resting-state fMRI data from the Human Connectome Project. We computed and visualized using violin-plots the values of the subcortical map within each ROI, where the ROIs were defined using three co-registered atlases: FreeSurfer aseg atlas, a probabilistic atlas for segmentation of the thalamus, and the Harvard ascending arousal network atlas. We found that the thalamus and brainstem are highly positively interconnected within the cortical DMN. Thalamic nuclei known to modulate consciousness, such as CL, CeM, and Pf, show strong positive correlations with the DMN, while the strongest brainstem correlations with the DMN were observed within DR and MnR. We also found that activities in the putamen and globus pallidus are negatively correlated with the cortical DMN nodes. These observations may provide empirical evidence of the proposed "mesocircuit" model in humans.

Poster Number 21

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In vivo microscopic assessment of idiopathic pulmonary fibrosis using endobronchial optical coherence tomography: A prospective diagnostic study

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Idiopathic pulmonary fibrosis (IPF) has the worst prognosis of all fibrotic interstitial lung diseases (ILD) and a mortality rate higher than many cancers. Early, accurate diagnosis of IPF is essential to determine the most effective therapy for patients, but often requires invasive surgical lung biopsy (SLB) to identify salient microarchitectural features such as microscopic honeycombing (< 2mm), not visible by high-resolution computed tomography (HRCT). Unfortunately, SLB has high risks of associated morbidity and mortality in this patient population. We aim to determine whether endobronchial optical coherence tomography (EB-OCT) can serve as a novel, low-risk, minimally-invasive method for in vivo microscopic diagnosis of IPF without surgery or tissue removal. In this prospective diagnostic study, EB-OCT was performed in 31 patients undergoing concurrent SLB for ILD diagnosis. Four patients were excluded from analysis due to non-diagnostic histopathology (n=3) or minor EB-OCT malfunction (n=1). Of the remaining 27 patients, an average of 6 distinct peripheral lung sites (range 1-9 sites, across both lungs and in multiple lobes) were imaged in 9.5 minutes (SD 4.22). There were no adverse events associated with EB-OCT. EB-OCT was 100% sensitive (95% CI: 75.8-100.0) and 100% specific (76.9-100.0) for both the histologic diagnosis and clinical follow up diagnosis of IPF. There was high agreement between EB-OCT and histopathology for the diagnosis of specific ILD fibrosis pattern (weighted κ: 0.87, 0.72-1.0).

Here we show the potential of EB-OCT as a microscopic complement to HRCT and alternative to SLB for the diagnosis of IPF and non-IPF ILD.

Poster Number 22

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Quantitative, rapid, and reproducible molecular MRI of tumor apoptotic response to oncolytic virotherapy

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The highly invasive nature of many cancer types and the toxicity of most systemic chemotherapies represent significant challenges for cancer therapies. An especially promising approach for overcoming these challenges is the use of oncolytic viruses that selectively kill only cancer cells while sparing the surrounding normal cells. Non-invasive imaging of the underlying molecular processes is an essential tool for achieving the full potential of this biological therapeutic, enabling the assessment of viral spread, innate immunity, and therapeutic response. However, previous methods for imaging oncolytic virotherapy had poor sensitivity and specificity or required the administration of radioactive or metal-based contrast-materials. Here, we present a new noninvasive method for quantitative and rapid molecular imaging of oncolytic virotherapy treatment response. The method combines proton exchange-based MRI with deep learning, yielding quantitative biomarker maps of protein and lipid/macromolecule concentrations as well as intracellular pH. The benefit of using this method was demonstrated in a glioblastoma mouse brain tumor model, where it allowed the early detection of apoptotic response to oncolytic virotherapy, in excellent agreement with histology and immunohistochemistry findings. The method was translated to clinical scanners and produced reproducible and quantitative 3D molecular maps of the human brain across 3 different imaging sites. The acquisition of 4 biomarker volumetric maps was achieved in less than 12 min; thus, it could potentially be incorporated within routine imaging sessions. The method is directly applicable to a wide range of additional pathologies where the intracellular pH or the lipid/metabolite concentration is altered, including stroke and neurological disorders.

Poster Number 23

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Near-infrared and shortwave-infrared imaging of endogenous lipofuscin to monitor non-alcoholic fatty liver disease (NAFLD) in human liver biopsy samples

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Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease with a global prevalence of 25%. Currently, clinicians are faced with both a limited ability to screen for NAFLD and monitor the progression/regression of the disease. We recently developed a novel imaging technique in the near-infrared (NIR) and short-wave-IR (SWIR) spectrum to quantify the endogenous autofluorescent pigment lipofuscin to monitor liver disease in NAFLD mouse models. We hypothesize that this technique can serve as a diagnostic and/or prognostic imaging tool in human patients with NAFLD. To test this hypothesis, we quantified the NIR/SWIR lipofuscin signal in formalin fixed paraffin-embedded (FFPE) human liver biopsies (n=17) and assessed non-alcoholic steatohepatitis (NASH) grade and stage using the Brunt criteria. Indeed, the NIR/SWIR autofluorescence of lipofuscin is detectable in FFPE human NAFLD liver biopsy samples. Our data reveals a 4-fold decrease in lipofuscin in patients with NASH cirrhosis compared to non-cirrhotic NASH (*** $P<0.001$). Patients with mild NASH grade show approximately 3-fold more lipofuscin compared to moderate (** $P<0.0042$) and severe (* $P=0.05$) grades. In summary, we show that lipofuscin can be used to monitor liver necroinflammation and fibrosis in FFPE human NAFLD liver biopsy samples with a potential to discern NAFLD disease stages. Further studies with additional patient samples are needed to obtain granularity in distinguishing among intermediate disease stages, as well as determining the pathological drivers of lipofuscin accumulation. With ongoing investigations, we anticipate that lipofuscin will be a biomarker for monitoring patients with NAFLD and other chronic liver diseases.

Poster Number 24

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Multi-modal real-time imaging enabled by a Dual Function Antibody Conjugate (DFAC) for image-guided tumor therapy

INVESTIGATORS: M. A. Saad, T. Hasan

Precision imaging, utilizing molecular targeted agents, is an important tool in cancer therapeutics, mostly employed in diagnostics, treatment planning/optimization, and monitoring response to therapy. While there are limitations associated with single mode imaging probes, multi-modal molecular imaging probes for complementary imaging technologies provide attractive alternatives. However, there are several challenges associated in designing molecular probes carrying contrast agents for complementary multi-modal imaging. In this study, we demonstrate the synthesis and evaluation of a Dual Function Antibody Conjugate (DFAC) for imaging and treatment of tumor tissues. The proposed DFAC comprises of an anti-EGFR antibody conjugated with a fluorophore (AF647) and photoacoustic contrast agent (IRDye800) for enabling real-time multi-modal imaging through complementary imaging technologies – fluorescence for high sensitivity and photoacoustic imaging for high spatial resolution. Through a series of in vitro and in vivo experiments we demonstrate the potential of the DFACs in targeting EGFR over-expressing tumor cells and successful delineation of tumor margins from healthy tissue, thus highlighting the potential of the DFAC as molecular-targeted probes for guiding tumor resection surgeries. The proposed DFAC can be easily adapted to incorporate different contrast agents, drugs and photosensitizers. We further demonstrate that by replacing the fluorophore with a photosensitizer (Benzoporphyrin derivative) the DFAC can be exploited, not only for its diagnostic and tumor margin delineation application, but also as a targeted therapeutic (photodynamic therapy) probe for the treatment of microscopic residual tumors, often left after surgical resection.

Poster Number 25

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Automatic Reconstruction of Cerebellar Cortex from Standard MRI Using Diffeomorphic Registration of a Novel Digital Atlas (ARCUS)

INVESTIGATORS: M. S. Hamalainen, B. R. Rosen

As cumulating evidence points to a wider range of functional tasks and neurological conditions that involve the cerebellum than previously known, the interest for examining the cerebellum with non-invasive neuroimaging techniques is growing. However, the standard methods of computational neuroanatomy for segmenting and reconstructing the cerebral cortex work poorly for the cerebellar cortex at the resolutions attainable with contemporary MRI technology because of its extremely intricate folding, making detailed and topologically correct reconstructions of the geometry of the cerebellar cortical surface unfeasible. Recently, a detailed digital surface reconstruction of the human cerebellar cortex was achieved from an ex-vivo specimen. By a combination of manual labeling and image processing of this model, we created to our knowledge the first digital, sub-folia resolution, cerebellar surface and volume atlas. These novel anatomical data enable a new reconstruction technique of the cerebellar cortex that is presented here, where this surface atlas is morphed to subject space based on standard in-vivo MRI data. The result is an approximate reconstruction of the cerebellar cortex that requires only standard-resolution MRI data and can be used e.g., in functional neuroimaging, for integrating topographic population data or for visualizing topographic data on flattened surface patches.

Poster Number 26

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Histologic parcellation and validation of Human Entorhinal Cortex in ex vivo MRI

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The entorhinal cortex is a critical area for memory processing and has clinical relevance as one of the earliest cortical areas affected with hyper-phosphorylated tau protein (neurofibrillary tangles) in Alzheimer's disease. As neurofibrillary tangles accumulate in the medial temporal lobe and beyond, Alzheimer's disease symptoms emerge and progressively worsen. In the neuroimaging field, the entorhinal cortex is often considered only in its entirety or divided into a medial and a lateral region. However, neuroanatomical differences support further division into smaller distinct subfields and there is evidence that some subfields of the entorhinal cortex succumb to neurofibrillary tangles earlier than other subfields.

In this work, we acquired high resolution MRI scans of ex vivo human brain samples and performed histological sectioning and staining of the samples. Using cellular information from the histologically stained tissue, we generated an MRI segmentation of the entorhinal subfields, bridging the gap between imaging and ground truth histology and yielding quantitative anatomical results. The overall goal of this work and these data is to build and validate an MRI atlas of the entorhinal cortex to be integrated into the FreeSurfer MRI atlas for in vivo application. The availability of a more sensitive tool such as the entorhinal subfield atlas will allow for better characterization of these vulnerable cortices longitudinally, as well as of how the individual differences that develop may contribute to resilience or susceptibility to Alzheimer's disease, potentially opening the door to detection of Alzheimer's disease changes in preclinical stages.

Poster Number 27

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Near-infrared fluorescence imaging with Feraheme-Alexa750 and TLR4-ZW800-1C dyes for atherosclerotic plaque detection in murine models

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Early detection and intervention of inflamed plaques can improve coronary artery disease morbidities by preventing serious and often fatal symptoms. We aim to locate these plaques with near-infrared fluorescence imaging by using particles that target anti-inflammatory macrophages. Two groups of atherosclerotic mice were fed a high-cholesterol diet for eight weeks and received streptozotocin to induce diabetes for accelerating plaque progression. Both groups undergone surgical ligation of the left carotid artery to mimic stenosis while keeping the right carotid artery intact. At week eight, one group was injected with the Feraheme-Alexa750 while the other with the TLR4-ZW800-1C molecular compounds. Fluorescence imaging was acquired of both in vivo and ex vivo carotid arteries. We show that both compounds were successful in highlighting the diseased left carotid artery while exhibiting minimal signal in the control right carotid artery (left to right signal-to-background ratio=1.7 ($p=0.0262$) and 1.2 ($p<0.01$) for Feraheme-Alexa750 and TLR4-ZW800-1C respectively even at short 100 ms exposure time). Near-infrared fluorescence imaging with these molecular compounds shows promise in the detection of atherosclerotic plaques, particularly for intravascular imaging applications. Continued investigation will include larger sample sizes and bigger animals using intravascular near-infrared fluorescence imaging.

Poster Number 28

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Registration of the Anatomic Skull Model to Dual Fluoroscopic X-ray Images Using Deep Learning

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Registration of 3D anatomic structures to their 2D dual fluoroscopic X-ray images is a widely used motion-tracking technique. However, the implementation of deep learning is often limited by a paucity of medical images and ground truths. We proposed a transfer learning strategy for 3D-to-2D registration using deep neural networks trained from an artificial dataset. In this strategy, digitally reconstructed radiographs (DRRs) and 2D skull landmarks were automatically created from craniocervical CT data of a female subject. They were used to train a residual network (ResNet) for landmark detection and a cycle generative adversarial network (GAN) for style translation between DRRs and actual X-rays. Landmarks on the X-rays detected by the ResNet were used in triangulation optimization for 3D-to-2D registration of the skull in a dual-fluoroscope imaging setup. The registration accuracy was evaluated in multiple scenarios of craniocervical motions. In walking, learning-based registration for the skull had angular/position errors of $3.9 \pm 2.1°/4.6 \pm 2.2$ mm. However, the accuracy was lower during neck flexion-extension ($8.9 \pm 3.6°/11.9 \pm 6.5$ mm), lateral bending ($14.1 \pm 6.2°/12.4 \pm 7.4$ mm), and axial rotation ($8.9 \pm 4.0°/8.0 \pm 3.9$ mm), due to overly small skull regions imaged on the dual fluoroscopic images at end-range positions. The results showed that the proposed strategy can tackle the complicated registration scenario when the skull was only partially captured on the dual fluoroscopic X-rays, and it has potentials to extend to widespread registration scenarios. Further improvement is warranted for accurate and reliable automatic 3D-to-2D registration.

Poster Number 29

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Post-transcriptional regulation of RNA methylation by stress signaling

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Canonical translation is reduced in stress conditions like quiescence and chemoresistance due to low mTOR activity and phosphorylation of eIF2α. In these low mTOR and phospho-eIF2α conditions, non-canonical translation mechanisms help cells make specific proteins that are required for growth and survival. Here we show that in leukemic and breast cancer cells treated with chemotherapy, mTOR is inhibited and eIF2α is phosphorylated. We show that in mTOR-inhibited and phospho-eIF2α conditions, m6A RNA methyltransferases METTL3 and METTL14 are translationally upregulated, which causes increase in m6A RNA methylation. M6A modification of RNAs can alter post-transcriptional expression of specific genes that are important in cancer progression. Additionally, we show that in chemotherapy treated patient samples and tumor spheroids, eIF2α is phosphorylated and METTL3 and METTL14 are increased. We identified METTL3 regulators using biotin tagged antisense affinity purification of METTL3 mRNA to identify associated translation factors. We find that non-canonical translation factors form a specialized complex that associate with and promotes METTL3 translation. We performed methylated RNA-IP (me-RIP), RNA and proteomic analysis to identify m6A targets. Increased METTL3 levels downregulate antiviral defense response and cell cycle genes necessary for proliferation. Furthermore, knocking down METTL3 decreases resistance to chemotherapy by increasing cell cycle and antiviral defense response genes. Likewise, treating cells with drugs that overcome the effect of eIF2α phosphorylation, reverses the increase of METTL3 and METTL14, thereby making these cells more sensitive to chemotherapy. Together our data suggests that chemotherapy induces stress signals that promote METTL3 and METTL14 translation that in turn promote chemosurvival.

Poster Number 30

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Health Outcomes and Quality of Life in a Multi-institutional Prospective Phase II Trial of Proton Radiotherapy for Pediatric Rhabdomyosarcoma

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Background: It is fundamental to improve pediatric rhabdomyosarcoma survivor's quality of life (QoL). We report its health outcomes in a prospective phase II trial of proton radiotherapy (PRT).

Methods: Patients <25 years-old with rhabdomyosarcoma treated with PRT and standard chemotherapy were enrolled between 02/2005 and 11/2019 at two institutions. CTCAEv3.0 graded toxicity. PedsQLv4.0 QoL inventory was offered during treatment and annually thereafter and evaluated by clinical variables.

Results: 103 patients with embryonal/FOX01- (80.6%, n=83), alveolar/FOX01+ (19.4%, n=20), low- (23.3%, n=24), intermediate- (72.8%, n=75), high-risk (3.9%, n=4) rhabdomyosarcoma, in Intergroup Rhabdomyosarcoma Study group I/II (n=12, 11.7%), III (n=86, 83.5%), and IV (n=5, 4.9%), had a median: 6 years (0.1-14.4) follow-up, age 4.2 years (0.6-24.6) at PRT, 50.4 GyRBE (36.0-59.4 GyRBE) total dose. Significant predictors for local failure were tumor size >5cm (HR 2.6, p=0.04), and age <2 or >10 at PRT (HR 2.6, p=0.05). 5-year EFS, OS, and LC were 66.6%, 76.3%, and 80.9%, respectively; 22 (21.4%) died, all from tumor progression. Excluding recurrences (n=30), late toxicity (>90 days post-PRT) assessments were available for 83.6% (n=61) of patients, with maximum grade 2 (50.8%, n=31), 3 (23.0%, n=14), without 4. 35.9% (n=37) had baseline and follow-up PedsQL data. Mean total mean core (64.7), physical (61.9), and psychosocial (66.6) summary scores increasing over time to 77.6, 83.8, and 74.3 (p=0.003, 0.0001, and 0.06), respectively.
Conclusions: Our data suggest that PRT was an effective treatment that led to excellent outcomes in children with rhabdomyosarcoma, with limited associated toxicity and QoL increase over time.

Poster Number 31

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The RNA binding protein FXR1 regulates chemosurvival via ribosome modifications

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The FXR1 (Fragile-X-Mental-Retardation-syndrome-Related protein 1) gene encodes for an RNA-binding protein that regulates RNA stability and translation. FXR1 chromosomal location is often amplified in several cancers and is correlated with poor survival outcomes. Quiescent (G0) leukemic cells are a transient non-proliferative subpopulation that are chemoresistant. Under these conditions, we found that FXR1 levels increase. Importantly, we find that depletion of FXR1 increases the susceptibility of leukemic cells to chemotherapy, implicating FXR1 in modulating chemosurvival. We find that FXR1 is required for ribosome levels. Depletion of FXR1 in leukemic cells leads to reduced levels of rRNAs, snoRNAs that are responsible for rRNA processing and several ribosomal proteins. Consistent, with these findings, global translation decreases along with 40S and 60S ribosome subunits. Conversely, FXR1 overexpression leads to increased levels of ribosome components, establishing the role of FXR1 in global translation regulation. Mechanistically, we find that FXR1 regulates the activity of key proteins involved in rRNA transcription and processing, including DDX21, PolR1D and NOLC1. We find that FXR1 overexpression leads to snoRNA directed modification changes on rRNA (2′-O-methylation of ribose). Such ribosomal modifications can promote non-canonical translation. Consistently, we find that FXR1 overexpression results in non-canonical translation. This allows translation of specific mRNAs that are important for chemosurvival. Together, our data suggest that the increased FXR1 in G0 and several cancers, facilitates ribosomal changes, and the consequent synthesis of pro-survival proteins promotes chemosurvival.

Poster Number 32

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DNASE2-mediated degradation in homeostatic control of chemotherapy-induced cytosolic DNA load

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Chemotherapy causes DNA damage and disrupts many cellular processes. High therapeutic doses can kill cancer cells, and yet we have observed that survivor cells post drug treatment display an altered phenotype of elevated pro-inflammatory genes and increased cytosolic DNA accumulation that distinguish them from normal cells. At low doses with limited cytotoxicity, acute treatment of human cancer cells A549 and HeLa using cytarabine (or ara-C, a nucleoside analog) or doxorubicin (a topoisomerase II inhibitor), showed DNA damage as marked by □-H2AX expression using flow cytometry and immunofluorescence. We also observed an increased cytosolic DNA load that triggered the DNA sensing STING pathway and upregulated type I interferon genes, in a dose-dependent manner. Interestingly, there was a concomitant increase in DNASE2A expression as examined by immunoblot and DNASE2A enzymatic activity by a plasmid nicking assay. DNASE2A is the major acidic lysosomal endonuclease that preferentially degrades double-stranded DNA, and we have previously shown its key homeostatic role in clearing damaged nuclear DNA from the cytosol via autophagic transport and thereby avoiding inflammation. We hypothesize that by inhibiting the activity of DNASE2A or intervening in the autophagic transport of intrinsic DNA load in cancer cells, we can achieve optimal innate immune responses at reduced chemotherapeutic doses to benefit tumor therapy.

Poster Number 33

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Tumor-agnostic microfluidic isolation of circulating tumor cells from leukapheresis products

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Circulating tumor cell (CTC)-based liquid biopsies provide unique opportunities for cancer diagnostics, treatment selection, and response monitoring, but even with advanced microfluidic technologies for rare cell detection, the very low number of CTCs in standard 10 mL peripheral blood samples limits their clinical utility. Clinical leukapheresis can concentrate mono-nuclear cells from almost the entire blood volume, but such large numbers and concentrations of cells are incompatible with current rare cell enrichment technologies. Here, we describe an ultra-high throughput microfluidic chip, LPCTC-iChip, that rapidly sorts through an entire leukapheresis product of over 6 billion nucleated cells, increasing CTC isolation capacity by two orders of magnitude (86% recovery with 105 enrichment). Using soft iron-filled channels to act as magnetic micro-lenses, we intensify the field gradient within sorting channels. Increasing magnetic fields applied to inertially-focused streams of cells effectively deplete massive numbers of magnetically labeled leukocytes within microfluidic channels. The negative depletion of antibody-tagged leukocytes enables isolation of potentially viable CTCs without bias for expression of specific tumor epitopes, making this platform applicable to all solid tumors. Thus, the initial enrichment by routine leukapheresis of mononuclear cells from very large blood volumes, followed by rapid flow, high gradient magnetic sorting of untagged CTCs, provides a technology for noninvasive isolation of cancer cells in sufficient numbers for multiple clinical and experimental applications.

Poster Number 34

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The Impact of COVID-19 Pandemic on International Practice Patterns in Breast Radiation Oncology: A Case-Based Survey Study from 54 Countries

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Introduction: The COVID-19 pandemic presented challenges in resource allocation and breast cancer (BC) treatment decisions. Our study aims to understand changes in international practice patterns of radiation oncologists (RO) treating BC during the COVID-19 pandemic.

Methods: IRB-approved 58-question survey containing 6 clinical scenarios was distributed through professional radiation oncology societies electronically between July 17-November 8 2020. The 6 cases included: 1) Low-grade ductal carcinoma in situ (DCIS), 2) Low-risk BC treated with lumpectomy, 3) Low-risk BC treated with mastectomy with reconstruction 4) BC treated with neoadjuvant chemotherapy and mastectomy with reconstruction 5) BC treated with mastectomy and adjuvant chemotherapy but without reconstruction 6) Metastatic BC with enlarging breast mass. All 6 cases surveyed specific treatment recommendations pre- and during the height of the COVID-pandemic.

Results: A total of 1,103 breast RO across 54 countries completed the survey, with majority (52%) of respondents from United States(n=285), Japan(n=117), Italy(n=63), Canada(n=58), and Brazil(n=56). 70% of RO reported no change in their treatment recommendation during the pandemic, with the exception of the palliative case, where 57% changed their recommendation (Yes=636; No=467, $p<0.0005$) and low-grade DCIS, with a relatively equal split (Yes=528; No=575, $p=0.157$). Treatment changes during the pandemic included omitting, delaying, or abbreviating radiotherapy treatment regimens. Compared to the US, United Kingdom RO were more likely to use ultrahypofractionated radiotherapy for low-risk BC during COVID-19 (90% v. 3%, $p<0.005$).

Conclusion: This is the first international survey of breast radiation oncologists during the COVID-19 pandemic, demonstrating differences in international healthcare delivery systems and resource allocations.

Poster Number 35

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Targeting fatty acid metabolism in Glioblastoma

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We recently demonstrated that Glioblastoma (GBM) cancer stem cells (GSCs) depend on adaptive activation of de novo fatty acid synthesis under endoplasmic reticulum (ER) stress. Stearoyl CoA Desaturase 1 (SCD1) is a key enzyme responsible for the conversion of saturated fatty acids (FA) to unsaturated FA, and is essential for regulating ER homeostasis in GSCs.

Pharmacological disruption of SCD activity is toxic due to the accumulation of saturated FA and the activation of cell death signaling mediated by the ER sensor inositol-requiring enzyme 1 (IRE1). This also results in mRNA decay of key DNA damage repair genes and impairs the ability of GSCs to repair radiation- or chemotherapy-induced DNA damage. Therefore, the combination of SCD inhibition with the alkylating agent temozolomide (TMZ) is highly effective in GBM.

Pharmacological inhibition of SCD delivered via the nasal route in mice had a remarkable therapeutic effect in patient-derived orthotopic GSCs mouse models. However, the modest brain permeability of the currently available SCD inhibitors precludes their clinical translation. To overcome this challenge, we have recently acquired a first-in-class, clinically relevant SCD inhibitor. This compound underwent extensive pharmacokinetic and pharmacodynamic studies which confirmed brain permeability, efficacy, and safety in small animals and non-human primates. We show that both the SCD inhibitor alone and, in particular, the combination of this SCD inhibitor with TMZ are effective in preclinical orthotopic GSCs mouse models with different aggressiveness and molecular signature. Our results support the clinical investigation of this new class of SCD inhibitors in patients diagnosed with GBM.

Poster Number 36

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Single EV Imaging for Early Cancer Detection

INVESTIGATORS: S. W. Ferguson, R. Weissleder

Tumor-derived extracellular vesicles (EVs) represent promising biomarkers for monitoring cancers. Recently, we developed a mathematical model to estimate key parameter values and future requirements for EV testing and predicted that emerging single EV methods would allow blood-based detection of cancers smaller than 1 mm3 in humans. This breakthrough in sensitivity positions EVs as an ultra early and significantly more abundant biomarker than other blood-based 'liquid biopsy' strategies such as cell-free DNA. For several cancers, such as pancreatic adenocarcinoma, the majority of patients are diagnosed at late stages. For these advanced tumors, surgical resection, and thus the possibility of curative intervention, is diminishingly small-- with 5-year survival rates <5%. Conversely, when detected early, in situ carcinoma has a 5-year survival rate of 84%. Building upon previous work, here we show a robust and facile methodology for the imaging detection of highly-specific pancreatic cancer markers on single EVs using antibody detection. This is accomplished by a novel total-EV labeling approach and commercially available purification strategies that significantly reduce background noise. The entire process uses ~10ul of serum and is imaging ready in ~5 hours. In clinical samples we are able to detect EVs harboring mutant KRAS as well as Mucin1 in 11 out of 11 patient samples. This represents a significant improvement over previous gold-standard bead-based bulk EV assays that only stratified 6 of the 11 clinical samples from healthy subjects using Mucin1 as a biomarker. Additional opportunities to detect co -localized cancer marker 'signatures' exist to further increase specificity in diagnoses.

Poster Number 37

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Ectopic expression of the Meiotic Protein SYCP2 in Breast Cancer Promotes Homologous Recombination and Endows Broad Resistance to DNA Repair-Targeted Drugs

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Drugs targeting the DNA damage response (DDR) pathway are widely used in cancer therapy, but resistance to these drugs remains a major clinical challenge. Here, we show that SYCP2, a component of the meiotic synaptonemal complex, is ectopically expressed in breast cancer and associates with broad resistance to DDR-targeted drugs. SYCP2 overexpression correlates with poor prognosis in breast cancer patients and with decreased survival in a clinical trial of antibody-conjugated topoisomerase I inhibitor. SYCP2 overexpression is sufficient to enhance homologous recombination (HR) and confer drug resistance. Mechanistically, SYCP2 promotes RAD51 localization to DNA breaks by interacting with RAD51 through a BRC-like domain. SYCP2 promotes HR independently of BRCA1, suggesting that SYCP2 is a BRCA-independent determinant of DNA damage sensitivity. Thus, SYCP2, which enhances HR, is potentially a biomarker for breast cancer diagnosis, a predictor of drug response, a prognostic marker for patient outcome, and a target in breast cancer therapy.

Poster Number 38

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Do Cohabitants Reliably Complete Questionnaires for Patients in a Terminal Cancer Stage when Assessing Quality of Life, Pain, Depression, and Anxiety?

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Background: Patients with bone metastases are often unable or unwilling to complete quality of life (QoL) questionnaires and cohabitants could play a role as a reliable alternative. However, the extent of reliability in this complicated patient population remains undefined and the influence of the cohabitant's own condition on their assessment of patient QoL is unknown.

Question: This study examined (1) whether QoL scores, measured by EuroQol-5D 5-level (EQ5-5L) and the Patient-Reported Outcomes Measurement Information System (PROMIS) in three domains (anxiety, pain interference, and depression), reported by patients differ markedly from scores as assessed by their cohabitants and (2) assessed whether the cohabitants' PROMIS-depression scores correlate with differences in measured QoL results.

Methods: The study included 47 pairs of patients with bone metastases and cohabitants who independently completed both questionnaires, with respect to the patients' symptoms. The cohabitants also completed the PROMIS-Depression survey, with respect to their own symptoms.

Results: No clinically important differences were found between scores of patients and their cohabitants for all questionnaires, and the agreement between patient and cohabitant scores were moderate to strong (Spearman correlation range 0.52-0.72). However, despite the good agreement in QoL, the cohabitants' own depression scores were correlated with increased differences in the anxiety and depression domains, p=.014 and p=<.001 respectively.

Conclusion: The present findings support the viability of using cohabitants as reliable raters for the QoL of patients with bone metastases. However, the cohabitants' condition may influence their judgement in assessing emotional domains such as anxiety and depression.

Poster Number 39

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Thymic Stromal Lymphopoietin (TSLP) Suppresses Tumor Development in the Lung

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Lung cancer remains the leading cause of cancer deaths in the United States as well as worldwide. Recurrence rates for patients diagnosed with lung cancer remain high. Immunotherapies for lung cancer patients have only been approved for late and advanced stages. The current immunotherapies rely on the cytotoxic potential of tumor infiltrating cytotoxic T lymphocytes for their effects. However, there is great potential in harnessing the immune system in the early stages of lung cancer development to reduce recurrence rates of these malignancies. Using a mouse model of lung adenocarcinoma carrying a KrasG12D mutation, we investigated the effects of thymic stromal lymphopoietin (TSLP) cytokine overexpression on early lung cancer development. TSLP is an epithelium-derived alarmin and its overexpression has previously been shown to suppress skin and breast cancer development. Herein, we demonstrate that TSLP induction leads to suppression of lung cancer development through differentiation of the tumor cells, rather than cytotoxicity. Our findings demonstrate that immunotherapy against the early stages of lung cancer development prior to exhaustion of immune cells can trigger a long-lasting immunity that may contribute to decreasing recurrence rates.

Poster Number 40

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Placental growth factor promotes tumor desmoplasia and treatment-resistance in intrahepatic cholangiocarcinoma

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Objective: Intrahepatic cholangiocarcinoma (ICC)—a rare liver malignancy with limited therapeutic options—is characterized by aggressive progression, desmoplasia and vascular abnormalities. The aim of this study was to determine the role of placental growth factor (PlGF) in ICC progression.

Design: We evaluated the expression of PlGF and Neuropilin (Nrp)-1 in specimens from ICC patients and assessed the therapeutic effect of genetic or pharmacologic inhibition of PlGF in orthotopically-grafted ICC mouse models. We evaluated the impact of PlGF stimulation or blockade in ICC cells and cancer-associated fibroblasts (CAFs) using in vitro 3-D co-culture systems.

Results: PlGF levels were elevated in human ICC stromal cells and circulating blood plasma, and were associated with disease progression. Single cell RNA sequencing showed that the major impact of PlGF blockade in mice was enrichment of quiescent CAFs, characterized by high gene transcription levels related to the Akt pathway, glycolysis and hypoxia signaling. PlGF blockade suppressed Akt phosphorylation and myofibroblast activation in ICC-derived CAFs. PlGF blockade also reduced desmoplasia and tissue stiffness, which resulted in re-opening of collapsed tumor vessels and improved blood perfusion, while reducing ICC cell invasion. Moreover, PlGF blockade enhanced the efficacy of standard chemotherapy in mice bearing ICC. Conclusion: PlGF blockade leads to a reduction in intratumoral hypoxia and metastatic dissemination, enhanced chemotherapy sensitivity and increased survival in mice bearing aggressive ICC.

Poster Number 41

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Proton Radiation Therapy for Pediatric Chordomas

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Chordomas are rare, malignant tumors that arise from ectopic remnants of the embryonic notochord. Chordomas have an incidence of approximately 1 in 1 million with approximately 325 new cases in the United States every year. Pediatric chordomas are very rare and comprise less than 10% of all chordoma cases. Treatment for chordomas generally involves high-dose proton radiation therapy (PRT) with or without surgical resection. At the MGH Proton Center, between 1980 and 2019, more than 1000 patients with skull base chordomas were treated with PRT. This resource, to our knowledge, exceeds the largest published studies of this disease by nearly an order of magnitude and has enabled novel clinical insights into the disease. In this study, we report outcomes for 194 pediatric patients with skull base chordomas treated with protons. Median prescription dose was 77.4 Gy (RBE) (range, 59.3 – 83.3). At a median follow-up of 8.7 years (range, 0.6 – 35.6) from the date of diagnosis 55 patients recurred (36 local, 13 distant and 6 iatrogenic). The 5- and 10-year overall survival (OS) rates were 83% and 77%, respectively. The 5- and 10-year progression-free survival (PFS) rates were 75% and 71%, respectively. On multivariate analysis, chondroid and cellular pathological subtype, the administration of chemotherapy prior to RT and the lower clivus location of the tumor were prognostic factors for OS and PFS. In conclusion, high rate of disease control with minimal toxicity can be achieved with a combined-modality strategy integrating maximal safe resection and high dose PRT.

Poster Number 42

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Combining Regorafenib and PD1 blockade increases CD8 T-cell infiltration by inducing CXCL10 expression in hepatocellular carcinoma

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Background and purpose: Combination therapy of inhibiting vascular endothelial growth factor (VEGF) and PD1 pathway has shown efficacy in multiple cancers, but the mechanism is still unknown. We examined the efficacy and the mechanism of combining regorafenib (a multikinase anti-VEGFR inhibitor) with PD1 blockade in orthotopic mouse model of hepatocellular carcinoma (HCC).

Methods: We investigated the effects of regorafenib (5, 10 or 20mg/kg daily) combined with anti-PD1 antibodies (10mg/kg intraperitoneally thrice weekly) in orthotopic HCC mouse model with liver damage. We checked tumor vasculature and immune microenvironment using immunofluorescence, flow cytometry, RNA-sequencing, ELISA and pharmacokinetics/pharmacodynamics analyses in mice and blood samples from patients with cancer.

Results: Combining regorafenib and anti-PD1 antibodies increased survival in a regorafenib dose-dependent manner. Combination therapy improved regorafenib delivery inside the tumors by normalizing tumor vasculature and increased CD8 T-cell infiltration and activation at an intermediate dose of regorafenib. We tested the effect of combination therapy in mice without functional T-cells (Rag1-deficient mice) and found no therapeutic effect in this model. Regorafenib increased the transcriptional level and protein expression of CXCL10 in both murine HCC and HCC patients' blood. Since CXCR3 is a receptor for CXCL10 and is expressed on tumor-infiltrating lymphocytes, we used Cxcr3-deficient mice and revealed that CXCR3 induced CD8 T-cell infiltration into tumor and prolonged survival after combination therapy.
Conclusions: Combination therapy of regorafenib and anti-PD1 antibodies can increase the expression of CXCL10, which improves both survival and tumor infiltration of activated CXCR3+CD8 T-cells by normalizing tumor vasculature.

Conclusions: Combination therapy of regorafenib and anti-PD1 antibodies can increase the expression of CXCL10, which improves both survival and tumor infiltration of activated CXCR3+CD8 T-cells by normalizing tumor vasculature.

Poster Number 43

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Characterization of murine breast tumor lymph node metastasis at single-cell resolution reveals cancer cell heterogeneity

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Advances in cancer therapy have significantly improved patient outcomes. However, treating metastatic disease remains a major challenge. The sentinel lymph node is often the first site of metastasis and is associated with worse prognosis across most solid tumors. Using a mouse model of breast tumor that develops spontaneous lymph node metastasis, we performed single-cell RNA sequencing of the primary tumor and tumor-draining lymph node (TDLN) to measure how cancer cells adapt to the lymph node microenvironment. Our study identified a heterogenous of epithelial and mesenchymal phenotypes of cancer cells in the TDLN, whereas the cancer cells in the primary tumor maintained an epithelial-mesenchymal hybrid phenotype. The mesenchymal-like cancer cells were predominantly located at the margin of the metastatic lymph node lesions and had increased expression of genes for chemokines involved in myeloid cell activation and migration. In contrast, the epithelial-like cancer cells resided in the center of the metastatic lymph node lesions, expressed high levels of genes encoding MHC class II. Our data show that IFN-? induced JAK/STAT signaling is essential for the elevation of MHC class II in epithelial-like cancer cells. Interestingly, the co-stimulatory molecules, such as CD80, CD86, Icosl, and CD40, were absent in the epithelial-like cancer cells, which may lead to CD4+ T cell tolerance. Our data suggest that the epithelial-like breast cancer cell phenotype induced by the lymph node microenvironment may prevent immune attack against lymph node metastases. These data provide the basis for new opportunities to therapeutically stimulate anti-cancer responses against lymph node metastasis.

Poster Number 44

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Comprehensive immune checkpoint and homologous repair deficiency (HRD) profiles of endometrioid and serous endometrial carcinomas.

INVESTIGATORS: V. Mazina, W. B. Growdon, S. Johnstone, S. Hill, B. Rueda

Endometrial cancer mortality has increased over the past three decades despite advances in surgical technology, genomic profiling, and the advent of immunotherapy. Therapies for recurrent disease have limited durability; while promising anti-tumor activity has been observed utilizing immune checkpoint blockade in recurrent endometrial cancer, over 50% of women do not respond despite harboring the molecular signatures associated with response. Improved response to immune checkpoint blockade has been observed in ovarian tumors when poly-ADP-ribose polymerase (PARP) inhibitors have been used simultaneously suggesting synergistic activity. The degree of homologous repair deficiency (HRD) has only been characterized on a limited basis in endometrial cancer but much pre-clinical data has demonstrated that genomic events, such as PIK3CA mutation and loss of PTEN create genomic instability mimicking HRD, and therefore, we believe that combination therapy with PARP inhibitors and immune checkpoint blockade is a promising future therapeutic strategy. We hypothesized that a subset of endometrial tumors harbors a significant degree of HRD that associates with expression of immune checkpoint proteins. We performed IHC staining for immune checkpoint protein PD-1 and its ligand PDL-1 on a cohort of 50 recurrent and high grade endometrioid and serous endometrial carcinomas, and associated PD-1/PDL-1 expression with clinical outcomes and present these results here. The next step will be to utilize an FDA approved platform to the degree of HRD in these tumors; these data will provide preclinical rationale for clinical trials investigating the role of dual immune checkpoint and PARP inhibition in women with recurrent endometrial cancer.

Poster Number 45

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Interplay between checkpoint molecule B7-H3 and human leucocyte antigen (HLA) class I expression: relevance to the clinical course of pancreatic ductal adenocarcinoma (PDAC)

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Background: HLA class I expression defects may provide malignant cells with an immune escape mechanism and have been associated with poor prognosis in various cancers. However, this association is not universal across studies. Whether this discrepancy reflects the modulation by the checkpoint molecule B7-H3 of the role of HLA class I in PDAC is unknown.

Methods: Resected PDACs (1998-2011) were immunohistochemically analyzed for HLA-A, HLA-B/C and B7-H3 expression, and immune cell infiltration. Gene correlation was analyzed using public databases.

Results: Of the 130 PDACs, HLA-A and HLA-B/C expression was defective in 75% and 59%, respectively. HLA class I and B7-H3 expression were positively correlated at both the protein ($p=0.006$) and mRNA ($p<0.001$) levels, possibly because of the shared transcriptional regulator RFX5. High B7-H3 expression (HR=2.1;$p=0.011$) and low CD8+ cell density (HR=2.1;$p=0.008$) were predictors of poor overall survival (OS), but HLA class I was not, despite its known role in tumor cell elimination by cognate T-cells. Therefore, we investigated whether its role was influenced by B7-H3, which inhibits cytotoxic T-cells. Indeed, defective HLA-A ($p=0.027$) and HLA-B/C ($p=0.004$) expression correlated with poor OS only among patients with low B7-H3 expression. Conversely, B7-H3 expression correlated with OS only when HLA class I expression was high ($p=0.001$).

Conclusions: Our findings may explain the inconsistent association between HLA class I expression and malignant disease prognosis. The negative impact of B7-H3 expression on PDAC prognosis emphasizes the need to develop B7-H3-blocking antibodies. These reagents may be very effective provided tumors have high HLA class I expression.

Poster Number 46

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Chk1/ATR as a Therapeutic Target in Kit-driven Melanoma

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Acral and mucosal melanomas (AMM's) arise from sun-protected sites, disproportionately impact darker-skinned individuals and exact a higher mortality than the more common types of cutaneous melanoma (CM). AMM's also exhibit a higher prevalence of amplifications and point mutations of KIT compared to typical CM's. However, therapeutic gains have been difficult as neither molecular nor immune therapies appear to be particularly efficacious against KIT-altered tumors. There is a crucial need for a robust pre-clinical system that mimics human KIT-melanomas and that allows for research into both the biogenesis of AMM's and potentially new treatment paradigms. We used mouse KIT p.K641E mutation, the counterpart of the most common KIT mutation p.K642E in human, to create a multi-stage murine cellular model of human KIT melanomas that recapitulates transformation and tumorigenesis (i.e. mKitK641E lines). Compared to its vector-controlled cells (mVec), mKitK641E cells proliferated more rapidly, exhibited greater chromosomal aberrations, and sustained 3D spheroid forming capability and aggressive tumor growth in C57BL/6J mice. We validated the functional dependence of these cells on KitK641E with both genetic and pharmacologic suppression. Gene expression profiling revealed cell cycle, DNA replication/RNA processing and ribosomal biogenesis pathways associated with KitK641E transformation and subsequent metabolic reprogramming with tumorigenesis. Surprisingly, we found a selective vulnerability to Chk1/ATR inhibition in the KitK641E activated cells. As supported by the pathway analysis, we subsequently proved that KitK641E induces profound DNA replication stress. These results indicate a novel role of replicative stress in KIT melanomagenesis and implicate possible new therapeutic strategies with Chk1/ATR inhibitors.

Poster Number 47

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CD64-chimeric receptor T cells in combination with B7-H3 monoclonal antibody effectively target B7-H3 expressing cancer cells

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BACKGROUND: In search of a simple, effective, and economical immunotherapeutic strategy for solid tumors, we have developed the following effector mechanism. T cells are transduced with CD64 chimeric receptor (CR), which has a high affinity to monomeric IgGs. B7-H3 was selected as a target since this antigen is expressed by most types of cancer and has a very low expression in normal tissues.

METHODS: CD64-CR contains an intracellular domain derived from CD28 and

CD3ζ, a transmembrane domain derived from CD28, and an extracellular domain derived from human CD64. A Mus musculus leader sequence is also incorporated for efficient expression on the cell membrane. CD64-CR T cells in combination with B7-H3 were cocultured with colorectal cancer cells. CD64-CR T cell cytotoxicity and activation were determined by cell viability assays, CD107a lytic degranulation assays.

Results: CD64 T cells in combination with B7-H3 mAb efficiently eliminated colorectal cancer cells in vitro. Similar results have been obtained with head and neck cancer cells. We also found that CD64-CR enhances the antitumor activity of T cells, even without the presence of mAb. In contrast, CD64-CR T cells showed minimal effects on non-tumor human cells such as fibroblasts and myoblasts.

Conclusion: CD64-CR T cell-based therapy appears to be effective for the elimination of solid tumors. CD64-CR T cells can target multiple antigens on a tumor through combination with different TA-specific mAbs and can be used for the treatment of different types of cancer as they can be combined with mAbs specific for any TA.

Poster Number 48

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A Unique Ability of PARP Inhibitor to Induce Trans-Cell-Cycle DNA Damage Underlies Its Efficacy in BRCA-Deficient Cancer Cells

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The use of inhibitors of PARP (PARPi) for the treatment of BRCA-deficient cancers has significantly improved patient survival over the last decade. While PARPi clearly induces synthetic lethality in BRCA-deficient cells, how PARPi generates lethal DNA damage in the absence of BRCA1/2 functions is still not fully understood. In this study, we asked when PARPi-induced DNA damage is generated during cell division and monitored the effects of PARPi over multiple cell cycles. We found that PARPi does not interfere with DNA replication significantly in the first cell cycle, but cells encountered severe replication problems in the second S phase. We show that this phenomenon results from PARPi-induced ssDNA gaps generated behind replication forks (RFs) in a first S phase. These gaps can persist unto a second S phase under maintained PARPi exposure, leading to RF-gap collision, RF collapse and formation of double strand breaks (DSBs). In contrast to BRCA-proficient cells, BRCA-deficient cells fail to repair collapsed replication forks, and are unable to activate ATR and suppress origin firing in the second S phase. This ultimately leads to continuous DNA synthesis and elevated DSB accumulation in BRCA1-deficient cells. Thus, PARPi selectively kills BRCA-deficient cells by progressively inducing ssDNA gaps and fork-gap collisions over multiple cell cycles, revealing a unique feature of PARPi that distinguishes it from other DNA-damaging drugs. Overall, our study provides a new model that explains the ability of PARPi to induce lethal DNA damage in BRCA-deficient cells, providing a molecular basis for improving PARPi therapy.

Poster Number 49

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Willingness to come back for care: preferences of patients with delayed colonoscopies due to COVID-19

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Many patients have had their screening colonoscopies delayed or cancelled due to the COVID pandemic, creating a sizable backlog of cases. Efforts are underway to reschedule these patients and resume colonoscopies. However, patients' attitudes and concerns about seeking cancer screening tests in this environment are largely unknown. The purpose of this study was to assess patient's anxiety, concerns, and willingness to screen, in support of the Massachusetts General Hospital (MGH) Gastroenterology (GI) department as they prioritize patients on the waitlist.

We identified patients who had their screening or surveillance colonoscopy at MGH delayed or cancelled due to the COVID pandemic, spoke English, and were between the ages of 45-75. A random sample of 200 of these patients were surveyed regarding their anxiety, colon cancer and COVID risk perceptions, cancer worry, willingness to screen, and preference for alternative screening options including stool testing or delaying.

We found that patients have considerable variability in their preferences for rescheduling screening colonoscopies and in their concern about their delayed procedures and returning to clinical sites during the COVID-19 pandemic. We need to use a patient-centered approach to engage patients to consider alternative screening options or postponing their procedures when clinically appropriate, and prioritize colonoscopies based on preferences and risk.

Poster Number 50

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cGAS suppresses genomic instability as a decelerator of replication forks

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The cyclic GMP-AMP synthase (cGAS), a sensor of cytosolic DNA, is critical for the innate immune response. Here, we show that loss of cGAS in untransformed and cancer cells results in uncontrolled DNA replication, hyperproliferation, and genomic instability. While the majority of cGAS is cytoplasmic, a fraction of cGAS associates with chromatin. cGAS interacts with replication fork proteins in a DNA binding-dependent manner, suggesting that cGAS encounters replication forks in DNA. Independent of cGAMP and STING, cGAS slows replication forks by binding to DNA in the nucleus. In the absence of cGAS, replication forks are accelerated, but fork stability is compromised. Consequently, cGAS-deficient cells are exposed to replication stress and become increasingly sensitive to radiation and chemotherapy. Thus, by acting as a decelerator of DNA replication forks, cGAS controls replication dynamics and suppresses replication associated DNA damage, suggesting that cGAS is an attractive target for exploiting the genomic instability of cancer cells.

Poster Number 51

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Unraveling the contribution of ACTL6A, a chromatin remodeling factor, to immune evasion in head and neck squamous cell carcinoma

INVESTIGATORS: S. F. Zhou, L. W. Ellisen

The gene encoding ACTL6A, a subunit of the SWI/SNF complex, is frequently amplified in head and neck squamous cell carcinoma (HNSCC). Our data suggest that elevated ACTL6A in HNSCC deregulates SWI/SNF function and enhances EZH2-mediated transcriptional repression. Anti-PD-1 immune checkpoint inhibitor (ICI) therapy has resulted in remarkable clinical response, but most of HNSCCs develop resistance to ICIs. We hypothesize that amplified ACTL6A promotes EZH2-mediated repression via H3K27me3 at key loci of immune responses genes, resulting in immune cold tumors that are resistant to ICI therapy. Here we show that inhibition of the ACTL6A/EZH2 axis enhances tumor cell antigen presentation and immune cell recruitment and subsequently sensitizes resistant tumors to anti–PD-1 therapy. ACTL6A deletion markedly increased tumor-free survival in the 4NQO autochthonous HNSCC model. In the syngeneic transplantable SCC model, although we observed significant anti-tumor effects of either inducible shRNA knockdown (KD) of endogenous ACTL6A or treatment with the catalytic EZH2 inhibitor (EPZ) or anti-PD-1 alone, combining either shACTL6A or EPZ with anti-PD-1 completely abrogated tumor progression. Correspondingly, we observed a >4-fold increase in the proportion of T cells and CD8+ T cells with the combination with anti-PD-1 and either shACTL6A or EPZ. Importantly, combination treatment of ACTL6A KD with anti-PD-1 contributed to long-term immune memory. Thus, inhibition of the ACTL6A/EZH2 axis activates the endogenous tumor immune microenvironment (TME) and enhance ICI therapy. ACTL6A expression identifies a tumor subset that could be therapeutically targeted with EZH2 inhibitors to modulate the TME and thereby promote synergy with Immune Checkpoint Inhibitors.

Poster Number 52

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Genetic Susceptibility to Stress Associates With Higher Amygdalar Activity and Greater Myocardial Infarction Risk

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Background: Chronic psychological stress is strongly linked to cardiovascular disease (CVD). Metabolic activity of the amygdala, a stress-associated brain center, robustly associates with high inflammation and CVD risk. We hypothesized that a validated polygenic risk score for neuroticism (nPRS), a broad trait measure of vulnerability to stress, independently associates with 1) heightened amygdalar activity (AmygA) and 2) increased myocardial infarction (MI) risk.

Methods: Individuals (N=14349; median age 64 yrs, 46%male) were identified from the Partners Biobank where genome wide nPRS and principle components of ancestry (PCI) were calculated. Using validated 18FDG PET/CT imaging methods, AmygA was measured (N=995) as a target-to-background-ratio in individuals with clinical PET/CT imaging. MI was adjudicated using International Classification of Diseases (ICD) diagnoses. CVD risk factors and psychiatric history were obtained using ICD codes and questionnaires. Independent t-tests and linear and logistic regression were employed.

Results: A total of 1708 (12%) individuals experienced MI. nPRS associated with AmygA (standardized β [95% CI]: 0.07 [0.01, 0.13], p=0.02) after adjustment for age, sex, and 10 PCI. It remained significant after additional adjustment for psychiatric history (p=0.02). AmygA also predicted MI in an adjusted model (OR: 1.50, p=0.006). Importantly, after adjustment for age, sex, and 10 PCI, nPRS significantly associated with MI incidence (standardized OR [95% CI]: 1.12 [1.05, 1.17], p<0.001) and remained significant after additional adjustment for CVD factors (p=0.006) and psychiatric history (p=0.007).

Conclusions: Here we show for the first time that a genetic susceptibility to stress (high nPRS) associates with greater stress-related neurobiological activity, and MI.

Poster Number 53

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Alcohol's beneficial effect on cardiovascular disease is partially mediated through modulation of stress-associated brain activity

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Background: Chronic stress associates with major adverse cardiovascular events (MACE) via neural mechanisms. We hypothesized that moderate alcohol intake decreases stress-related neurobiological (amygdalar metabolic activity, AmygA) and that this neural effect mediates the beneficial impact of alcohol on MACE.

Methods: Data were obtained from a Partners Biobank healthcare survey of 50,559 participants with median age 60 years [IQR 45, 70]. Of these, a subset of 752 had undergone 18F-fluorodeoxyglucose positron emission tomography imaging. Alcohol intake was classified as low (<1 drink/week), moderate (1-14 drinks/week) or high (>14 drinks/week). AmygA was measured as the ratio of amygdalar to pre-frontal cortical activity. MACE was determined using International Classification of Disease (ICD) codes.

Results: Of the 50,559 participants, 6,648 (13.1%) experienced a MACE. Moderate alcohol intake associated with reduced MACE risk after controlling for cardiovascular risk factors (smoking history, hypertension, diabetes, physical activity, BMI, Charlson Index), socio-economic risk factors (employment status and educational attainment) and psychological risk factors (history of depression/anxiety and history of insomnia) in a logistics regression model with an odds ratio [95% confidence interval (CI)]: 0.870 [0.816, 0.928], p<0.0001. In the 752 participant subset, moderate alcohol intake also associated with decreased AmygA (standardized β [95% CI]: -0.088 [-0.031, -0.003], p=0.018) in adjusted analyses. Mediation analysis demonstrated that reduced AmgyA in part mediated alcohol's beneficial effect on MACE, (log odds [95% CI]: -0.035 [-0.088, -0.001], p<0.05).

Conclusion: Moderate alcohol intake attenuates MACE risk in part by lowering stress-related neurobiological activity. New therapies with similar stress reduction but fewer toxicities are needed.

Poster Number 54

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Deep Learning Approach for the Automated Characterization of Cardiac Mechanics

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There is an urgent need for the early recognition and treatment of subclinical cardiac dysfunction which precedes changes in ejection fraction and the development of cardiovascular disease (CVD). Myocardial strain analysis from cinematic magnetic resonance imaging (cine-MRI) data could provide a more thorough characterization of left-ventricular function than ejection fraction (EF), but technical sources of variation including segmentation and motion estimation have limited its wide clinical use. We designed and validated a deep learning (DL) workflow to generate both volumetric parameters and strain measures from cine-MRI data, consisting of segmentation and motion estimation convolutional neural networks developed and trained using healthy and cardiovascular disease (CVD) subjects (n=150). Volumetric parameters included left-ventricular EF, mass, and volume, and strain analysis included both global and regional (i.e., polar maps) assessments. DL-based volumetric parameters were correlated (>0.98) and without significant bias relative to parameters derived from manual segmentations in 50 healthy and CVD subjects. Measures of end-systolic global strain from these cine-MRI data showed no significant biases relative to a tagging-MRI reference method on 15 healthy subjects. Applications in CVD subjects without reduced left-ventricular EF showed both global and asymmetric strain abnormalities at a group-level. At an individual level we showed polar map segments with strain reduction matched infarcted regions, and that strain reduction can be diffused or focal.

In conclusion, we developed and evaluated a DL-based, end-to-end fully-automatic workflow for global and regional myocardial strain analysis to quantitatively characterize cardiac mechanics of healthy and CVD subjects based on ubiquitously acquired cine-MRI data.

Poster Number 55

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Polygenic risk score and deep learning using chest radiographs: Complementary prediction of incident coronary artery disease

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Background: Individual genomic and phenotypic characteristics may improve cardiovascular risk prediction beyond traditional clinical risk factors (TCR) such as age, sex, diabetes mellitus, hypertension, and hyperlipidemia. We assessed the prognostic value of a polygenic risk score (PRS) and a chest radiograph (CXR) based deep learning (DL) risk score to predict incident coronary artery disease (CAD).

Methods: The Mass General Brigham (MGB) Biobank is a prospective cohort of adult patients within the MGB healthcare system. A 6.6M variant CAD PRS using externally derived weights and an externally derived DL model (CXR-Risk) predicting long term mortality from CXR images were calculated for individuals with array-derived genotypes and CXRs. International Classification of Disease code-based incident CAD outcomes were defined as the first event after CXR and assessed by Cox Proportional Hazards.

Results: Of 7,237 patients without known CAD (mean age 63.4 [SD 8.0] years; 3,259 [45.0%] male), 585 (8.1%) developed incident CAD over median follow-up of 4.7 [IQR 2.7-7.2] years. Adjusted for TCR and the first five principal components of ancestry, both CAD PRS (HR 1.19 per SD, 95% CI 1.09-1.31, $p<0.001$) and CXR-Risk (HR 1.27 per SD, 95% CI 1.15-1.39, $p<0.001$) were independently associated with incident CAD. Incorporation of both improved incident CAD discrimination (c-statistic 0.67 vs 0.65, $p<0.01$) beyond TCR alone. CXR-Risk addition significantly improved a model with both TCR and CAD PRS (c-statistic 0.67 vs 0.66, $p = 0.02$).

Conclusion: Advanced genomic (CAD PRS) and phenotypic (DL based CXR-Risk) measures independently predict and complimentarily improve risk prognostication for incident CAD.

Poster Number 56

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Shared Decision Making in Cardiology: How a Decision Aid and a Heart Valve Team Impact Patients with Severe Aortic Stenosis Considering Valve Replacement

INVESTIGATORS: A. Selberg, K. Valentine, F. Marques, S. Elmariah, L. Flannery, N. Langer, K. Sepucha

Clinical guidelines recommend that patients with severe aortic stenosis (AS) with an indication for aortic valve replacement participate in shared decision making (SDM) with a multidisciplinary heart valve team. However, data evaluating the efficacy of SDM and of the HVT are limited. The purpose of this study was to identify how a decision aid impacts patient knowledge, SDM, communication, decisional conflict, treatment preferences and treatment choice in patients with severe AS considering valve replacement, via either surgical (SAVR) or transcatheter (TAVR) intervention.

Eligible patients were randomly assigned to receive either the decision aid (DA) in the intervention arm or to usual care in the control arm. Patients in both arms completed a short survey after their clinic visit to assess measures of knowledge, SDM, decisional conflict, and treatment preferences. English-speaking, intermediate-risk patients with symptomatic severe AS being considered for either TAVR or SAVR were deemed eligible.

We found that the use of the DA in patients with severe AS deciding between TAVR and SAVR increased patient knowledge and improved communication. Patients also reported greater SDM after exposure to both members of the heart valve team and were twice as likely to receive their preferred treatment when the treatment preference was elicited after the patient had seen both Heart Valve Team specialists (cardiologist and cardiac surgeon) as compared to when it was elicited after visiting only one member. These observations highlight the importance of incorporating an SDM approach and of the multidisciplinary Heart Valve Team in the management of severe AS.

Poster Number 57

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An age-specific regulation via CD38 pathway mediates the airway smooth muscle hypercontractility in early life

INVESTIGATORS: Y. Bai, A. Guedes, X. Ai

Early-life environmental exposure could impact lung function by age-specific mechanisms during postnatal lung development. In line with this concept, we found age-specific airway smooth muscle (ASM) hypercontractility by cholinergic overstimulation only in the mouse pups but not adults. In the present study, we further investigated whether and how the age-related contractile regulation was applied to human ASM.

Utilizing human precision-cut lung slices (PCLSs), we verified the methacholine (MCh)-induced ASM hypercontractility exclusively in young children but not teenager or adult group. An increment of ASM mass did not accompany this airway hypercontractility. Microarray analysis of ASM from the murine model of neonatal asthma revealed the regulation potentially via CD38/cADPR pathway. Utilizing primary ASM cells, we demonstrated a higher CD38 expression in young children's cells than adults' cells. Moreover, MCh-treatment further increased the CD38 expression in young children but not adult cells via activating PI3K/Akt. Upregulating CD38 via MCh treatment or plasmid transfection led to the augmentation of Ca2+ responses to constrictive stimuli. In contrast, suppressing the CD38 pathway with CD38 knockout, CD38 siRNA transfection, or pharmacological agents abolished the Ca2+ dependent contractile regulation of mouse or human ASM in early life.

Overall, our results supported an age-specific ASM regulation via the CD38/cADPR pathway in human ASM. This regulation provides a unique pathogenic mechanism of airway hyperresponsiveness in early life. Targeting the CD38 pathway would introduce a novel therapy to reduce the airway obstruction in this time period.

Poster Number 58

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The evolutionarily conserved TLDc domain defines a new class of (H+)V-ATPase interacting proteins

INVESTIGATORS: M. Merkulova, D. Brown

The H+-ATPase (V-ATPase), or proton pump, is found within all cells, where it is responsible for the acidification of intracellular compartments. Furthermore, V-ATPases are expressed at the plasma membrane of proton-secreting cells in several organs and its dysfunction leads to serious diseases. Our lab focuses on kidney intercalated cells (ICs), which maintain systemic acid-base balance by constantly adjusting the amount of V-ATPase at their cell surface in response to blood pH variations. Failure of this important process causes distal renal tubular acidosis (dRTA). However, there are genetic cases of dRTA for which mutations in V-ATPases have not been identified, suggesting that there are other unidentified "dRTA" genes that regulate kidney V-ATPase activity. Despite this, very little is known about any protein-protein interactions that regulate V-ATPase function in the kidney or indeed in other organs. However, we recently identified a novel class of five V-ATPase interacting proteins that are defined by the presence of the highly conserved "TLDc" domain (NCOA7, OXR1, TLDC1, TLDC2, TBC1D24), and are known to play a protective role against oxidative stress in cells. Importantly, NCOA7 knockout mice have sub-functioning ICs, leading to dRTA, similar to mice lacking specific subunits of the V-ATPase itself. This strongly suggests a physiological role for the TLDc protein family in V-ATPase regulation. Therefore, we hypothesize that the TLDc family of proteins have a regulatory role in kidney function and their loss may contribute to dRTA by causing dysregulation of the V-ATPase, leading to an acidification defect.

Poster Number 59

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Identification of Arc Expression in induced Pluripotent Stem Cells (iPSCs)

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Arc (Activity-Regulated Cytoskeleton-Associated Protein; Arg3.1) is an immediate-early gene (IEG) with activity-dependent expression in neurons, where it functions as a master regulator of synaptic plasticity. Arc is rapidly upregulated in response to neuronal activity and it is trafficked from the nucleus to sites of synaptic activity. While dysregulation of Arc and associated networks are implicated in numerous neurological disorders, such as schizophrenia, autism spectrum disorder, and Alzheimer's disease, there have been few studies examining the role of Arc outside of the nervous system. Arc expression has been detected in a number of other tissues outside the brain, and recent studies have revealed several previously unexplored non-neuronal functions of Arc, including regulation of the heat stress response. We examined Arc in induced pluripotent stem cells (iPSCs) and discovered that Arc has high levels of expression in iPSCs. Consistent with our data in human neurons, Arc was localized outside the nucleus. Baseline expression of Arc appears to decline during iPSC differentiation along the neuronal lineage. While our studies show previously unidentified Arc expression in iPSCs, further research is required to elucidate the functional role of Arc in iPSCs.

Poster Number 60

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Cytoskeletal regulation of a transcription factor by DNA mimicry

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A long-established strategy for transcription regulation is the tethering of transcription factors to cellular membranes. In contrast, the principal effectors of Hedgehog developmental pathway, the Gli transcription factors, are regulated by microtubules in the primary cilium and the cytoplasm. How Gli is tethered to microtubules remains unclear. Here we uncover that DNA mimicry by the ciliary kinesin Kif7 underlies the recruitment of Gli to microtubules, revealing a new mode of tethering DNA binding proteins to the cytoskeleton. We find that the Gli-binding domain of Kif7 is a rheostat that regulates the kinesin-microtubule binding in response to Gli concentration. Thus, the kinesin-microtubule system is not a passive Gli tether but a regulatable platform tuned by the kinesin-transcription factor interaction. We exploited the unique DNA-mimicry-based Gli-Kif7 interaction to create a tool that inhibits Gli nuclear localization, revealing a strategy for precisely controlling the localization and inhibiting erroneously activated Gli in human cancers.

Poster Number 61

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tiRNA signaling via stress-regulated vesicle transfer in the hematopoietic niche

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The bone marrow represents a particularly stress responsive tissue as it is the site of production of blood and immune cells. The basis for its stress response has largely focused on classic ligand-receptor pairing. However, this is likely a relatively late evolutionary development. We provide evidence for a more primitive signaling system whereby extracellular vesicles (EV) traffic from bone marrow stromal cells in vivo to myeloid progenitor cells in a stress-regulated manner.

Through the use of GFP mesenchymal reporter models, we show that osteoblastic cells are major producers of EVs labeled with endocytic markers that are taken up mostly by granulocyte-macrophage progenitors (GMPs), a process that is augmented by infectious and genotoxic stress.

Small RNA analysis of EV RNA demonstrated the enrichment of stress induced tRNA fragments (tiRNAs) compared to cellular RNA. EV labeled GMPs (GMPGFP+) demonstrated a 2 fold enrichment in reads mapping to tRNAs with 12 specific tRNAs present at significantly higher levels. GMPGFP+ demonstrated enrichment in protein translation and proliferation functions that were validated using in vivo protein translation and cell cycle analysis.

The transfection of 2 out of 10 synthetic oligos corresponding to the sequence of the top ten tiRNAs that are enriched in GMPGFP+ resulted in increased protein translation, cellular proliferation and enhanced myeloid differentiation of GMPs.

Finally, the increased production of osteoblastic EVs enhanced myeloid recovery post irradiation and improved the survival of C. albicans infected mice. These data support a novel mode of tiRNA mediated signal transfer in the bone marrow niche.

Poster Number 62

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Poster Number 63

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Metabolic reprogramming of the retinal pigment epithelium drives TGFβ2-induced epithelial-mesenchymal transition

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Transforming growth factor-beta 2 (TGFβ2) is a key orchestrator of retinal wound healing through induction of epithelial-mesenchymal-transition (EMT) in retinal pigment epithelial cells (RPE). Here we show a previously unrecognized function of TGFβ2 in modulating mitochondrial morphology and metabolic function in human RPE cells. Treating ARPE-19 (human RPE cell line) with TGFβ2 (10 ng/ml) induced defects in mitochondrial network integrity with increased sphericity and fragmentation. Correspondingly, TGFβ2 reduced expression of genes regulating mitochondrial dynamics, reduced citrate synthase activity and intracellular ATP content. High-resolution respirometry showed that TGFβ2 reduced mitochondrial oxidative phosphorylation (OXPHOS) levels consistent with reduced expression of NDUFB5, a key gene of Complex I of the electron transport chain. The reduced mitochondrial respiration was associated with a compensatory increase in gene expression of glycolytic enzymes (PFKFB3, PKM2, LDHA) and glycolytic reserve. TGFβ2 induced a severe suppression of PGC-1α gene expression. Treatment with the selective small molecule activator of PGC-1α, ZLN005, blocked TGFβ2-induced upregulation of mesenchymal genes (αSMA, Snai1, CTGF, COL1A1), vimentin protein expression and TGFβ2-induced migration using the scratch wound assay. Our data show that EMT is accompanied by mitochondrial dysfunction and a profound metabolic shift towards reduced OXPHOS and increased glycolysis that may be driven by PGC-1α suppression. The PGC-1α promoter, ZLN005, effectively blocks EMT in RPE and thus serves as a novel therapeutic avenue for treatment for subretinal fibrosis.

Poster Number 64

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The differences in epidermal calcium gradients in various skin types

INVESTIGATORS: K. J. Stalnaker, C. Fuchs, R. R. Anderson, J. Tam

Calcium is a critical component of skin physiology as it is a master regulator of keratinocyte differentiation and barrier function in the epidermis. Calcium also is an essential part of wound healing, acting as a signal to begin response to the injury. Our research investigates whether this calcium gradient is the same in the volar skin of the foot. A common clinical problem is the treatment of diabetic foot ulcers. Because volar skin heals differently it presents various challenges particularly in diabetic patients where foot ulcers can become a recurring problem. In our work we examined the calcium profile of skin biopsies from weight bearing volar, non-weight bearing volar, dorsal foot skin, snout, and flank skin of pigs. Our results demonstrate that the calcium gradient in volar skin has a distinctly different profile from that of flank skin. Calcium content in tissue samples was visualized using a calcium-sensitive dye, imaged by confocal microscopy, and quantified by digital image analysis. The calcium profile of normal skin shows a sharp peak in the stratum granulosum which declines in the stratum corneum. Comparatively, volar skin has a flatter profile with a small peak near the end of the stratum corneum. The potential role the differing calcium gradients may have in wound healing and morphological differences are currently being investigated.

Poster Number 65

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Multiplex characterization of cytoplasmic chromatin fragments in senescence

INVESTIGATORS: Y. Tan, Z. Dou

Senescence-associated secretory phenotype (SASP) together with its concomitant chronic inflammation is a major culprit for aging, which justifies the removal of senescent cells for anti-aging purpose.

A turning point in the perception of senescent cells as a therapeutic target for aging was the finding in mice that the targeted removal of senescent cells resulted in increased life span and beneficial effects on health. However, how to precisely target senescent cells in human, which lack unified molecular markers, has remained a challenge.

We have lately discovered that chromatin which is traditionally viewed as wholly nuclear entity is able to move to the cytoplasm upon senescence. These cytoplasmic chromatin fragments (CCF) comprise DNA and heterochromatic histone markers. CCF activate the cytosolic DNA sensor cGAS and trigger the SASP program via the cGAS-STING innate immunity pathway. As a distinctive feature of senescence, targeting CCF will be a precise anti-aging intervention. However, it remains unknown how to intervene against CCF given its undefined components and elusive signaling pathways that mediate its formation.

I reason that identifying CCF composition through multiplex methodologies is the critical priming step to research upstream mechanisms, as well as, developing downstream intervention strategies. I have developed multiple novel approaches to thoroughly unravel the DNA and protein composition of CCF. With all the well-established tools and state-of-the-art technologies, this project holds the promise to decipher the CCF composition, which will lay the foundation of both mechanistic and translational exploration of CCF, whereby novel precision anti-aging therapies may be fermented.

Poster Number 66

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ABHD1/12-mediated TEADs depalmitoylation regulates Hippo pathway transcriptional output

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TEAD domain (TEAD) transcription factors bind with the transcription co-activator YAP/TAZ and regulate the transcriptional output of Hippo pathway, thus controlling organ size and tumorigenesis. Our lab previously identified that TEADs underwent autopalmitoylation, which was required for TEADs' binding to YAP/TAZ and related to transcriptional output of Hippo pathway. Here we showed that endogenous TEADs underwent dynamic autopalmitoylation with rapid turnover rate (half-life was about 2-3 hours). We found that depalmitoylation of TEAD is regulated by alpha/beta hydrolase domain proteins (ABHD1/12). This dynamic process is tightly governed by cell density, ABHD1 and ABHD12. We found that TEADs palmitoylation levels were negatively correlated with cell density and positively correlated with transcriptional activities of YAP/TAZ. Furthermore, ABHD1/12 decreased TEADs autopalmitoylation levels in a cell density dependent manner. Overexpression of ABHD1 or ABHD12 significantly promoted the turnover of TEADs palmitoylation. While silencing of ABHD1 or ABHD12 with shRNAs or treatment with ABHD12 specific inhibitor DO264 significantly increased TEADs autopalmitoylation in concomitant with increased transcriptional activities of YAP/TAZ. Immunoprecipitation and immunofluorescent staining revealed the direct binding of TEADs with ABHD1 and ABHD12. Interestingly, the inhibitory effects of ABHD1 and ABHD12 on TEADs autopalmitoylation suppressed the output of Hippo pathway in YAP-dependent cancer cell lines, such as H226 mesothelioma cell line. Thus, ABHD1/12 are critical for the tight regulation of TEADs autopalmitoylation and transcriptional output of Hippo pathway. Further studies would be of interests and significance to explore the regulatory role of ABHD1/12-TEADs axis ex vivo and in vivo.

Poster Number 67

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FMRP Facilitates the Demethylation of Damage Induced RNA m5C by TET1 to Promote Transcription-Coupled Repair

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RNA modifications regulate a variety of cellular processes including DNA repair in cancer. The RNA methyltransferase TRDMT1 generates methyl-5-cytosine (m5C) on mRNA at DNA double-strand breaks (DSBs) in transcribed regions, promoting transcription coupled-homologous recombination (TC-HR). Here, we show that Fragile X mental retardation protein (FMRP) interacts with both the m5C writer TRDMT1 and the m5C eraser ten eleven translocation protein 1 (TET1). The m5C writer TRDMT1, reader FMRP, and eraser TET1 are recruited to transcriptionally active sites of DSBs in a sequential manner in cells. FMRP displays a higher affinity for DNA:RNA hybrids containing m5C-modified RNA than hybrids without RNA m5C and facilitates demethylation of m5C by TET1. Importantly, both the chromatin and RNA bindings of FMRP are required for TET1-mediated RNA m5C demethylation. Since unresolved R-loop and m5C prevent completion of DSB repair, loss of FMRP compromises demethylation of damage induced m5C, R-loop resolving, and BRCA-independent TC-HR in cells. Moreover, FMRP low cancer cells are relatively radio-sensitive. Together, our study present a new m5C reader, FMRP, acts as a coordinator for between m5C writer and eraser to promotes DNA repair and cell survival in cancer cells.

Poster Number 68

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Modeling the effects of social stigma on PTSD screening dynamics

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Post-traumatic stress disorder (PTSD) is a mental health disorder characterized by failure to recover after experiencing a traumatic event that is shocking, scary, or dangerous. The effects of PTSD are debilitating and when left untreated, individuals suffering from PTSD may lose their careers, families, or commit suicide. Among populations at elevated risk of developing PTSD, accurate screening is critical to ensuring that proper care and treatment are received. To this end, much research aimed to identify an optimal screening threshold for PTSD. Yet, few have considered the role that stigma plays in screening decisions. PTSD screening heavily relies on self-reported measures, and stigma may cause individuals to under-report these measures. Hence, understanding the intricate dynamics between stigma and screening is critical to establishing optimal screening protocols. In this research, we formulate a mathematical model that captures the long-term dynamics between screening and stigma. This model considers how missed diagnoses or increased treatment affect stigma, and how stigma may affect screening efficacy in future cohorts. Using simulation, we find that thresholds designed to minimize short-term societal costs may inadvertently increase stigma and cause future screenings to be less accurate – thereby incurring greater long-term costs. Conversely, screening policies which aim to reduce the long-term screening costs incur greater short-term costs, but reduce stigma in the long-term, resulting in more accurate future screenings. Our findings highlight the importance of accounting for stigma in PTSD screening and understanding the impact of screening decisions in the long-term.

Poster Number 69

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Reconstruction of metagenome-scale species-resolved models of the gut microbiota metabolism in Inflammatory Bowel Disease

INVESTIGATORS: I. M. Goodchild-Michelman, A. R. Zomorrodi, A. Colarusso

Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition of the intestinal tract that affects over three million Americans each year. Numerous studies have associated microbial species and microbially-derived metabolites in the gut and IBD. Nevertheless, the exact microbial mechanisms of IBD pathogenesis are still unknown as association studies do not necessarily point to underlying causal mechanisms. To better understand the functional role of microbial species in IBD pathogenesis, we aimed to reconstruct genome-scale models (GEMs) of metabolism for bacterial species in the gut microbiota of IBD and non-IBD subjects. To this end, we used taxonomic profiling data obtained from amplicon or metagenomic sequencing of fecal samples collected during the Human Microbiome Project from IBD and non-IBD subjects and reconstructed GEMs for all microbial species in these samples. We then integrated GEMs of species present in each sample into a community model representative of the fecal microbiota in that sample. These community GEMs are being used to computationally simulate the metabolic activity of individual microbial species and inter-species metabolic interactions in IBD and non-IBD subjects. By comparing the predicted species-level metabolite secretion and uptake fluxes, we will determine how microbiota-derived metabolites and inter-species metabolite exchanges are different between the IBD and non-IBD models. These analyses will enable us to trace back microbial species that are responsible for the production of metabolites implicated in IBD pathogenesis. Overall, our studies will provide unprecedented insights into species and metabolite-level biomarkers of IBD, which can guide future experimental studies for IBD diagnosis or treatment.

Poster Number 70

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Single-cell RNA sequencing analysis of fibroblast transcriptomes from pancreatic ductal adenocarcinoma samples treated with neoadjuvant FOLFIRINOX-based therapy suggest therapeutic resistance in the cancer-associated fibroblast subtype

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Understanding fibroblasts, the largest cellular component of the ductal adenocarcinoma (PDAC) tumor microenvironment (TME), may illuminate new therapeutic targets. Using single-cell RNA sequencing technology (scRNA-seq), we characterized PDAC fibroblasts in the context of benign pancreatic tissue and FOLFIRINOX-based neoadjuvant therapy (NAT).

scRNA-seq analysis was performed on 156,072 cells from 12 PDAC tumors (six untreated, six post-NAT), four pancreatitis, and three normal pancreas samples. Dimensionality reduction yielded 16 cell clusters, which were annotated for expression of specific cell type markers such as COL1A1 and MMP2 in fibroblasts. Three subtypes emerged from the fibroblast cluster based upon tissue sample origin, which included one subtype restricted to the benign pancreas and another reactive pancreatic fibroblast (RPF) that expanded in chronic inflammation and malignancy. The third subtype, cancer-associated fibroblasts (CAFs), existed only in tumors, however, RPFs were the dominant subtype in 67% of PDAC samples. Transcriptomic analysis of benign pancreatic fibroblasts, RPFs, and CAFs demonstrated progressive upregulation of genes encoding extracellular matrix components (collagens, FN1, POSTN, and CTHRC1), and unbiased trajectory analysis suggested a common lineage between these subtypes. Comparing untreated and post-NAT tumor fibroblasts revealed an upregulation of DNA damage response pathways (p53, ATM, and FOXO mediated transcription pathways) in RPFs and not CAFs, suggesting RPFs are more chemosensitive than CAFs.

From scRNA-seq analysis of pancreatic tissue we show that CAFs are a chemoresistant fibroblast subtype and evolve from a resident fibroblast precursor. Additional characterization of CAF programs within the PDAC TME may identify adjunctive therapeutic targets for patients with CAF-dominant tumors.

Poster Number 71

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A collaborative annotation strategy for AI doctor: The initial step

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Well-organized annotated datasets are the key to training a high-performance artificial intelligence (AI) model for various image classification and detection tasks. Especially in the context of developing supervised deep learning algorithms for medical diagnosis, designing an efficient annotation strategy is essential due to the data-deficiency nature. However, there has been a lack of tools to annotate the medical data and to analyze the annotations efficiently. In this work, we introduce a newly developed web-based tool for collaborative annotation of medical image data and provide the analysis on the chest posteroanterior (PA) dataset as an example. A new annotation strategy is also proposed to realize an optimized annotation process based on the AI performance and annotation time weighted analysis. The proposed approach can improve the performance of deep learning algorithms that rely on a large number of high-cost data, further opening a new horizon for evaluating the value of data.

Poster Number 72

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cDNA-detector: detection and removal of cDNA contamination in DNA sequencing experiments

INVESTIGATORS: M. Qi, U. Nayar, L. Ludwig, J. McDonald, N. Wagle, E. Rheinbay

DNA sequencing experiments are broadly applied in biological research, but DNA contamination from exogenously introduced gene constructs or other sources in the generating laboratory in the form of complementary DNA (cDNA) can be present in such datasets. Sequencing reads from contaminating cDNA can lead to spurious peak calls in read enrichment studies, false genomic variant calls, and even cause incorrect scientific conclusion. Surprisingly, there is very little awareness and computational solutions to this problem in the next-generation sequencing (NGS) analysis community.

We here present a new computational tool implemented in Python, cDNA-detector, to identify and remove exogenous cDNA contamination in DNA sequencing experiments. We demonstrate that cDNA-detector can detect cDNA with high sensitivity and specificity in different types of DNA-sequencing experiments (ATAC-seq, ChIP-seq, whole-exome sequencing). We compare cDNA-detector to an existing tool and show that the current method is much more sensitive, and further identify cDNA contamination occurrences in public datasets.

Poster Number 73

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Computational investigation of eco-evolutionary dynamics in cheater and cooperator populations of S. cerevisiae using genome-scale models of metabolism

INVESTIGATORS: M. R. Rayle, A. R. Zomorrodi

Ecological interactions and metabolite exchanges between microbes shape the dynamics and equilibria of microbial consortia in fundamental ways. It has been recently recognized that ecological dynamics (i.e., changes in population density) may occur on the same time scale as evolutionary dynamics (i.e., changes in genotypes frequencies). This implies that ecological and evolutionary dynamics can interact and affect each other -- a phenomenon called eco-evolutionary feedback. Understanding eco-evolutionary feedback is particularly important for human microbiome studies, as measuring the absolute microbial abundances (population densities) is not yet feasible using existing microbiome-profiling techniques. While theoretical models for studying eco-evolutionary dynamics exist, they are based on abstract models, which do not take into account community-specific omics data. Here, we report on the development of new mechanistic computational models for studying eco-evolutionary dynamics in microbial communities through integrating eco-evolutionary game theory models with GEnome-scale metabolic Models (GEMs). As a proof-of-concept study, we apply these models to a well-characterized experimental system consisting of a wild-type cooperator and a mutant cheater strain of Saccharomyces cerevisiae growing on sucrose as the sole carbon source to infer the eco-evolutionary dynamics and stable equilibrium states of this system. We found that either cheaters or cooperators may dominate the entire community or both may co-exist at equilibrium, depending on the shared nutrient availability in the communal space, cost of cooperation to the wildtype, and population density. Our studies lay the foundation for studying the complex dynamics and non-intuitive equilibrium states of synthetic and human-associated microbial communities at high mechanistic resolution.

Poster Number 74

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External validation of a deep learning algorithm for plus disease classification on a multinational Retinopathy of Prematurity dataset

INVESTIGATORS: J. Kalpathy-Cramer, Imaging and Informatics in Retinopathy of Prematurity (https://i-rop.github.io/)

Deep learning (DL) algorithms have shown to perform well for classifying plus disease in Retinopathy of Prematurity (ROP). However it is common for DL algorithms to have reduced performance on external datasets compared to the datasets that they were originally trained on. In this study, we demonstrate the efficacy of a DL algorithm, trained on a North American (iROP) population, on two external multinational datasets.

Retcam fundus images were obtained from India and Thailand through databases hosted by partner institutions, Aravind Eye Hospital (AEH) and Khon Kaen University (KKU) respectively. Both the Indian and Thai datasets were additionally labelled by 2-3 North American experts and gold standards were obtained through mutual consensus among all raters for each dataset. The performance of iROP-DL model, previously trained on Retcam images from iROP population, was evaluated on both the external datasets after screening out all the non posterior-pole (PP) images.

Compared to the original training and testing iROP population, applying DL algorithms on the two external datasets is prone to several challenges, owing to demographic or phenotypic differences (samples with considerable pigmentation), or differences in acquisition methodology (multiple views of the retina, anterior segment photos, etc). Here we show low performance before PP-filtering (AUC's 0.88 & 0.78 for India and Thai respectively), which improved considerably after PP-filtering (AUC's 0.89 & 0.84) and later by using consensus labels (AUC's 0.97 & 0.95). UMAP visualization further substantiates our point and highlights segregation of external datasets from the iROP dataset owing to the remaining ethnic/phenotypic differences.

Poster Number 75

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MIAAIM: multi-modal image alignment and analysis by information manifolds

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High-dimensional technologies that generate very large, layered datasets are becoming increasingly widespread and informative in biology and medicine. The adequate integration and analysis of large datasets across multiple imaging modalities remains challenging. Here, we present a new computational method designed to address multi-modal integration and analysis of two such high-dimensional imaging methods, imaging mass cytometry (IMC) and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry imaging (MSI), in combination with classical histopathology. Multi-modal image alignment and analysis by information manifolds (MIAAIM) combines the registration and analysis of these imaging modalities by utilizing information theoretic concepts as they pertain to manifold learning and by implementing correlation network analyses to identify single-cell molecular niches distributed across tissue. We highlight the ability of MIAAIM to provide unique insights to single-cell microenvironmental niches in the context of diabetic foot ulcers.

Poster Number 76

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Novel roles of Irf6 in craniofacial morphogenesis revealed by tissue-specific ablation in conditional knockout mouse model

INVESTIGATORS: S. H. Carroll, N. Myers, E. C. Liao

Interferon regulatory factor 6 (IRF6) is a key gene variant associated with syndromic and non-syndromic orofacial cleft. Most studies on Irf6 has focused on its role in the palatal shelf epithelium and periderm during palate fusion, as well as its role in keratinocyte differentiation. However, in addition to a secondary palate cleft and skin dysfunction, the Irf6 knockout mouse has a dysmorphic anterior palate and lip with changes to mesenchymal tissue composition. Therefore, this study aimed to delineate tissue-specific function of Irf6 during craniofacial morphogenesis, particularly to understand Irf6 function in epithelial vs. mesenchymal cell types. Irf6 is expressed in mesenchymal tissue of the frontonasal prominences and palate shelves, as well as in the neural crest. Whereas ubiquitous ablation of Irf6 led to pups with severe epithelial adhesions and dysfunctional skin causing perinatal lethality, pups with neural crest cell-specific ablation of Irf6 using a Wnt1-Cre driver were viable, although significantly smaller and with mild craniofacial dysmorphology, specifically a foreshortened midface and maxillary asymmetry. Tissue-specific ablation of Irf6 has allowed us to study its role in craniofacial development, independent of its role in epithelium and periderm. Additional Cre driver lines will allow fine-tuning of cell and tissue specific Irf6 ablation. Future research utilizing this newly generated Irf6 conditional knockout mouse model will facilitate important inroads into the etiology of Irf6 and craniofacial dysmorphologies.

Poster Number 77

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Plantar skin – an evolutionary coup that came at a cost

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Diabetes is at epidemic proportions and is associated with many co-morbidities, one of the most common and severe of which is diabetic foot ulcers (DFUs), which results in an extensive burden to the US healthcare system and the 5-year mortality rates are higher than many cancers. Since most studies in DFUs focus on diabetes-specific effects, we elected to investigate whether there are intrinsic differences between plantar and non-plantar skin that may contribute to inferior healing capacities in foot skin. We used RNA-Seq to identify unique aspects of the gene expression profile in plantar skin. Calcium ion binding was one of the molecular functions that was essentially different from other skin sites and within the 50 most regulated genes the S100 family was very prominent. We verified our RNA-Seq data at the protein level as some proteins of the S100 family were expressed up to 20-fold higher in plantar versus other skin sites. Moreover, pathways involved in cell adhesion, locomotion and inflammatory response were differentially regulated. Histological analysis revealed tremendous differences in epidermal structures and proteins, calcium concentration profile, as well as keratinocyte morphology. Currently we are investigating the hypothesis that plantar skin has evolved to specialize in weight-bearing by prioritizing vertical growth and tight adhesion of keratinocytes at the cost of the horizontal migration needed for wound closure and healing.

Poster Number 78

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Thymus Regeneration driven by Ccl19-expressing stromal mesenchymal cells

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T cell neogenesis declines sharply after puberty due to thymic atrophy and contributes to poor immune recovery post-injury and to age-related immune decline. We comparatively assessed non-hematopoietic cells comprising thymic stroma in settings of thymic vigor or the dysfunctional states post-irradiation and with advanced age. Among the mesenchymal cells that predominately comprise stroma, three subsets were defined, one of which was particularly deficient in settings of thymic dysfunction. This population distinctively expressed periostin and T cell regulating genes. When isolated and adoptively transferred, the cells durably engrafted atrophic thymus, recruited early T progenitors to the thymus and enhanced new T cell generation. These cells increased peripheral T cells as well as host response to neoantigens. The chemokine, Ccl19, produced by periostin+ thymic stromal cells was necessary for increased T cell neogenesis and was sufficient to convey proregenerative properties when ectopically expressed in other mesenchymal cells. The mesenchymal stroma of the thymus plays critical roles in T neogenesis and may be used to therapeutically augment T cell immunity in settings of thymic dysfunction.

Poster Number 79

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Single-cell RNA sequencing of ovaries reveals transcriptional networks underlying follicular quiescence regulated by Mullerian Inhibiting Substance

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Women are born with a limited number of primordial follicles constituting their ovarian reserve. Primordial follicles activation is an irreversible process leading to ovulation or atresia. Mullerian inhibiting substance (MIS/AMH), produced by granulosa cells of growing follicles, is an important regulator of ovarian reserve maintenance by providing negative feedback to primordial follicle. We hypothesized that MIS inhibits folliculogenesis by imposing granulosa cells quiescence. Postnatal day 1 mice were injected with AAV9-MIS or empty vector control, euthanized at day 6, and ovaries were dissociated to perform single-cell RNA sequencing. We catalogued a cell atlas of the neonatal ovary along with gene expression signatures associated with MIS treatment and confirmed cell-specific signatures by qPCR and in situ (RNAish). We confirmed MIS receptor (MISR2) expression in pregranulosa and granulosa cells in mouse ovaries by RNAish. In these cells, we identified a unique cell-specific signature induced by MIS. By comparing gene expression in quiescent and activated follicles in the control to that of granulosa cells treated by MIS we defined a quiescent gene signature involving important pathways of stemness, immediate-early genes, cytokine signaling. MIS treatment resulted in a preantral follicle maturation defect in which oocyte development occurred without concomitant expansion and differentiation of granulosa cells. This was supported by the modest transcriptional perturbation associated with MIS in oocytes, suggesting the inhibition of folliculogenesis is driven by effects on granulosa cell differentiation.

To conclude, treatment with MIS imposed quiescence to primordial follicles granulosa cells leading to a suppression of ovarian activity independently of oocyte maturation.

Poster Number 80

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Severity of genetic defects predicts pubertal severity in men with Idiopathic Hypogonadotropic Hypogonadism: Clinical implications

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Introduction: Idiopathic Hypogonadotropic Hypogonadism (IHH) is a rare genetic disorder characterized by absent pubertal development due to absent/abnormal GnRH secretion. Over 70 genes have been implicated in its pathogenesis. Currently, it is not known whether severity of genetic defects in IHH genes correlate with severity of pubertal phenotypes. Understanding this relationship may aid inform genetic counseling and clinical care.

Methods: A total of 1459 IHH subjects underwent exome sequencing as part of a genetics study. A subset of 201 men with detailed pubertal phenotypic data were selected for analysis. Inactivating and homozygous mutations in 36 high-confidence IHH genes were characterized as severe while missense mutations were characterized as mild. Testicular volume (TV) < 4ml was deemed severe; TV ≥ 4ml deemed mild. Proportions of IHH subjects with severe or mild pubertal phenotypes harboring severe or mild genetic defects were compared.

Results: A total of 106 subjects had a severe pubertal phenotype, of whom 48% harbored severe variants; 95 subjects had mild pubertal phenotype, of whom 36% harbored severe variants. At a variant minor allele frequency of < 0.001 (0.1%), IHH men with severe variants showed a significant trend towards having severe pubertal phenotypes ($p = 0.063$).

Conclusions: By subclassifying causal variant severity and the degree of phenotypic severity, we demonstrated a significant trend towards correlation between severe variants and smaller TV. Since TV is an important determinant of long-term fertility outcomes in IHH men, patients harboring severe causal mutations may benefit from earlier initiation of fertility therapy to increase TV.

Poster Number 81

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Higher Peptide YY Levels are Associated with Lower Bone Mineral Density in Low Weight Adolescent Girls with Avoidant/Restrictive Food Intake Disorder

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Background: Avoidant/Restrictive Food Intake Disorder (ARFID) is a condition characterized by lack of interest in eating/food, sensory sensitivity, and/or fear of aversive consequences of eating. Little is known regarding associations between appetite regulating hormones and bone mineral density (BMD) in ARFID.

Methods: Among 10 adolescent low-weight females with ARFID and 14 healthy controls (HC), we performed cross-sectional analysis to compare BMD and fasting PYY levels, and regression analyses to determine associations.

Results: ARFID and HC females were 15.1±2.9 (mean±SEM) and 17.1±3.8 years old, respectively (p=0.178), with mean BMI Z-scores of -1.74±0.21 kg/m2 and 0.24±0.18 kg/m2 (p<0.0001). Total body BMD Z-scores were significantly lower in ARFID than in HC (-1.59±1.19 vs. -0.41±1.11, p=0.022), and lumbar BMD Z-scores were numerically lower in ARFID vs. HC (-1.13±1.40 vs. -0.44±0.86, p=0.212). Mean PYY levels trended higher in ARFID (104.6±39.9 pg/ml, n=8) vs. HC (71.4±24.5 pg/ml, n=9) (p=0.054). In combined analysis including all participants PYY was negatively associated with lumbar BMD and BMD Z-scores (r=-0.52 and -0.54, p=0.038 and 0.031, n=16). In multivariable analysis, PYY was a primary determinant of lumbar BMD adjusted for age and height (p=0.035, β= -0.36) and a borderline significant predictor of lumbar BMD Z-scores adjusted for height (p=0.064, β=-0.5). Restricting analysis to the ARFID group, PYY remained a significant predictor of lumbar BMD (p=0.031, β=-0.97) adjusted for age and height.

Conclusion: Female adolescents with low-weight ARFID have lower BMD and trend toward higher PYY levels compared with HC. Higher PYY may contribute to the lower BMD observed in ARFID.

Poster Number 82

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Long-acting Opioid Use is Associated with Opioid-induced Adrenal Insufficiency

INVESTIGATORS: L. Nachtigall MD, B. Biller MD, K. Miller MD

Opioids can cause adrenal insufficiency and disrupt endogenous cortisol diurnal variation. There is limited understanding of risk factors for adrenal insufficiency and the impact of opioids on the validity of morning serum cortisol level for diagnosis of adrenal insufficiency. We performed a retrospective analysis of cosyntropin stimulation tests performed at Mass General Brigham from 5/2015 to 10/2020. Risk factors for adrenal insufficiency were examined in patients receiving opioids (N=122). The validity of a morning cortisol <3 µg/dL for the diagnosis of adrenal insufficiency was investigated in patients with opioid exposure and control patients (N=1363). Patients with adrenal insufficiency were more likely than those without to have been prescribed a long-acting opioid (65.5% vs. 40.8%, p=0.020) but not to have a higher one-year cumulative opioid exposure (20250 vs. 7875 mg, p=0.162), higher daily morphine milligram equivalents (108 vs. 58 mg, p=0.332) or duration of exposure (7 vs 7 months, p=0.615). For opioid-exposed and unexposed hospitalized patients, the positive predictive value (PPV) for adrenal insufficiency of a morning cortisol level < 3 µg/dL was 39.1% (CI 22.2-59.2%) and 45.5% (CI 29.8-62.0%), respectively. For outpatients, the PPV for opioid-unexposed patients was 90.0% (CI 59.6 – 98.2%). Here we show among patients receiving opioids for at least 2 weeks, long-acting opioid preparations are associated with increased risk for adrenal insufficiency. For the diagnosis of adrenal insufficiency in hospitalized patients, whether or not they are receiving opioids, serum morning cortisol <3 µg/dL is not an accurate diagnostic test.

Poster Number 83

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Genetic and Socioeconomic Factors are Independently Associated with Type 2 Diabetes

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Genetics and socioeconomic status (SES) are key drivers of increased type 2 diabetes (T2D) risk, but the extent to which they impact disease in an additive or non-additive manner is unknown.

We examined the association between globally expanded polygenic score (gePS) and multiple area-based socioeconomic risk estimates (here: census tract percent high school education) and T2D prevalence based on a machine-learning algorithm using cross-sectional electronic health record (EHR) data from 27,771 participants of European ancestry in the Mass General Brigham Biobank.

The age and sex-adjusted odds of T2D was 1.72 (95% CI 1.63-1.81, p<0.001) per SD increase of T2D gePS and 1.36 (95% CI 1.31-1.42, p<0.001) per SD increase in socioeconomic risk in separate models. In a combined model, these associations persisted, with no evidence of interaction. Persons in the highest quintiles of both genetic and socioeconomic risk had almost 3-fold risk of T2D compared to those of middle quintile risk (OR 2.67, 95% CI 2.08-3.44, p<0.001). The T2D risk of individuals in the highest quintile of genetic risk but the lowest quintile of socioeconomic risk was indistinguishable from that of those in the middle quintile for both risk factors (OR 1.13, 95% CI 0.85-1.51).

These data provide evidence for the independent associations of genetic risk and SES with T2D and suggest that high SES is associated with reduced T2D risk, even among those at increased genetic risk, raising the hypothesis that interventions targeting elements of SES (e.g. education) may reduce risk of T2D in those with high genetic risk.

Poster Number 84

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Sequential therapy with recombinant human IGF-1 followed by risedronate increases spine bone mineral density in women with anorexia nervosa

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Anorexia nervosa is complicated by low bone mineral density (BMD) and increased fracture risk associated with low bone formation and high bone resorption. The spine is most severely affected. Low bone formation is associated with relative IGF-1 deficiency. Our objective was to determine whether bone anabolic therapy with recombinant human (rh) IGF-1 followed by antiresorptive therapy with risedronate would increase BMD more than risedronate or placebo in women with anorexia nervosa. We conducted a 12-month, randomized, placebo-controlled study of 90 women with anorexia nervosa and low areal BMD (aBMD). Participants were randomized to 3 groups: 6 months of rhIGF-1 followed by 6 months of risedronate ("rhIGF-1/Risedronate")(n=33), 12 months of risedronate ("Risedronate")(n=33), or double placebo ("Placebo")(n=16). Outcome measures were spine [1°endpoint: PA spine], hip, and radius aBMD by DXA and vertebral, tibial, and radial volumetric (v)BMD and estimated strength by high-resolution peripheral quantitative CT (for extremity measurements) and multi-detector computed tomography (for vertebral measurements). Mean age, BMI, and BMD were similar among groups. At 12 months, mean PA spine aBMD was higher in the rhIGF-1/Risedronate group than Placebo (p=0.03). Mean lateral spine aBMD was higher in the rhIGF-1/Risedronate than the Risedronate or Placebo groups (p<0.05). Vertebral vBMD was higher in the rhIGF-1/Risedronate than Placebo group (p<0.05). Sequential therapy with rhIGF-1 followed by risedronate increased lateral spine BMD, the site most severely affected in women with anorexia nervosa, more than risedronate or placebo. Strategies that are anabolic and antiresorptive to bone may be most effective at increasing BMD in this population.

Poster Number 85

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Olfactory Performance in Youth with Full and Subthreshold Avoidant/Restrictive Food Intake Disorder

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Avoidant/restrictive (A/R) food intake disorder (ARFID) is characterized by restrictive eating defined by lack of interest in food, sensory sensitivity, and/or fear of aversive consequences of eating resulting in a failure to meet adequate nutritional and/or energy needs. The complex psychopathology that differentiates ARFID from other eating disorders highlights the need to explore the role of sensory systems in disease etiology. Olfaction has an important role in eating behavior. More specifically, poorer olfaction is associated with decreased food intake and appetite. This relationship has yet to be examined in individuals with ARFID. We hypothesized that higher levels of PYY, which signals satiety, would be associated with poorer olfactory performance. We evaluated a cross-sectional sample of children and adolescents with full and subthreshold ARFID (n=82, 46.2% female, mean age 15.8±3.8). We measured olfactory performance with the Sniffin' Sticks test (Burghardt®, Wedel, Germany) which captures odor discrimination, identification, and threshold scores, with higher scores indicative of stronger olfactory performance. We measured fasting serum total PYY by ELISA (Millipore, Billerica, MA). Statistical analyses included Spearman's correlations. We found that greater fasting serum PYY levels were associated with significantly poorer performance on the odor threshold test (r=-0.4, p=0.003) only, and that being older was positively associated with odor discrimination (r=0.29, p=0.01) and identification (r=0.35, p=0.001). Olfactory performance may be related to number of odor exposures, which may increase with age. Future research should investigate whether high levels of PYY and poor olfactory performance are causes, consequences, or correlates of A/R eating.

Poster Number 86

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Changes in marrow adipose tissue in relation to changes in bone parameters following estradiol replacement in adolescent and young adult females with functional hypothalamic amenorrhea

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Context: Low energy availability causes disruption of hypothalamic gonadotropin-releasing hormone secretion leading to functional hypothalamic amenorrhea (FHA) and hypoestrogenism, which in turn contributes to decreased bone mineral density (BMD) and increased bone marrow adipose tissue (MAT). Transdermal estradiol administration in physiologic doses increases BMD in adolescents and adults with FHA. However, the impact of estrogen replacement on MAT in relation to changes in BMD has not been studied in adolescents. We hypothesized that physiologic estrogen replacement would lead to decreases in MAT, associated with increases in BMD.

Methods: We studied 15 adolescent and young adult females with FHA (14-25 years). All participants received a17β-estradiol transdermal patch at a dose of 0.1 mg/day (applied twice weekly) for 12 months. Participants also received cyclic progestin for 10-12 days each month. We quantified MAT (lipid/water ratio) of the fourth lumbar (L4) vertebral body and femoral diaphysis by single proton (1H)-magnetic resonance spectroscopy, and compartmental volumetric BMD of the distal radius and tibia using high-resolution peripheral quantitative computed tomography.

Results: Transdermal estradiol therapy over 12 months resulted in a decrease in MAT at the L4 vertebra from 0.92 ± 0.55 at baseline to 0.63 ± 0.29 at 12-months(p=0.008), and an increase in radial and tibial cortical vBMD(p=0.006, p=0.0003). Changes in L4 MAT trended to be inversely associated with changes in radial cortical vBMD (rho= -0.47, p=0.08).

Conclusion: We show that in adolescent and young adult girls with FHA, MAT decreases following transdermal estrogen therapy and these changes are associated with increased cortical vBMD.

Poster Number 87

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Dehydroepiandrosterone Sulfate (DHEAS) Levels Predict Weight Gain in Women with Anorexia Nervosa

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Anorexia nervosa (AN) and atypical AN (weight loss and psychological features of AN but BMI>18.5 kg/m2) are serious disorders complicated by endocrine dysregulation. Studies have suggested that higher urinary free cortisol (UFC) may predict weight gain in women with AN. We hypothesized that serum levels of dehydroepiandrosterone sulfate (DHEAS) would correlate with UFC and be a predictor of weight gain in women with AN.

We prospectively studied 34 women with AN and atypical AN, mean (±SD) age 27.4±7.7 years, who received placebo in a 6-month randomized trial. At baseline, mean weight was 51.3±4.9 kg, mean DHEAS level was 173±70 µg/dL (0.7±0.3 times mean normal for age) and mean UFC for subjects who completed testing (n=15) was 20±18 µg/24h (normal 0-50 µg/24h). Eighteen subjects gained weight over 6 months (range 0.1-10.3 kg). Higher DHEAS levels at baseline predicted weight gain ($r=0.61$, $p<0.001$) and an increase in fat mass ($r=0.40$, $p=0.03$), appendicular lean mass ($r=0.38$, $p=0.04$) by DXA, and abdominal fat by CT ($r=0.60$, $p<0.001$). Associations remained significant after controlling for age, baseline BMI, OCP use, and SSRI/SNRI use. UFC did not predict change in weight or body composition. DHEAS levels were positively associated with UFC ($r=0.61$, $p=0.02$).

Conclusion: In women with AN, higher DHEAS levels are a predictor of weight gain and increases in fat mass, skeletal muscle mass, and abdominal fat. Serum DHEAS correlates with UFC, a predictor of weight gain in prior studies. DHEAS may be a more practical biomarker of recovery, as 24-hour urine collections are challenging.

Poster Number 88

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PYY Levels and Relationship to Appetite Across Different Presentations of Anorexia Nervosa

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While the pathophysiology of anorexia nervosa (AN) is not well understood, the anorexigenic hormone peptide YY (PYY) may play a role.

In a cross-sectional study of 106 females [26 with atypical AN (atypAN), 11 with binge/purge type (AN-BP), 29 with restricting type (AN-R) and 40 healthy controls (HC), age 10-22 yrs], we hypothesized that PYY levels would be high in all AN presentations vs. HC and associated with appetite. Fasting blood was drawn for PYY, and visual analog scales were administered to assess appetite.

PYY levels were higher in atypAN, AN-BP, and AN-R than HC ($ps \leq 0.045$). Hunger and desire to eat one's favorite food were lower in atypAN and AN-BP vs. HC ($ps \leq 0.042$). The relationship between PYY and hunger was negative in AN-BP ($p= -0.71$, $p=0.012$), positive in AN-R ($p=0.40$, $p=0.035$), and not significant in atyp AN ($p=0.02$, $p=0.90$). The relationship between PYY and desire to eat favorite food was negative in AN-BP at trend level ($p=-0.56$, $p=0.071$), positive in AN-R ($p=0.52$, $p=0.005$), and not significant in atypAN ($p=0.09$, $p=0.65$).

In all AN presentations, PYY levels were higher than HC. Appetite was lower in atypAN and AN-BP than HC. Higher fasting PYY levels were associated with lower appetite in AN-BP and greater appetite in AN-R, while no relationship was found in atypAN. The absence of an association in atypAN, which includes females who do not meet low weight criteria for AN-R or AN-BP, may reflect opposing relationships in those who restrict vs. binge/purge. Future research is required to further understand the differences in relationships between PYY levels and appetite across AN presentations.

Poster Number 89

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Inverse relationship between postprandial oxytocin levels and amygdala brain volume in anorexia nervosa compared to healthy controls

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Background: The relationship between postprandial change in anorexigenic oxytocin (OXT) and subjective appetite differs between anorexia nervosa (AN) and healthy controls (HC), suggesting a disconnect between OXT and appetite regulation in AN. Gray matter volume of amygdala and hippocampus, areas rich in OXT receptors, are important in food reward and smaller in AN. We hypothesized that the relationship between postprandial change in OXT and bilateral amygdala and hippocampus volume will differ between AN and HC.

Methods: We performed a cross-sectional study in 47 females (22 restrictive AN; 25 HC). Peripheral OXT levels were measured fasting, 30-, 60- and 120-min postprandial. Fasting T1-weighted brain MRI scans were performed and gray matter brain volumes extracted from the bilateral amygdala and hippocampus. Linear regression models were used to determine differences between AN and HC of minimum pre- to postprandial percent change in OXT on amygdala and hippocampus volume.

Results: The association between OXT percent change and bilateral amygdala was lower in AN than HC ($p=0.03$) with a significant interaction (left $p=0.05$, right $p=0.02$), indicating a positive correlation between OXT percent change and left ($p=0.02$) and right ($p=0.04$) amygdala in AN, and no correlation in HC ($p>0.4$). No significant associations were found for OXT and the bilateral hippocampus ($p>0.4$).

Discussion: Our results indicate an association between OXT percent change and bilateral amygdala gray matter volume in AN but not in HC. Further research investigating the biological link between OXT secretion and brain structural differences in AN and impact on appetitive behavior will be important.

Poster Number 90

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Insulin Resistant Gestational Glucose Intolerance Is Associated With Pregnancy-Related Hypertension

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BACKGROUND: Women with gestational diabetes (GDM) and gestational glucose intolerance (GGI, abnormal initial GDM screening) have increased risks of adverse perinatal outcomes. We aimed to determine if GGI/GDM subtypes based on insulin resistance and/or deficiency are at differential risk for adverse outcomes.

METHODS: We applied homeostatic model assessment (HOMA2) to fasting glucose and insulin levels at 16-20 weeks' gestation to assess insulin resistance and deficiency. We defined GGI/GDM as glucose loading test (GLT)≥140mg/dL (n=245). We classified women with GGI/GDM into subtypes: insulin resistance (IR), insulin deficiency (ID), or mixed pathophysiology (MP). We compared odds of adverse outcomes in each subtype to odds in women with normal glucose tolerance (NGT, n=1538) using logistic regression (adjusted for age, race/ethnicity, marital status, BMI).

RESULTS: Of women with GGI/GDM, 120 (49.0%) had the IR subtype, 75 (30.6%) had ID, 39 (15.9%) had MP, and 11 (4.5%) had neither IR nor ID. We found increased odds of large for gestational age infants (LGA) in women with IR compared to NGT, which were attenuated with BMI adjustment (OR 1.39, $p=0.25$). There was a trend toward increased odds of LGA in women with ID (OR 1.89, $p=0.08$) and no increased odds in MP compared to NGT. The odds of pregnancy-related hypertension in the IR subtype were increased (OR 1.67, $p=0.04$) compared to NGT; women with ID or MP did not have increased odds.

CONCLUSION: Insulin resistant GGI/GDM is a high-risk subtype for pregnancy-related hypertension. Using HOMA2 to delineate subtypes may provide opportunities for personalized approaches to GGI/GDM.

Poster Number 91

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Contribution of Copy Number Variation in Idiopathic Hypogonadotropic Hypogonadism

INVESTIGATORS: M. Stamou*, H. Brand*, M. F. Lippincott, I. Wong, L. Plummer, R. Balasubramanian, M. Talkowski, S. Seminara

Introduction: While the role of single nucleotide variants (SNVs) in causal genes for Idiopathic Hypogonadotropic hypogonadism (IHH) is known, the contribution of copy number variants (CNVs) to IHH has not been systematically studied. Here, we examined the prevalence of CNVs in a large IHH cohort and their associated phenotypic spectrum.

Methods: Exome sequencing from 1,441 IHH probands was analyzed for rare CNVs and SNVs in IHH genes. IHH subjects were evaluated for reproductive and non-reproductive phenotypes (kidney, eye, cardiovascular, bone and neurological abnormalities).

Results: (i)CNV prevalence: Three percent of the IHH probands (46/1441 probands) harbored CNVs in 19/36 IHH genes (26 deletions/ 20 duplications). The majority of CNVs disrupted either ANOS1 (26%) or FGFR1 (17%). Intriguingly, CHD7 (a gene with SNVs in ~12% of IHH patients) did not harbor any CNVs. (ii)Phenotypic analysis: CNVs were more common in IHH subjects with anosmia (Kallmann syndrome, N=33) compared to normosmic IHH (N=13). More than half of the subjects carried at least 1 non-reproductive feature (N=26/46). A syndromic presentation with multiple non-reproductive phenotypes was more common in IHH subjects harboring multigenic CNVs compared to IHH subjects with single IHH gene CNV or IHH SNV (61% vs.12% or 18%, respectively; p 0.0006).

Conclusions: CNVs in known IHH genes contribute to 3% of the IHH genetic architecture. IHH subjects with larger multigenic CNVs displayed phenotypes consistent with contiguous gene syndromes. The absence of genic CNVs in genes frequently found to carry SNVs requires additional analysis to establish the biologic or mechanistic reasons for their rarity.

Poster Number 92

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Developing CRISPR/Cas-based Gene Therapies for Neurofibromatosis type 1 (NF1)

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Neurofibromatosis type 1 (NF1) is a tumor predisposition genetic disorder with an incidence of 1 in 3,500. Plexiform neurofibromas (PNs) are among the most serious symptoms of NF1. Although benign, they cause significant morbidity, and a substantial fraction transform into typically fatal malignant tumors. NF1 is caused by loss of neurofibromin (NF1), a RAS-GAP which negatively regulates the RAS signaling pathway. >1,000 pathogenic NF1 germline mutations have been described. PNs arise due to somatic mutation of the remaining wild type NF1 allele in Schwann cells (SCs), resulting in bi-allelic NF1 inactivation and dysfunctional regulation of RAS. Recent development of genome-editing technologies, such as CRISPR, base editing, and prime editing, could revolutionize genetic disorder treatments. We are investigating the feasibility of using genome editing as a therapeutic approach to correct NF1 mutations in PNs. Using CRISPR/Cas variants developed in our lab, we are focusing on three pathogenic NF1 mutations: a four base pair deletion, a nonsense point mutation, and a splicing mutation (found in 4% of NF1 patients). We designed a dual approach to test genome editing strategies: (i) using patient-derived cell lines–both lymphoblastoid cell lines (LCLs) and immortalized tumor-derived SCs with germline (NF1-/+) mutations and (ii) generating de novo SC models with patient-specific mutations using CRISPR. These NF1 cell models are subsequently being used to devise and test base editing and prime editing for efficient and accurate mutation correction. Our goal is to develop efficient, personalized, safe, and effective genome editing strategies to treat NF1 tumors.

Poster Number 93

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Human genetic analyses of organelles highlight the nucleus in age-related trait heritability

INVESTIGATORS: R. Gupta, K. J. Karczewski, D. Howrigan, B. M. Neale, V. K. Mootha

Aging is associated with defects in many organelles, but an open question is whether the inherited risk for age-related disease is enriched within loci relevant to each organelle. Here, we begin with a focus on mitochondria, as mitochondrial dysfunction is a hallmark of age-related disease. We report a striking lack of enrichment of mitochondria-relevant loci across GWAS for 24 age-related traits. Analyses of nine additional organelles reveal enrichment only for the nucleus, particularly nuclear transcription factors. Consistent with these results, natural selection appears to exert stronger purifying selection against protein-truncating variants for transcription factors compared to mitochondrial pathways, underscoring the importance of inherited variation in gene-regulation in age-related traits.

Poster Number 94

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Overcoming the Challenges of Interpreting Complex and Uncommon MNS Alleles from Whole Genomes

INVESTIGATORS: J. B. Halls, S. Vege, J. Aeschlimann, H. Mah, M. Lebo, P. Kumar, W. Ouwehand, C. Westhoff, W. Lane

We have developed automated interpretive software (bloodTyper) for determining red blood cell antigens from whole genome sequencing (WGS) data and validated it against commonly typed antigens including the MNS blood group system antigens: M, N, S, and s. WGS data for the remaining allele variants in the MNS system is yet to be comprehensively evaluated and characterized. The MNS blood group system consists of three homologous genes: GYPA, GYPB, and GYPE which create difficulties for genetic assays. In addition, many MNS alleles represent complex structural variations which include the formation of hybrid genes composed of two or more of the system genes due to partial gene deletions and multi-step gene recombination steps. Here we show a set of samples that demonstrate WGS data for most other MNS alleles that are of critical importance in developing WGS genotyping algorithms. We developed a novel approach to identify these established variants using split reads, paired reads, and read depth interpretations of WGS data. Our analysis allowed us to establish the breakpoints of several alleles without previously described breakpoints. Additionally, we updated our automated interpretive software, bloodTyper, to identify these rare and complex MNS blood group alleles using a variety of methods to detect samples encoding phenotypes for U–, Uvar, Hil+, MINY+, Mur+, DANE+, Mi(a+), and MUT+. The samples also included complex structural variants and hybrid alleles.

Poster Number 95

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High-dimensional inverse probability (IP)-weighted adjustment for selection bias in the Mass General Brigham (MGB) Biobank

INVESTIGATORS: Y. H. Lee, Y. Sheu, J. W. Smoller

Most large-scale biobank studies are based on volunteer and convenience sampling, both of which are highly prone to selection bias. Selection mechanisms are highly heterogeneous and poorly understood, especially in hospital settings where recruitment is incredibly dynamic. It particularly concerns methods that combine the effects of multiple genetic variants, such as polygenic risk scores (PRS), since the association between the phenotype and participation might lead the score to be more strongly related to participation than each variant is. In the present study, we quantified the extent to which the standard PRS analysis (hereinafter, unweighted analysis) may produce biased estimates of polygenic loadings and propose an approach for statistical adjustment. We specified an inverse probability (IP) weighted model for selection using demographic and diagnostic features extracted from a high-dimensional dataset consisting of the full electronic health records on more than 2.6 million MGB patients. Overall, unweighted models overestimated the effect of PRS on a respective clinical diagnosis and made less accurate predictions than IP-weighted models. For example, IP-weighted adjustment for selection bias substantially increased the prediction accuracy of PRS for hypertension and coronary artery disease, thereby implying potential real-world consequences if not appropriately addressed. With a growing interest in the clinical implementation of PRS, proper measurement and adjustment for selection bias are crucial for a safe and effective translation of genetic findings.

Poster Number 96

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PRS-CSx: Improving Polygenic Prediction in Ancestrally Diverse Populations

INVESTIGATORS: Y. Ruan, Y. A. Feng, C. Chen, M. Lam, A. Sawa, A. R. Martin, S. Qin, H. Huang, T. Ge, Stanley Global Asia Initiatives

Polygenic risk scores (PRS) have attenuated cross-population predictive performance. As existing genome-wide association studies (GWAS) were predominantly conducted in individuals of European descent, the limited transferability of PRS reduces its clinical value in non-European populations and may exacerbate healthcare disparities. Recent efforts to level ancestry imbalance in genomic research have expanded the scale of non-European GWAS, although they remain under-powered. Here we present a novel PRS construction method, PRS-CSx, which improves cross-population polygenic prediction by integrating GWAS summary statistics from multiple populations. PRS-CSx couples genetic effects across populations via a shared continuous shrinkage prior, enabling more accurate effect size estimation by sharing information between summary statistics and leveraging linkage disequilibrium (LD) diversity across discovery samples, inheriting computational efficiency and robustness from PRS-CS. We show that PRS-CSx outperforms alternative methods across traits with a wide range of genetic architectures and cross-population genetic correlations in simulations. We further assessed the prediction accuracy of PRS-CSx on quantitative traits using discovery data from the UK Biobank (European N=350K), Biobank Japan (East Asian N=62k-158k), PAGE (African and American Latino, N=20k-49k), and independent testing samples from different populations in UKBB. PRS-CSx outperformed all alternative methods across target populations. Lastly, PRS-CSx best predicted schizophrenia in EAS cohorts: the relative increase in prediction accuracy against PRS-CS based on a single population and alternative methods based on multiple populations ranges from 52% to 126%. In sum, PRS-CSx showed improved prediction accuracy in non-European populations and will be a valuable tool to reduce healthcare disparities for underrepresented populations.

Poster Number 97

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Systematic discovery of thousands of gene-environment interactions for cardiometabolic serum biomarkers

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Gene-environment interaction (GEI) analysis enables precision medicine by revealing how genetic variants modify the effects of environmental exposures on cardiometabolic risk. Ideally, these interactions could be mapped comprehensively across all genetic variants, exposures, and outcomes, but this approach is computationally intensive and statistically underpowered. Recent studies have shown that variance-quantitative trait loci (vQTLs), or genetic variants that associate with differential variance (rather than mean) of a biomarker, often reflect underlying GEIs. Here, we sought to first identify vQTLs for cardiometabolic traits, then use this smaller genetic search space to uncover novel GEIs across thousands of environmental exposures. A two-stage, multi-ancestry analysis was conducted in 355,790 unrelated participants from the UK Biobank. First, we performed a genome-wide vQTL scan for each of 20 serum metabolic biomarkers (including lipids, lipoproteins, and glycemic measures), prioritizing 539 independent genetic variants. Next, we collected over 2000 variables corresponding to socioeconomic, lifestyle, and clinical exposures, and conducted an interaction analysis for each combination of exposure and vQTL. The subsequent GEI analysis revealed 1,533 significant interactions passing a stringent significance threshold. Many involved anthropometric exposures; for example, BMI was associated with a greater increase in triglycerides in risk allele carriers at variant rs738408 in the PNPLA3 gene ($p = 2.6E-16$). Other interactions had behavioral implications; variants near the fatty liver-associated gene TM6SF2 modified the impact of fish intake on cholesterol ($p = 2.9E-8$). This catalog of vQTLs and interactions can inform future mechanistic studies and provides a knowledge base for genome-centered precision approaches to cardiometabolic health.

Poster Number 98

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Function of Bmi1 in neural differentiation via polycomb repressive complex 1 (PRC1)

INVESTIGATORS: J. Zhu, S. K. Marr, M. Damle, E. McCaslin, R. E. Kingston

Polycomb repressive complex 1 (PRC1) are key players in establishing and maintaining gene expression patterns by regulating histone modification and chromatin structure. In mammals, PRC1 is very diverse and can be sub-classified as canonical PRC1 (cPRC1) and non-canonical PRC1 (ncPRC1). While cPRC1 compacts chromatin and generates phase separation, ncPRC1 monoubiquitinates lysine 119 at histone H2A (H2AK119ub1). Although these complexes' catalytic functions are well known, how they cooperate in controlling gene expression in vivo, especially during cellular differentiation, is not. To answer this question, we study Bmi1 (Pcgf4), one of the core PRC1 members, in mouse embryonic stem cells (mESCs) neural differentiation. Bmi1 can form either cPRC1 or nPRC1 with different PRC1 subunits. Using CRISPR, we have generated Bmi1 knockout mutants and point mutants that only form ncPRC1, but not cPRC1. To our surprise, loss of Bmi1 causes no significant phenotype during mESCs neural differentiation. However, abolishing the capability of Bmi1 to form cPRC1 results in gene misregulation and a consequent blockage towards a neural cell fate. We hypothesize that the presence of Mel18, another PCGF protein that is highly homologous to Bmi1, can compensate Bmi1 loss-of-function but not its malfunction. Our results indicate that cPRC1 is required for the establishment of gene expression pattern during cellular differentiation.

Poster Number 99

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A hesitancy triangle: The role of social determinants, information, and motivators in vaccine hesitancy

INVESTIGATORS: A. F. Brown, S. D. Carson, S. L. Vassar, STOP COVID-19 CA; NIH CEAL; UCLA CTSI; NHLBI

Vaccine hesitancy in Black, Indigenous, Latinx/o, and other marginalized communities stems from various factors including distrust in healthcare and research and structural barriers to care. These same communities have been disproportionately affected by the SARS-CoV-2 2019 (COVID-19) pandemic, experiencing more infections, hospitalizations, and deaths compared to whites. To examine and address these disparities, the National Institutes of Health funded the Community Engagement Alliance Against COVID-19 Disparities in 11 states. The California collaborative, the Share Trust Organized Partner COVID-19 California Alliance (STOP COVID-19 CA), includes 11 academic institutions and 73 community partners. Using community engagement best practices, this collaborative is working to identify and develop strategies to address vaccine hesitancy in marginalized communities. The group shares vaccine hesitancy and readiness narratives described in town halls, focus groups, and community meetings, as well as potential engagement strategies to inform stakeholders for future intervention development. The group will also facilitate a series of healing circles that acknowledge past and present abuses and provide marginalized participants a safe space to discuss concerns. Three themes have emerged that elucidate factors contributing to vaccine hesitancy and provide emergent opportunities for public health interventions: social determinants interfere with willingness to vaccinate; disinformation and a lack of culturally and linguistically-appropriate information contributing to confusion and mistrust; and the use trusted messengers and influential experiences may be important motivators of vaccine uptake. This presentation outlines preliminary findings shared in February 2021 with key local, state, and national stakeholders for rapid intervention in marginalized communities in the state of California.

Poster Number 100

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Association of Neighborhood Factors with Pediatric Asthma Disparities

INVESTIGATORS: E. Aryee, J. M. Perrin, D. Iannuzzi, K. A. Kuhlthau, N. M. Oreskovic

Neighborhood characteristics such as quality of housing and built environment can influence the health of its residents. Low-income children are more vulnerable to asthma and asthma-related outcomes; however, few studies have used nationally representative data to examine associations of neighborhood factors and asthma. In this study, we examined the association between neighborhood factors and asthma prevalence and severity among low-income children in a large national sample.

Methods: We calculated prevalence of reported asthma by presence or absence of neighborhood factors using the 2018 National Survey of Children's Health data. Study sample included 8,653 low-income children. We used logistic regression to compare rates and severity of asthma by types of neighborhood, adjusting for age, sex, race, parent education, and insurance.

Results: Asthma prevalence among low-income children whose parents reported living in unsupportive neighborhoods was 11.0% compared to 7.8% for children living in supportive neighborhoods ($p = 0.005$). Children living in neighborhoods with dilapidated housing versus non-dilapidated housing had asthma prevalence of 12.2% and 8.9% respectively ($p = 0.009$), while those living in neighborhoods with vandalism had a prevalence of 13.9% compared to 8.9% for those in neighborhoods without vandalism ($p = 0.02$). Parents of low-income children in unsupportive neighborhoods were 1.4 times more likely than those in supportive neighborhoods to report having a child with moderate vs mild asthma ($p = 0.03$).

Conclusion: Low neighborhood support, dilapidated housing and vandalism were associated with higher asthma prevalence in low-income children. Neighborhood support was also associated with higher asthma severity in this population.

Poster Number 101

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Lung Cancer Strategist Program: A Novel Care Delivery Model to Improve Timeliness of Diagnosis and Treatment in Patients at High-Risk for Healthcare Disparities

INVESTIGATORS: J. M. Copeland, J. R. Armitage, P. J. Catalano, J. S. Weissman, Y. L. Colson, C. S. Lathan, C. de Forcrand

Background: The diagnosis and treatment of lung cancer is challenged by complex diagnostic pathways and fragmented care that can lead to disparities for vulnerable patients. Here we show, patient triage through our care delivery model facilitates assessment of benign versus malignant lesions as well reduces time to diagnosis and treatment within vulnerable patient populations at high-risk for treatment delay.

Methods: A multi-institutional, multidisciplinary conference was convened to address the complexity of lung cancer care in vulnerable patients at high-risk for treatment delay. The resulting care delivery model, the Lung Cancer Strategist Program (LCSP), emphasized expedited surgery and early oncologic consultation for vulnerable patients newly diagnosed with a lung nodule. A retrospective review was performed in the first 100 LCSP patients to evaluate the efficiency and oncologic outcomes of our care model, this was compared to 100 matched vulnerable patients that received routine surgical and oncologic referral.

Results: In the 78 LCSP and 41 routine referral patients managed with ongoing surveillance, LCSP patients had a shorter time from suspicious finding to work-up (3 vs. 26 days, $p < 0.001$) and from diagnosis to establishing a surveillance management plan (12.5 vs. 39 days, $p < 0.001$). In the 22 LCSP and 59 routine referral patients treated for intrathoracic malignancy, LCSP patients had fewer hospital visits (4 vs 6, $p < 0.001$) and diagnostic studies (4 vs 5, $p=0.01$) with a shorter time to diagnosis (30.5 vs. 48 days, $p=0.02$) and treatment (40.5 vs. 68.5 days, $p=0.02$).

Poster Number 102

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How Patient Centering, Scan Length, and Arm Position Affect Radiation Dose in Chest CT for COVID-19 in Four Countries?

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We assessed centering, scan length, and positioning of patients undergoing chest CT for suspected or known COVID-19 pneumonia and investigated their effect on associated radiation doses. With respective approvals from institutional review boards, we compiled CT imaging and radiation dose data from four hospitals belonging to four countries (Brazil, Iran, Italy and USA) on 400 adult patients who underwent chest CT for suspected or known COVID-19 pneumonia. We recorded patient demographics and volume CT dose index (CTDIvol) and dose length product (DLP). From thin-section CT images of each patient, we estimated the scan length and recorded the first and last vertebral bodies at the scan start and end locations. At the level of tracheal bifurcation, we measured patient off-centering and anterior-posterior and lateral dimensions as surrogates of patient size. Arm position (raised over shoulders or by the side of body) was also recorded. The extent and frequency of patient mis-centering did not differ across the four CT facilities (>0.09). The frequency of patients scanned with arms by their side (11-40% relative to those with arms up) had greater mis-centering and higher CTDIvol and DLP at 2/4 facilities ($p= 0.027$-0.05). Despite lack of variations in effective diameters ($p=0.14$), there were significantly variations in scan lengths, CTDIvol and DLP across the four facilities ($p<0.001$). Most frequent scan start, and end positions were C7 and L1 vertebrae. Mis-centering, over-scanning and arms by the side are frequent issues with use of chest CT in COVID-19 pneumonia and are associated with higher radiation doses.

Poster Number 103

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The impact of genital psoriasis among psoriatic patients

INVESTIGATORS: P. L. Gorrepati, G. P. Smith

Genital dermatoses can severely affect patients' quality of life, including their sexual life.1 We sought to examine the impact of genital psoriasis among psoriatic patients, their response to treatments, and how often genital psoriasis is evaluated by dermatologists. A cross-sectional qualitative study was completed. Patients 18 years or older seen for psoriasis at the Massachusetts General Hospital Outpatient Dermatologic Clinic were recruited. Fifty-five subjects were enrolled in the study and completed a 23-item questionnaire. Only 23.6% of participants had ever been asked if they had a genital rash by a dermatologist. 60% of participants indicated a dermatologist had never examined the genital area. However, 43.6% of participants indicated they had had at some point some form of itching, burning, pain, or involvement in the genital areas. 29.1% of participants described that their overall psoriasis has caused some form of sexual difficulty for them. 50% of participants with involvement in their genital area indicated experiencing some form of sexual difficulty. When looking at the gender breakdown of sexual difficulty, 62.5% of female patients indicated significant sexual difficulty compared to just 25% of male patients. This qualitative study of patients with psoriasis showed that even though almost half of the patients enrolled in the study had a rash or some form of symptoms in their genital regions, a majority of patients had never had their genital area examined by a dermatologist for psoriasis nor had they ever been asked if they had a genital rash.

Poster Number 104

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The trajectory of racial/ethnic disparities in the use of cancer screening before and during the COVID-19 pandemic: A large U.S. academic center analysis

INVESTIGATORS: F. O. Marcondes, D. Cheng, E. T. Warner, S. C. Kamran, J. S. Haas

Cancer screening rates declined sharply early in the COVID-19 pandemic. The impact of the pandemic may have exacerbated existing disparities in cancer screening due to the disproportionate burden of illness and job loss among racial/ ethnic minorities, and potentially, uneven resumption of care between different racial/ ethnic groups. Using electronic health record data from Mass General Brigham (MGB), we assessed changes in rates of breast, cervical, colorectal and lung screening before and during the pandemic. Among patients who received primary care in an MGB-affiliated primary care practice, cancer screening rates were calculated as the number of individuals who received a screening test for each cancer type over the number of individuals due for each test, during each month between April 2019-November 2020. We conducted an interrupted time-series analysis to test for changes in screening rates by race/ethnicity before and during the pandemic. Prior to the pandemic, Asian women were less likely to receive breast cancer screening ($p<0.001$), and Latinx and Black individuals were less likely to screen for lung cancer ($p<0.001$ and $p=0.02$) – all relative to White individuals. Our results did not show significant improvement or worsening of racial/ethnic disparities for any cancer screening type as screening resumed. However, as of November 2020 rates of screening for breast cancer were lower than pre-pandemic levels for Latinx individuals, and rates of lung cancer screening rates did not return to baseline for Latinx, Black or White individuals. Further monitoring of disparities in cancer screening is warranted as the pandemic evolves

Poster Number 105

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Pro-Inflammatory Diet and Risk of Incident Gout: Three Prospective Cohort Studies of US Men and Women Over 30 Years

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Introduction: Pro-inflammatory diet is associated with increased risk of cardiovascular disease and type-2 diabetes. Evidence suggests chronic inflammation may drive the development of gout (cardiometabolic arthritis affecting 9 million US adults), but the role of inflammatory diet on gout risk is unknown.

Objectives: Examine relation between dietary inflammatory potential and risk of gout in large cohorts of US women and men over 30 years.

Methods: We studied 164,090 women from Nurses Health Study I & II (1986-2016; 1989-2017) and 40,598 men from Health Professionals Follow-up Study (1986-2016), without gout at baseline, who completed validated questionnaires every 4 years. Using the empirical, food-based dietary index of inflammatory potential (EDIP), pre-defined based on circulating levels of IL-6, C-reactive protein, adiponectin, and TNFαR2, we prospectively examined the association between EDIP score and incident gout, adjusting for potential confounders, and stratified by alcohol intake, as alcohol has anti-inflammatory properties, but is associated with higher gout risk, particularly beer.

Results: We documented 2,874 incident gout cases over 5,124,940 person-years. In pooled multivariable-adjusted analyses, those with the most inflammatory diets (highest EDIP quintile) had 59% higher gout risk than the least inflammatory (multivariable RR=1.59; 95% CI=1.41–1.79, p-trend<0.001). This remained positive after adjusting for BMI, a likely causal intermediate (RR=1.27, p-trend<0.001), and was stronger among alcohol non-drinkers than drinkers (RR=2.37 vs. 1.57).

Conclusions: Findings support a role for chronic inflammation in development of gout, independent of adiposity. Diets with lower inflammatory potential (i.e., less red/processed meat, tomatoes) may modulate systemic and metabolic inflammation, potentially reducing gout risk.

Poster Number 106

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Gender Minority Enrollees in the Massachusetts All Payer Claims Database

INVESTIGATORS: A. McDowell, V. Fung

Gender minority (i.e., transgender or gender diverse) individuals experience significant health inequities. Access to gender affirming health care services, including hormone therapy and surgical procedures, has been associated with significant improvements in well-being for gender minority (GM) individuals. However, many states allow health insurers to explicitly exclude coverage of gender affirming health care services. In recent years, Massachusetts has enacted non-discrimination policies to increase coverage of these services for GM individuals enrolled in private health insurance plans and MassHealth. We used the Massachusetts All Payer Claims Database (APCD) to study GM children and adults across insurance types during a period with pivotal changes regarding gender affirming care coverage. Importantly, we assess whether codes for gender identity-related diagnoses (which are used to identify GM patients and are often required for coverage of gender affirming services) increased after these coverage changes.

We show that, among all enrollees in the APCD in 2012-2016, 0.048% (n=6,495) had at least one gender identity-related code and were thus included in our GM cohort. The number of TGD individuals with their first gender-identity related code increased each year for all insurance types, though growth in new codes was greatest following implementation of non-discrimination policies. The proportion of individuals with gender-identity-related diagnosis codes in the 2012-2016 Massachusetts APCD is notably higher compared to similar studies using health insurance claims data, a finding which may be attributable to our ability to observe younger individuals in this dataset or to more generous coverage of gender affirming care in Massachusetts.

Poster Number 107

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Managing Neonatal Opioid Withdrawal Syndrome (NOWS): PRN Pharmacologic Agent Choice

INVESTIGATORS: T. M. Morrison, K. D. MacMillan, P. Melvin, D. Schiff, R. Singh, J. Murzycki, M. Van Vleet, R. Rothstein, T. O'Shea, M. Gupta, E. Wachman

Managing Neonatal Opioid Withdrawal Syndrome (NOWS): PRN Pharmacologic Agent Choice

Background: The incidence of Neonatal Opioid Withdrawal Syndrome (NOWS) increased five-fold between 2004 and 2014. Methadone (MTD) and morphine (MOR) are the most commonly administered first-line agents for NOWS treatment. The optimal PRN pharmacologic agent for NOWS has not previously been examined.

Objective: To compare NOWS hospitalization outcomes in infants treated with PRN MTD and MOR.

Methods: This was a multi-centered, retrospective cohort of infants pharmacologically treated for NOWS. Data was collected from four Massachusetts hospitals between 2017 and 2020. Infants born >/= to 36 weeks gestation with prenatal opioid exposure treated with PRN MTD or MOR were included. Mixed effects logistic and linear regression models were employed to evaluate differences in PRN treatment failure (defined as a switch to scheduled dosing), length of stay (LOS), and number of PRN doses administered in infants treated with PRN MTD vs MOR using MOR as the reference group.

Results: There were 76 infants in the MTD group and 49 in the MOR group with no significant differences in baseline characteristics. There was a significant difference in number of PRN doses, but not in LOS or PRN failure rates between PRN MTD vs MOR in adjusted models. Breastfeeding was associated with decreased LOS, and care within a NICU/SCN was associated with greater odds of PRN failure.

Conclusions: There were no significant differences in most NOWS hospitalization outcomes. Results suggest that attention to maternal factors, infant care location, and breastfeeding may improve hospitalization outcomes.

Poster Number 108

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Deep learning to identify high-risk smokers for lung cancer screening computed tomography

INVESTIGATORS: V. K. Raghu, T. Mayrhofer, H. Aerts, U. Hoffmann, M. T. Lu

Background: Lung cancer screening with computed tomography (CT) reduces lung cancer death. Centers for Medicare and Medicaid Services (CMS) eligibility criteria for lung cancer screening require detailed smoking information and miss many incident cancers. A deep learning-based risk score using chest radiograph images may identify more high-risk smokers who may benefit from lung cancer screening CT.

Methods: A convolutional neural network (CXR-LC) predicting 12-year incident lung cancer from the chest radiograph image, age, sex, and whether currently smoking was developed in 41,856 persons aged 55-74 from the Prostate, Lung, Colorectal & Ovarian trial (PLCO). The final model was tested in held-out smokers from PLCO (n=5,615, 37.9% CMS eligible, 12-year follow-up), with external testing in the National Lung Screening Trial (NLST, n=5,493, all CMS eligible, 6-year follow-up). Discrimination for incident lung cancer was assessed using the area under the ROC curve (AUC). Sensitivity was compared at a fixed screening population size defined by CMS eligibility. Results are reported in test datasets only.

Results: CXR-LC had better discrimination for 12-year incident lung cancer than CMS eligibility in the PLCO test dataset (CXR-LC AUC 0.755 vs. CMS 0.634; $p < 0.001$). and was more sensitive than CMS eligibility at a fixed screening population size (74.9% vs. 63.8%, p=0.01) resulting in 30.7% fewer missed lung cancers. In NLST (all CMS eligible), CXR-LC maintained high discrimination (AUC 0.659 [0.62,0.70]).

Conclusions: CXR-LC identified smokers at high risk of incident lung cancer, beyond CMS screening eligibility and using information commonly available in the electronic medical record.

Poster Number 109

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Detection of Physiologic Abnormality Markers Post-ICU Transfer to Surgical Floors with Remote Surveillance

INVESTIGATORS: J. J. Rajotte, J. P. Wiener-Kronish, K. C. Safavi

The decision to transition a patient from the surgical ICU (SICU) to a lower intensity of care requires agreement between the surgical and critical care teams that it is medically safe to do so. A subset of patients will once again require ICU-level monitoring, resulting in readmission. Early recognition of patients warranting attention could be possible if patterns of decompensation were present in the electronic health record. In this pilot study, experienced intensivists selected a priori alerts that could identify an actively decompensating patient at the appropriate time to prevent readmission. We hypothesized that the presence of any of a predefined cadre of vital sign or laboratory abnormalities, such as tachycardia, desaturation, low hemoglobin, hypo or hyperglycemia, or hyperkalemia preceded readmission to the SICU. Of the 432 transfer events out of the ICU in an 18-month period, there were 350 unique patients. 66 (15.3%) of transfers resulted in readmission to the SICU, only 40 of which produced alerts on the floor. With respect to readmission, we report that the sensitivity of triggering any alert was 60.6 [95% CI 47.8 – 72.4] %, while the specificity was 11.2 [95% CI 8.2 – 14.9] %. Individual alert types proved to be fairly specific, but less sensitive. The frequency of specific alerts varied by the 15 floor destinations was studied. We suspect that alerts that are tailored by floor destination could improve the sensitivity and specificity of a remote surveillance system in a future study and decrease readmission rates without causing alarm fatigue.

Poster Number 110

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Depression, anxiety, and suicidal ideation among graduate students in doctoral degree programs: Systematic review and meta-analysis

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University administrators and mental health clinicians have raised concerns about depression and anxiety among graduate students in doctoral degree programs. Mental health problems are highly prevalent among undergraduate and professional degree students, but doctoral students face unique uncertainties and stressors. However, no study has systematically synthesized the evidence on mental health problems in this population. We searched PubMed, Embase, PsycINFO, ERIC, Business Source Complete, and the grey literature for studies of doctoral students in which depression, anxiety, and/or suicidal ideation were assessed using validated screening instruments. Thirty-two articles and reports, describing 29 unique studies, were included. Twenty (69%) studies were conducted in the United States. The median sample size was 172 students (IQR, 68–654; range, 6–6,405). Among 16 studies reporting the prevalence of depression across 23,469 doctoral students, the estimates ranged from 7-50%. Our pooled estimate of the proportion of doctoral students with depression was 0.24 (95% confidence interval [CI], 0.18–0.31; 95% predictive interval [PI], 0.04-0.54), with substantial between-study heterogeneity (I2 = 98.75%). In a meta-analysis of the nine studies reporting the prevalence of anxiety, the estimated proportion of doctoral students with anxiety was 0.17 (95% CI, 0.12-0.23; 95% PI, 0.02-0.41; I2 = 98.05%). We conclude that depression and anxiety are highly prevalent. Data limitations precluded our ability to estimate differences in prevalence across subgroups of students, determine drivers of depression and anxiety, or obtain a pooled estimate of suicidal ideation prevalence. Programs to support the mental health and wellbeing of doctoral students are urgently needed.

Poster Number 111

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Evaluation of an electronic platform to record lifestyle habits in subjects at risk of developing type 2 diabetes in a middle-income population

INVESTIGATORS: M. Sevilla-Gonzalez, B. Bourguet-Ramirez, L. Lazaro-Carrera

Background: Health-based therapy has proved positive results for Type 2 diabetes (T2D) prevention in high-income settings, but little is known about their effectiveness in low- and middle-income populations where the burden of T2D is substantial.

Objective: We sought to identify barriers, feasibility, usability and effectiveness of an electronic platform "Vida Sana", to record lifestyle habits in subjects at T2D risk in a middle-income (MI) setting.

Methods: This was a 3-months interventional study. We included Mexican prediabetes subjects with overweight or obesity. Feasibility was assessed by study retention. Usability was evaluated with the System Usability Scale (SUS). Effectiveness measures included changes in glycemic and anthropometric parameters from baseline to 3-months visit.

Results: The feasibility of Vida Sana was 42.8% (n=33 subjects), and the usability was 48.7% + 14.2. The most reported barrier for not using the platform was difficulty for accessing to the platform (36.3%). Vida Sana was effective for lowering fasting glucose (-3.1 mg/dL vs -0.11 + 8.08; p = 0.038) and at 2hr (-16.9 mg/dL vs 2.5 + 26.1; P = 0.045) , body fat percent (-1.3 (-2.2 – - 0.7) s -1.02 (-1.9 - -0.3); P =0.024), and waist circumference (-3.2 + 5.1cm vs -1.7 + 5.0; P=0.023) independent their age, sex, treatment and education attainment.

Conclusions: The use of an electronic platform was effective to improve glycemic and anthropometric parameters in a population at risk of T2D. Improving accessibility and ease of navigation could be objectives to improve the acceptance of mobile applications in MI population.

Poster Number 112

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Predictors of Premature Discontinuation of Opioid Use Treatment in the United States

INVESTIGATORS: C. A. Stafford, M. Jalali, K. Lich, B. Naumann

Discontinuation of treatment for problematic opioid use leads to relapse and higher risk of opioid poisoning. Rates of premature treatment exit average around 30%, which warrants exploration as opioid related deaths continue to rise. Especially considering effects of COVID-19 on substance use, it is imperative to understand the effect of risk factors for treatment discontinuation when treating people who use opioids. In this prognostic study, we built a machine learning model using the Treatment Episode Data Set–Discharge (TEDS-D). Included were 2,446,710 treatment episodes with discharge dates between January 1st, 2015 and December 31st, 2018 (the latest available data). Exposures included 31 factors around substance use history, treatment history, criminal involvement history, and demographic characteristics. The predicted outcome was premature treatment exit as defined by "reason for discharge" equal to "dropped out of treatment (left against professional advice)." We present that the main important factors in predicting treatment attrition are age of first substance use, service setting, primary source of payment for treatment, health insurance status, and geographical region. Our findings help inform interventions directed at treatment delivery to address varying propensity for treatment attrition across opioid users. For example, more hands-on attention may be given to individuals with an earlier age of substance use initiation or alterations may be made to the service setting to minimize attrition.

Poster Number 113

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Comparing Machine Learning Algorithms for Predicting ICU Admission and Mortality in COVID-19

INVESTIGATORS: S. Subudhi, A. Verma, A. B. Patel, C. C. Hardin, M. J. Khandekar, H. Lee, T. Stylianopoulos, L. L. Munn, S. Dutta, R. K. Jain

As predicting the trajectory of COVID-19 disease is challenging, machine learning models could assist in determining high-risk individuals and aid physicians in allocating essential medical resources. Our study compares the performance of 18 machine learning algorithms for predicting ICU admission and mortality among COVID-19 patients. It includes COVID-19 patient data from the multi-hospital Mass General Brigham (MGB) healthcare database. We developed and internally validated models using patients presenting to Emergency Department (ED) between March and April 2020 (n = 1144) and externally validated them using those individuals who encountered ED between May and August 2020 (n = 334) (temporally distinct cohort). We made distinct models based on two primary outcomes, namely predicting ICU admission (within 5 days of ED visit) and mortality (within 28 days of ED visit) among COVID-19 patients. Using clinical and laboratory parameters, ensemble-based machine learning algorithms performed better than other model types at predicting both 5-day ICU admission and 28-day mortality from COVID-19. CRP, LDH, and procalcitonin levels were important for ICU admission models whereas eGFR < 60 ml/min/1.73m2, ventilator use, and potassium levels were the most important variables for predicting mortality. Implementing ensemble-based models would be helpful in clinical decision-making for future COVID-19 outbreaks. This framework of model development can also be used for other pathogenic disease outbreaks.

Poster Number 114

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Deaths in LTC Facilities: A Step Towards Lifting the Pandemic Fog

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Long-Term Care Facility (LTCF) residents have been among the most likely to die during the COVID-19 pandemic. The exact numbers of deaths, however, are less clear, e.g., CDC-reported 3,900 LTCF deaths among 9,700 total COVID-related deaths versus state-reported 7,400 LTCF deaths among 12,200 total COVID-related deaths. The mortality information has not been validated and may suffer from attribution challenges because of incomplete testing and evolving knowledge about this new pathogen. We used 73,915 Massachusetts Death Certificates (2016-2020) to examine all-cause LTCF pandemic mortality. We fit 2016-2019 data with a seasonal time-varying spline to estimate expected 2020 LTCF death rates for comparison. We find that LTCF deaths were common pre-pandemic, but many more died during the pandemic, albeit half those in some early reports. In 2020, all-cause mortality was 23% higher than expected (2,899 excess deaths; 13.6 actual versus 11.1 expected deaths/day/10,000 residents). COVID was a cause in 3,800 death certificates. Between April-May 2020, there were 5,401 all-cause deaths (163% higher than expected; 95%CI:157%-165%), including 3,040 COVID-related deaths, and mortality rates peaked at 48 deaths/day/10,000 residents. Mortality waned between June-November 2020 (9.51% lower than expected; 95%CI:8.11%-10.4%) and waxed in December 2020 (6.21% higher than expected; 95%CI:4.28%-8.11%). While COVID-related deaths led to a mortality increase, concurrent decreases in non-COVID-related mortality moderated the net effect, leading to fewer all-cause excess deaths than COVID-related deaths. We also find higher excess death in older and Black or Hispanic residents, as well as residents in larger, urban, and non-for-profit facilities.

Poster Number 115

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Human enteric viruses shape disease phenotype through divergent immunomodulation

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Although the microbiome has been established as a critical regulator of health, the role of viruses that inhabit human intestine (collectively, the virome) is unknown. Metagenomic studies have shown that fecal virome is altered in inflammatory bowel disease (IBD). How the virome contributes to host homeostasis, how changes in virome composition impact inflammation, and which viruses are immunomodulatory is unknown. Here we asked how and if viromes derived from human healthy or IBD intestinal resections regulate host immunity differently. Interestingly, we found a direct role for the human intestinal virome in educating host immunity in a TLR/RLR/cGAS-dependent fashion. Healthy viromes elicited an anti-inflammatory/pro-survival response in macrophages. Conversely, the IBD virome triggered robust pro-inflammatory responses. Importantly, healthy enteric viruses were capable of inciting intestinal protection in vivo since mice with a "humanized virome" derived from non-IBD colon resections were rescued from intestinal inflammation. Conversely, "humanized" IBD virome mice exhibited marked expansion of tissue-resident lamina propria CX3CR1+ mononuclear phagocytes and exacerbated colitis. Notably, healthy viromes, or their immune products, successfully dampened the IBD-virome inflammatory response. Finally, mutations in IFIH1 encoding the virus receptor MDA5 abrogated responses to the healthy enteric virome and disrupted intestinal epithelial integrity in Ulcerative Colitis patients. Together, these studies demonstrate that human enteric viromes can autonomously and divergently shape host immunity and disease phenotype. Manipulation of the human enteric virome, or the host immune responses to it, may be beneficial in IBD.

Poster Number 116

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Transgenic β-cell-specific expression of CXCL12 synergizes with peripheral hematopoietic stem cell mobilization to suppress autoimmune diabetes

INVESTIGATORS: D. A. Alagpulinsa, A. Jajoo, M. H. Chapin II, M. C. Poznansky

Hematopoietic stem and progenitor cells (HSPCs) highly express CXCR4, the receptor for CXCL12. This keeps HSPCs predominantly anchored in the bone marrow (BM) where CXCL12 is constitutively highly expressed. HSPCs possess superior immunoregulatory properties and their infusion is being investigated for treatment of type 1 diabetes (T1D), a chronic disease that results from autoimmune-mediated destruction of insulin-producing beta cells of the pancreatic islets. We hypothesized that CXCL12 could be exploited to recruit and retain HSPCs in the islets to ameliorate immune destruction of beta cells while supporting their survival to prevent T1D. We found that CXCL12 levels are 3-fold higher in the BM of T1D NOD mice compared with age-matched non-diabetic C57BL/6 mice. In reverse, the activity of DPP4, an enzyme that inactivates CXCL12 to promote peripheral mobilization of HSPCs, is ~2-fold lower in the BM of T1D NOD mice compared with their age matched C57BL/6 mice. Indeed, we showed that peripheral mobilization of HSPCs using G-CSF plus a DPP4 inhibitor (diprotin A) is ~2.5-fold higher than using G-CSF alone, a commonly used mobilization agent. Intriguingly, ectopic β-cell-specific expression of Cxcl12 transgene under the control of the murine insulin promoter using a recombinant adeno-associated virus vector synergized with peripheral mobilization (G-CSF plus diprotin A treatment) to significantly suppress T1D in NOD mice (12-week-old) compared with either treatment alone ($p \leq 0.02$). This synergy was characterized by increased numbers of circulating HSPCs, reduced insulitis and improved metabolic function. Our findings demonstrate proof-of-concept of a novel "pushing" and "pulling" approach for T1D treatment.

Poster Number 117

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Topoisomerase repression by immune-restricted chromatin reader SP140 prevents development of immune disease

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Recognition of post-translational modifications on histones by chromatin 'readers' is a critical step in the regulation of gene expression for cell identity and responsiveness to environmental cues. Certainly, dysregulated chromatin readers and aberrant chromatin architecture are central events in cancer. Yet virtually nothing is known about how altered chromatin readers contribute to immunological disease. Speckled Protein 140 (SP140) is a chromatin 'reader' that is immune-restricted and loss-of-function mutations within SP140 associate with three complex immune disorders: Crohn's disease (CD), multiple sclerosis (MS) and chronic lymphocytic leukemia (CLL). We recently identified SP140 as a heterochromatin maintenance factor for macrophage gene expression and intestinal homeostasis but the mechanism of action of SP140 remains uncharacterized. Here we employed a global proteomic strategy and identified SP140 as a novel repressor of topoisomerases (TOP). We show that SP140 acts to disrupt topoisomerase-mediated DNA nicking and unwinding activity needed for transcription. In SP140 null mouse or human macrophages, or peripheral blood mononuclear cells from CD patients bearing loss-of-function SP140 variants, SP140-TOP complexes were disrupted resulting in unleashed TOP activity, and severely compromised lineage-defining and microbe-inducible immune cell transcriptional programs that could be rescued with pharmacological inhibition of TOP1 or TOP2. Furthermore, Sp140-/- mice that displayed exacerbated colitis were uniquely restored with FDA-approved TOP1 or TOP2 inhibitors in vivo. Collectively, our findings identify SP140 as a novel regulator of topoisomerases and TOP inhibition as a precision medicine strategy for manipulation of the aberrant immune transcriptome due to loss of SP140 chromatin reader.

Poster Number 118

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IL-33/regulatory T cell axis attenuates cutaneous fibrosis in systemic sclerosis

INVESTIGATORS: S. Y. Cheon, A. H. Ameri, R. Nazarian, S. Demehri

Fibrosis is the main pathological hallmark of systemic sclerosis (SSc), a rare but deadly autoimmune disease affecting the connective tissues of multiple organs. Immune pathways that escalate fibrotic pathology of SSc have been thoroughly mapped; however, there is a lack of understanding in how immune system, in association with alarmins, controls fibrosis in tissues. The immune role of IL-33, a member of the IL-1 family, and its receptor Interleukin 1 receptor-like 1 (IL1RL1), which is also known as Suppressor of Tumorigenecity (ST2), in dermal fibrosis has not yet been well elucidated. The purpose of this study is to test the hypothesis that Interleukin-33 (IL-33)/regulatory T cell (Treg) axis attenuates cutaneous fibrosis in systemic sclerosis (SSc). To investigate the immune mechanism of regulatory T cells in context of alarmins of skin fibrosis, we subjected WT C57BL/6 mice to subcutaneous bleomycin (BLM) or control PBS injection protocol to induce skin fibrosis, modeling skin sclerosis. After confirming elevation of IL-33 mRNA and protein levels in bleomycin treated skin, we treated ST2 deficient mice (ST2KO) with bleomycin. We show that the ST2KO mice has increased dermal fibrosis compared to its treated wild type counterpart. We were then able to demonstrate IL-33/Treg axis role in attenuation of dermal fibrosis via using a mouse model where where ST2 is only deleted specifically within Tregs. The lack of IL-33/Treg axis in this mice model led to increased development of dermal fibrosis when treated with bleomycin. In conclusion, the IL-33/Treg axis attenuates bleomycin-induced cutaneous fibrosis in mice.

Poster Number 119

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Enrichment of photodynamically-primed anti-tumor immune infiltrates in pancreatic cancer: Enabling enhanced immunotherapy

INVESTIGATORS: P. De Silva, M. A. Saad, A. P. Carmago, T. Hasan

Pancreatic ductal adenocarcinoma (PDAC) has a dismal 5-year survival rate. Immunotherapy has brought hope for cancer therapeutics. However, for PDAC patients, even modest success is limited to ~2% of patients, as most tumors lack immune infiltration necessary for effective immunotherapy. Thus, there is an unmet need to enhance the immunogenicity of PDAC. Photodynamic therapy (PDT) is an FDA approved modality that utilizes light, a photo-responsive non-toxic chemical called a photosensitizer, and oxygen to generate reactive molecular species that confer cytotoxicity or prime the tumors to be more responsive to traditional therapies. We aimed to investigate PDT-induced immunogenicity in PDAC. Using an immunocompetent mouse model of PDAC, we evaluated tumor infiltrating lymphocyte (TIL) infiltration in tumors as a response to PDT. There was a gradual increase of TIL infiltration at 1h time point to 5 days post-PDT. T cell subset analysis showed a higher infiltration of CD8+ T cells. We also observed proliferating T and B cells in the Splenic B cell follicles at 1h post-PDT, suggesting an early activation of an adaptive immune response. Activated dendritic cells were localized in the spleens treated with PDT compared to the untreated controls. Moreover, in the same tumors, there was a significant increase of PD1 and CTLA4 immune checkpoints expression on CD8+ T cells by day 5 post-PDT highlighting enhanced immunogenicity, which may benefit from immunotherapy. Our data shows a possibility of triggering an immediate and effective anti-tumor immune-response by PDT in PDAC tumors, enhancing the immunogenicity of this tumor type.

Poster Number 120

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The chromatin reader SP140 regulates innate immune responses to microbes and intestinal homeostasis

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Genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) within SP140 that associate with Crohn's disease (CD) in addition to multiple sclerosis (MS) and chronic lymphocytic leukemia (CLL). SP140 is a transcriptional regulator expressed exclusively in immune cells. It is part of the Speckled Protein family that also contains SP100, SP110, and SP140L and shares high homology with autoimmune regulator (Aire). We have previously shown that SP140 is a critical orchestrator of macrophage identity by occupying and repressing silenced lineage-inappropriate genes. However, the role for SP140 functional domains in regulating macrophages identity and transcriptional responses to microbial sensing remained unknown. Here we show how SP140 influences innate immune responses to microbes. By engineering domain mutants, we investigate how the chromatin-binding domains (SAND, plant homeodomain, and bromodomain) and the caspase activation and recruitment domain (CARD) within SP140 dictate macrophage cytokine production. Furthermore, we determine how SP140 localization to promyelocytic leukemia (PML)-nuclear bodies affects function. Global deficiency of SP140 results in exacerbated colitis in mice, and a loss of SP140 specifically in the hematopoietic compartment results in microbial dysbiosis. Collectively, our work provides functional characterization of the novel chromatin regulator SP140 and how disease variants may contribute to immunological disease.

Poster Number 121

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Exercise training boosts infiltration of CD8+ T cells and sensitizes tumors to immune checkpoint therapy

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Exercise can promote health and beneficial effects to cancer patients. However, how exercise training (ExTr) induces antitumor effects is not well understood. Here, we show underlying mechanisms by which ExTr exerts antitumor activity in three murine breast cancer models – E0771, MCa-M3C and EMT6 – grown in syngeneic female mice (C57BL/6, FVB, and Balb/c, respectively). Moderate intensity exercise training (45 min daily on a running treadmill) started when tumors were established (~100 mm3). We found ExTr consistently delays tumor growth, normalizes abnormal tumor vasculature (increasing vessel maturity, perfusion and reducing hypoxia). RNA sequencing analysis indicated a shift in metabolic signature towards oxidative metabolism and enhanced antitumor immunity. Flow cytometry and immunohistochemistry indicated that ExTr boosted tumoral infiltration and effector function of CD8+ T cells. The therapeutic benefits of ExTr depends on CD8+ T cells, as their depletion abrogates the exercise-mediated tumor control. Mechanistically, we found that ExTr enhances the CXCL9/11-CXCR3 chemokine system that recruits CD8+ T cells to tumors, whose antitumor effect is prevented in Cxcr3-/- mice (C57BL/6 background). Furthermore, ExTr prevents the development of distant lung metastasis, and sensitizes refractory tumors to immune checkpoint blockade (anti-PD1 alone or in combination with anti-CTLA4). Since exercise has already been recommended to cancer patients as supportive physiological intervention, proper ExTr prescription should be investigated in clinical trials and rapidly extended to clinical practice.

Poster Number 122

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Transgenic overexpression of human ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1, or hCD39) in pig to primate xenotransplantation models

INVESTIGATORS: K. Deng, K. M. Lee, Z. A. Habibabady, C. G. Rickert, K. J. Ahrens, J. M. O, D. Becerra, P. M. Patel, W. Westlin, M. Youd, N. Serifis, T. M. Coe, D. Cloonan, A. M. Azimzadeh, C. LeGuern, J. C. Madsen, S. C. Robson, J. F. Markmann, Triple knock out pigs and study were funded by eGenesis

Human CD39 (hCD39) is the gene of interest in xenotransplantation due to its reported capacity to prevent platelet aggregation by metabolizing extracellular ATP to AMP, reducing IL-6 and ALT and depleting resident CD4+ T lymphocyte in the graft. One heterotopic cardiac xenotransplantation using a TKO-hCD39 donor (n=1) survived >100 days. We studied the impact of endothelial cell activation and expression of hCD39 on survival of aortic endothelial cells (AEC) recovered from normal and genetically modified pigs. AECs from wild type (WT) (n=1), TKO.hCD39 (n=3), TKO (n=3) pigs and one human deceased donor were isolated and activated using LPS (1ug/mL), Ionomycin (1ug/mL), and PMA (50ng/mL) for 9, 12, 18 and 24 hours at 37°C. CD31 and hCD39 expression and viability were measured by flow cytometry. TKO.hCD39 AECs expressed more hCD39 than hAECs, while, as expected, no human CD39 was detected on WT or TKO AECs (Fig 1A). However, after nine hours of LPS challenge, hCD39 expression by the TKO.hCD39 AECs decreased to less than 50% of baseline, while expression of CD39 remained stable in the human AECs. At nine hours, substantial cell death, as well as decreased CD39, was observed in the activated TKO.hCD39. At 18 -24 hours, cell death measurements in the TKO.hCD39 AECs neared the maximal level observed . In the human AECs at 18 hours the signal representing dead hAECs was high. Overexpression of transgenic CD39 in the pig AEC did afford significant protection, potentially as levels of hCD39 decreased with cellular activation.

Poster Number 123

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Reconstruction of genome-scale models of macrophage metabolism in celiac disease at single-cell resolution

INVESTIGATORS: A. R. Zomorrodi

Celiac disease (CD) is an autoimmune digestive disease that progressively damages the small intestine upon consumption of gluten – a protein found in wheat, rye and barley. While the adaptive immune response to gluten in CD is well studied, the innate immune response, which plays a key role in early stages of CD development, is still poorly characterized. The innate immune response is primarily mediated by macrophages, which can polarize into pro- or anti-inflammatory states. While it is known that certain metabolic pathways are up- or down-regulated in each of these states, the mechanisms by which gluten or metabolites produced by the gut microbiota elicit an innate immune response by macrophages in CD are insufficiently characterized. To address this knowledge gap, we sought to reconstruct genome-scale models of metabolism for human intestinal macrophages in both healthy subjects and CD patients using single-cell gene expression data. Toward this end, we start with a genome-scale model of global human metabolism as the template and use single-cell RNA sequencing data from healthy subjects and CD patients to reconstruct metabolic models of macrophage metabolism in these subjects. Next, we will use these genome-scale models to computationally investigate what gluten peptides and microbially-derived metabolites in the gut are involved in the differentiation of intestinal macrophages to pro- or anti-inflammatory states and to explore what intracellular metabolic pathways get activated during macrophage polarization. Taken together, these studies provide a road-map for systematically identifying immunomodulatory metabolites and potential mechanisms that are critically linked to macrophage activation.

Poster Number 124

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Analysis of Oral Food Challenge Outcomes to Sesame

INVESTIGATORS: A. M. Swensen, C. E. Rosenberg, L. B. Robinson, E. S. Stieb, P. Hesterberg, W. G. Shreffler, Y. V. Virkud

Background: Sesame allergy, one of the most common food allergies, is burdensome and often misdiagnosed. Oral food challenges (OFCs) are the gold standard for diagnosing sesame allergy; however, this procedure is not without risk of anaphylaxis and is resource-intensive. Current diagnostic testing such as skin prick testing (SPT) and sesame-specific IgE (sIgE) have variable diagnostic performance and defined clinical parameters are needed. Objective: To describe sesame OFC outcomes and assess the predictive value of clinical testing. Methods: Retrospective chart review was performed on 117 sesame challenges completed by 113 patients, aged 1-38 years, who received care at the Massachusetts General Hospital allergy clinics (2008-2020). Results: Sesame OFCs included 70 (60%) passes, 14 (12%) indeterminates, and 33 (28%) failures, which is similar to challenge failure rates for other allergens (26%). Median eliciting dose was 60 mg sesame protein (range: 40-6000 mg). Nine (8%) individuals required epinephrine. Failed challenges were associated with larger sesame SPT (median SPT passes vs fails: 3 mm vs 7 mm, $P<0.001$), but not with sesame sIgE (median sIgE passes vs fails: 1.1 kU/L vs 2.17 kU/L) or history of prior sesame reaction. Fifty-four (77%) individuals passed sesame OFCs despite history of positive sesame sIgE and/or sesame SPT. No individual with sesame SPT greater than 8 mm passed their challenges. Conclusions: Sesame SPT is a more accurate predictor of sesame allergy compared with sesame sIgE and history of prior sesame reaction. A diagnostic cutoff of SPT of 8 mm may prove useful for guiding clinical decision making.

Poster Number 125

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High mobility group box 1 mediates corneal subepithelial infiltrates in adenovirus keratitis

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Ocular surface infection by viruses within human adenovirus species D (HAdV-D) causes epidemic keratoconjunctivitis (EKC). Subepithelial infiltrate (SEI) formation in the cornea is the most significant long-term complication of EKC, and causes reduced vision and photophobia in about one-third of cases. However, the mechanism of SEI formation after adenoviral infection of the cornea remains unknown. High-mobility group box protein 1 (HMGB1) is an endogenous danger signal molecule and a chemokine that variably regulates inflammatory responses. Although HMGB1 has been implicated previously in viral infections, and may play a role in bacterial keratitis, its role in EKC has not been studied. Herein, we infected hTERT-immortalized human corneal epithelial (THE) cells and primary human corneal fibroblasts (HCF) with HAdV-D37 or HAdV-C5, and evaluated the expression of HMGB1 by western blotting, immunofluorescence, and ELISA. HAdV-D37 infection resulted in HMGB1 translocation from the nucleus to the cytoplasm in both THE cells and HCF, but was secreted only by THE cells. HAdV-C5, a virus not associated with EKC, did not induce expression of HMGB1 from either cell type. Cytokine expression by HCF treated with rHMGB1 was analyzed using human cytokine protein arrays. Active recombinant HMGB1 treatment of HCFs triggered expression of several pro-inflammatory mediators. Our results suggest that HMGB1 secreted by adenovirus infected corneal epithelial cells could induce underlying stromal cells to express pro-inflammatory mediators, leading indirectly to the development of SEI. HMGB1 may be a viable therapeutic target for preventing the corneal stromal complications of EKC.

Poster Number 126

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Preliminary Analysis of the Effect of Genetic Modifications of the Porcine Genome on Inflammatory and Coagulation Responses During Ex-Vivo Porcine Liver Perfusion with Human Blood

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Background: Xenotransplantation using pig organs provides a promising solution to the shortage of donor organs, but this is limited by human xenoreactive antibodies that cause early graft rejection. Genome editing can eliminate xenoantigens in donor pigs to minimize the impact of these xenoantibodies. Herein, we describe the effect of various genetic modifications of the pig donor on the inflammatory and coagulation responses of human blood after ex-vivo perfusion of porcine livers.

Methods: Ex-vivo liver perfusion of wild type (WT) (n=2), GTKO.CD55 (n=1) and two variations of triple knock out (GTKO, ?4GalKO, CMAHKO) pigs with additional genetic modifications targeting complement, coagulation, and inflammation regulation (Pig 2.05; n=3, Pig 2.09; n=3) was completed using human whole blood and plasma. EDTA plasma samples were collected for human IL-1B, IL-6, IL-8, TNF-α, fibrinogen and D-dimer measurement by Luminex assay.

Results: Liver perfusion lasted for 5-7 hours for WT, 3 hours for GTKO.CD55, 8-11 hours for 2.05 and 12-14 hours for 2.09. Elaboration of IL1-B, IL-6 and TNF-α and IL-8 was lower in the 2.05 and 2.09 groups when compared to WT and GTKO. CD55 livers. Groups 2.05 and 2.09 showed decreased consumption of fibrinogen and decreased production of D-dimer compared to WT livers throughout ex-vivo perfusion.

Conclusions: Our preliminary data are encouraging and suggestive of reduction in pro-inflammatory cytokines, coagulation markers and liver survival prolongation compared to WT pigs. However, due the small numbers tested to date we cannot yet draw firm, statistically validated conclusions. Studies in progress are to further substantiate these findings.

Poster Number 127

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Distinct effector functions of NK cells in peripheral tissue

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Natural killer (NK) cells belong to the innate lymphoid cell (ILC) family and play an important role in controlling viral infections and malignant cells. NK cells express an array of activating and inhibitory receptors and a balance between these distinct signals dictates the outcome of NK cell response. Several Ly49 family receptors inhibit NK cells in response to MHC-I molecules on host cells (i.e. self-tolerance), while, Ly49H is an activating receptor with specificity for murine cytomegalovirus (MCMV) encoded protein m157. In the syngeneic transplant setting, NK cells from recipient mice reject MHC-I deficient (β2m-/-) donor cells in circulation while spare the β2m-/- solid organ transplant, however, the mechanism behind this altered NK cell response in peripheral tissues is unknown. Here, we utilized m157-Ly49h axis and MHC-I deficiency in the syngeneic skin transplantation model to investigate the requirements for NK cell activation in solid organs. Combination of m157 expression with loss of β2m failed to induce skin graft rejection despite the robust NK and T cell infiltration. Large numbers of skin recruited NK cells remained conventional having the full potential to be cytotoxic. However, MHC-mismatch, together with m157 activation, in the context of F1 skin transplantation, resulted in skin graft rejection which was dependent on both, NK and T cells. Transcriptome analysis by RNA sequencing of circulatory NK cells vs skin-infiltrated NK cells revealed distinct effector functions following the recruitment in the skin. Currently, we are studying the factors that induce this immediate switch in NK cells upon entering the skin.

Poster Number 128

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Exploiting Survival Mechanisms of Plasmodium to Combat Severe Malaria

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Malaria kills one child every 30 seconds reaching upto 3000 children a day with more than 70% of victims under the age of 5 years and is responsible for death and suffering of millions every year. Transmitted by mosquitoes, the malarial parasite invades the blood stream and hijacks red blood cells (RBCs), using them to provide shelter and food. This leads to fever, anemia, respiratory complications, multiple organ failure and death in severe cases. One of the great medical successes of the 20th century is the development of antimalarial drugs. However, malaria has adapted to most of the treatments and has become resistant. Currently, many patients with severe malaria cannot be effectively treated and, in such patients, the outlook is grim. To solve this problem, we took advantage of the malarial parasite's survival mechanisms by first feeding it a naturally occurring compound called ALA (5-aminolevulinic acid). ALA is selectively taken up by the infected versus the healthy RBCs. When the parasites are overloaded on ALA, they become light-responsive in the process. Then illuminating red-light resulted in proof-of-concept studies for treatment of malaria. The red-light exposure gave the infected RBCs a lethal "sunburn" and killed malaria under therapeutically relevant conditions. To bring this approach to the clinic, we propose using blood-donation-machinery to withdraw and extract blood, shine red light on it and return it to the patient. Thus, the results presented in this study have set the stage that will be applicable worldwide in areas affected by malaria.

Poster Number 129

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Clinical Risk Factors Predicting Cutaneous Abscess and MRSA Incidence for Cellulitis in Hospitalized Pediatric Patients

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Background: Cellulitis is a common skin infection that can be complicated by the formation of cutaneous abscess, a walled accumulation of pus, and that is frequently caused by methicillin-resistant Staphylococcus aureus (MRSA).

Methods: A single-center, retrospective chart review of 893 pediatric inpatients from 2007 through 2019 was conducted. Patients were excluded if they had intensive care unit stays or had complicated infection sites such as overlying recent surgery or indwelling hardware. Multivariate analysis for prediction of cutaneous abscess was conducted on all included patients and for prediction of MRSA incidence was conducted on all patients receiving a wound culture.

Results: 559 patients (63.3%) had cellulitis and met inclusion criteria, of which 216 patients (38.6%) had cutaneous abscess. 290 patients (51.9%) had wound culture obtained with 117 patients (40.3%) having MRSA growth. Independent predictors of cutaneous abscess were infection of the groin and buttocks (OR 2.80; 95% CI 1.09, 7.17), leukocytosis at presentation (OR 1.89; 95% CI 1.35, 2.66), and MRSA nasal carriage (OR 1.95; 95% CI 1.28, 2.98). Independent predictors of MRSA incidence were infection of the groin and buttocks (OR 5.86; 95% CI 1.00, 34.4) and MRSA nasal carriage (OR 29.3; 95% CI 12.9, 66.5).

Conclusion: Both infection of the groin and buttocks and MRSA nasal carriage emerged as predictors of cutaneous abscess and MRSA incidence in inpatient pediatric cellulitis and should be considered especially for evaluation for possible surgical management and for judicious selection of antibiotics.

Poster Number 130

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Clinical and radiologic characteristics in varicella zoster virus (VZV) reactivation

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Objective: This study describes clinical and magnetic resonance imaging (MRI) characteristics in varicella zoster virus (VZV) reactivation.

Methods: This is a retrospective case-series with confirmed VZV infection affecting the eye, brain or spinal cord from 2006-2020.

Results: Thirty-seven cases were included. The mean age was 64.7 years (SD: ?17.1 years), 21 (56.7%) were men, and 9 (24.3%) were immunocompromised. Cerebrospinal fluid (CSF) was available in 22/36 (61%) patients; median CSF white blood cell count was 11 cells/ul (range 0-154), protein 40 mg/dL (range 20-496) and glucose 65 mg/dL (range 46-103). CSF VZV PCR assays were positive in 6/21 (29%) and 8/9 vitreous or anterior chamber aqueous (90%) samples. Clinical involvement included the optic nerve in 12 (32.4%), other cranial nerves in 20 (54%), brain parenchyma in 12 (32.4%) and spine/nerve roots in 4 (10.8%) patients.

Twenty-four/26 immunocompetent patients (92%) had MRIs for review. Optic perineuritis (OPN) was observed in 8 (33%) as optic nerve sheath enhancement on T1 post-contrast fat-saturated sequences; all 8 experienced vision loss: 3 optic neuritis, 1 acute retinal necrosis, and 3 CNS vasculitis with 1 central and 1 branch retinal artery occlusion and 1 uveitis. In diplopic patients, findings included cavernous sinus enhancement and polycranial neuritis. Seven/8 immunosuppressed patients (88%) had greater radiographic neuroaxis involvement, including encephalitis, vasculitis and transverse myelitis; a single case had OPN.

Conclusion: Here we show that OPN is a common neuroradiographic finding in VZV-associated vision loss in immunocompetent patients. Optimizing MRI protocols to assess for OPN in such cases is warranted.

Poster Number 131

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COVID-19 Diagnostic Clinical Decision Support: A Pre-Post Implementation Study of CORAL (COVID Risk Calculator)

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Background: Isolation of hospitalized persons under investigation (PUIs) for COVID-19 reduces nosocomial transmission risk. Efficient PUI evaluation is needed to preserve scarce healthcare resources. We describe the development, implementation, and outcomes of an inpatient diagnostic algorithm and clinical decision support system (CDSS) to evaluate PUIs.

Methods: We conducted a pre-post study of CORAL (COvid Risk cALculator), a CDSS that guides frontline clinicians through a risk-stratified COVID-19 diagnostic workup, removes transmission-based precautions when workup is complete and negative, and triages complex cases to Infectious Diseases (ID) physician review. Pre-CORAL, ID physicians reviewed all PUI records to guide workup and precautions. Post-CORAL, frontline clinicians evaluated PUIs directly using CORAL. We compared pre- and post-CORAL frequency of repeat SARS-CoV-2 nucleic acid amplification tests (NAATs), time from NAAT result to PUI status discontinuation, total duration of PUI status, and ID physician work-hours, using linear and logistic regression, adjusted for COVID-19 incidence.

Results: Fewer PUIs underwent repeat testing after an initial negative NAAT post-CORAL than pre-CORAL (54% vs. 67%; aOR 0.53, 95% CI: 0.44-0.63, p<0.01). CORAL significantly reduced average time to PUI status discontinuation (adjusted difference: -7.4 [SE 0.8] hours/patient; p<0.01), total duration of PUI status (adjusted difference: -19.5 [SE 1.9] hours/patient; p<0.01), and average ID physician work-hours (adjusted difference: -57.4 [SE 2.0] hours/day; p<0.01). No patients had a positive NAAT within 7 days after discontinuation of precautions via CORAL.

Conclusions: CORAL is an efficient and effective CDSS to guide frontline clinicians through the diagnostic evaluation of PUIs and safe discontinuation of precautions.

Poster Number 132

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Cost-effectiveness of public health strategies for COVID-19 epidemic control in South Africa: A microsimulation modeling study

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Objective: To evaluate the clinical and economic outcomes and cost-effectiveness of COVID-19 public health strategies in KwaZulu-Natal, South Africa.

Methods: We developed a microsimulation model to assess the clinical outcomes and costs associated with different strategies to address COVID-19. Interventions assessed were Healthcare Testing (HT), where diagnostic testing is performed only for those presenting to healthcare centers; Contact Tracing (CT) in households of cases; Isolation Centers (IC), for cases not requiring hospitalization; Mass Symptom Screening and testing for symptomatic individuals (MS); and Quarantine Centers (QC), for household contacts testing negative. We evaluated two main epidemic scenarios over 360 days, with effective reproduction numbers (Re) of 1.5 and 1.2. We compared combinations of interventions, considering those with incremental cost-effectiveness ratio (ICER) < $3250/year-of-life saved (YLS) cost-effective. In sensitivity analyses, we varied Re, molecular testing sensitivity, and efficacies and costs of each strategy.

Results: With Re 1.5, HT+CT+IC+MS+QC reduced mortality by 94%, increased costs by 33%, and was cost-effective ($340/YLS) compared to HT. With Re 1.2, HT+CT+IC+QC was the least costly and most efficient strategy; HT+CT+IC was the next least costly strategy. HT+CT+IC+MS+QC was cost-effective in many sensitivity analyses; notable exceptions were when Re was 2.6 and when the efficacies of ICs and QCs for transmission reduction were substantially lower.

Conclusions: In South Africa, strategies involving household contact tracing, isolation, and mass symptom screening, with or without quarantining household contacts, would substantially reduce mortality and be cost-effective. The optimal combination of interventions depends on epidemic growth characteristics and practical implementation considerations.

Poster Number 133

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Healthcare worker perceptions of COVID-19 preparedness at antiretroviral therapy pick-up points in a decentralized HIV care program in South Africa

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Decentralized HIV care models have been proposed as a means to provide safe continuity of HIV care during the COVID-19 pandemic. The Central Chronic Medicines Dispensing and Distribution (CCMDD) program allows stable patients with HIV to collect antiretroviral therapy (ART) at community-based venues, such as private pharmacies and schools, in Kwazulu-Natal, South Africa. We evaluated healthcare worker perceptions of preparedness for safe delivery of ART through the CCMDD program during the COVID-19 pandemic. We administered semi-structured telephone interviews to healthcare workers at primary health clinics and community-based pick-up points participating in the CCMDD program at two time points: April-May 2020 and August 2020. We completed interviews with 136 healthcare workers, including 49 clinic staff in April-May 2020, and 63 clinic staff and 24 pick-up point staff in August 2020. Here we show a discrepancy in preparedness to safely delivery ART between staff in clinics and community-based pick-up points. In clinics, the proportion of staff reporting resources to screen patients for COVID-19, necessary information to perform their duties during the time of COVID-19, and access to necessary personal protective equipment increased significantly over time to 100%, 94%, and 89% by August 2020, respectively. At the same time point, only 54% of pick-up point staff reported access to COVID-19 screening resources, 67% to necessary information, and 50% to necessary personal protective equipment. While decentralized HIV care programs hold promise for continuity of care during a pandemic, community-based venues for medication delivery must be adequately supported to continue safe provision of care.

Poster Number 134

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Cryopreservation of infectious Cryptosporidium parvum oocysts by ultra-rapid vitrification

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Cryptosporidiosis, an enteric infection caused by Cryptosporidium parasites, is a major cause of acute infant diarrhea and diarrhea-associated mortality in the developing world. A major bottleneck to research progress is the lack of methods to cryopreserve Cryptosporidium oocysts, thus requiring continuous propagation of medically relevant species in laboratory animals. The obstacles to cryopreservation by traditional methods are oocyst sensitivity to ice formation and the impermeable nature of the oocyst wall preventing exclusion of water and inclusion of cryoprotectants (CPAs) necessary to protect cells during freezing. Here we demonstrate methods to both permeabilize and achieve ice-free cryopreservation by ultra-rapid vitrification of the zoonotic isolate of C. parvum. Water was excluded from oocysts using extracellular hyperosmotic gradient of trehalose, while permeability to CPAs was achieved by alteration of the oocyst wall structure with hypochlorite. Oocysts were frozen in liquid nitrogen using novel devices engineered to enable cooling rates as high as ~250,000°C/min, namely silica microcapillaries and polycarbonate high-aspect ratio cassettes. Cryopreserved oocysts exhibit high viability, robust in vitro excystation and infectivity to interferon-γ knockout mice comparable to that of unfrozen control. Importantly, oocyst viability and infectivity is not visibly changed after several weeks of cryogenic storage. The application of our cryopreservation method will permit the scientific community to share well characterized isolates and transgenic lines and will substantially relieve the effort of continuous passage in animals. Most significantly, our method will enable generation of uniform, optimized and standardized parasite preparations for the upcoming human challenge studies for the evaluation of therapeutics.

Poster Number 135

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A microscale, full-thickness, human skin-on-a-chip model for skin infections and antibiotic treatment

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Cellulitis, an infectious skin disease, accounts for $3.7 billion in ambulatory care costs with 14.5 million cases/year in the United States. Unfortunately, one-third of the cellulitis patients are often misdiagnosed because of a group of non-infectious skin diseases, pseudo-cellulitis, which mimic symptoms and signs of cellulitis without a microbe. Therefore, a novel tool is required for the objective diagnosis of cellulitis.

In this study, we present an ex vivo human skin-on-a-chip (SOC) model for studying immune responses to skin infection and antibiotic treatment. A micro-biopsy technique allowed for harvesting a full-thickness, human, microscopic skin tissue columns which were > 100 times smaller than traditional punch biopsy. This minimally invasive technique allowed for minimizing the pain and healing without a scar. A rational design of a microfluidic device allowed for neutrophil migration directly from a drop of whole blood (< 10 ul) without an isolation step. Combining microfluidics and micro-biopsy, we monitored the neutrophil's migration from the whole blood to healthy and Staphylococcus aureus inoculated skin samples. We found a positive correlation between the number of migrated neutrophils and the amount of bacteria on the skin. We used our platform to evaluate the antibiotic treatment by comparing the immune response between the penicillin-treated and non-treated skin columns. Our results suggests that neutrophils may be a potent biomarker for skin infection. We also envision that in the future, our platform will augment the physician's ability to differentiate infectious cellulitis from non-infectious pseudo-cellulitis.

Poster Number 136

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Studying early Mycobacterium Tuberculosis infection in situ using lung slice models

INVESTIGATORS: J. Liu, X. Ai, Prof. Deborah T. Hung

It is unknown what in early stage of the infection decides whether tuberculosis (TB) infection will be eliminated, contained as asymptomatic or developed into deadly active. Functional heterogeneity of alveolar macrophages (AM) in response to Mycobacterium Tuberculosis (Mtb) has been proposed responsible but remains an unresolved issue due to the lack of a model that retain in vivo AM phenotype and is readily investigable. We developed lung slice infection model that enables observation of early interaction between AM and Mtb in alveolar space. AMs in human, marmoset and mouse lung slices eliminated BCG (Bacillus Calmette–Guérin), the vaccine strain for tuberculosis, within five days post infection, consistent with the in vivo observation that BCG CFU of mouse alveolar macrophages dropped over one log in the first week after BCG administration intratracheally, during which alveolar macrophages were the predominant host cells of BCG. On the contrary, primary BAL (bronchoalveolar lavage) alveolar macrophages from marmoset and mouse permitted intracellular BCG to persist and grow, as well as other commonly used models including mouse bone marrow derived macrophage and J774 macrophage cell line. Intracellular growth of BCG in BAL alveolar macrophages can be prevented by co-culturing with lung slices, suggesting lung-derived signals are necessary for alveolar macrophages to properly kill BCG. Overall, the successful establishment of the lung slice model makes possible the investigation of early Tb infection in situ and boost the discovery of key mechanism underpinning the variable Tb outcomes.

Poster Number 137

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A Novel Diagnostic Test for Invasive Fungal Infections

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A rapid and accurate diagnostic method for invasive fungal infections remains a critical clinical need. We recently reported a new rapid molecular method for bacterial species identification directly from clinical samples by targeting highly abundant ribosomal RNA on a multiplexed hybridization platform called NanoString. Here, we extend this approach to fungi and demonstrate that we can readily distinguish 10 phylogenetically similar Candida species, including the emerging highly resistant pathogen Candida auris, within 4 hours. We also demonstrate our level of detection and species distinction averages around a single yeast cell. The clinical application of such a test could transform clinical fungal infection diagnostics from slow, low-yield fungal culture to rapid, accurate molecular-based testing which would ultimately impact timely clinical care.

Poster Number 138

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Impact of monocytes and macrophages on liver inflammation and injury in SARS-CoV-2

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SARS-CoV-2, which predominantly affects the lungs, is associated with elevated liver biochemistries in over half of patients. The contribution of macrophages during viral infection to the liver pathogenesis is increasingly being recognized. Cytokine storm, which is a common feature in SARS-CoV-2 infection, may be a critical factor in polarizing macrophages, and in shaping them to function in advancing liver damage. The aim of the present work is to identify the macrophage phenotype associated with SARS-CoV-2 infection, and the role of these cells in cytokine storm and liver injury. A total of 89 sera and PBMC samples were obtained from SARS-CoV-2 infected individuals at Massachusetts General Hospital (MGH). Key M1 and M2 polarization markers were measured by qRT-PCR. A Luminex assay was used to assess circulating cytokines. Analysis of mRNA revealed higher expression of the M2 markers (CD163 and ARG1), the M1 markers (TNFα, NOS2 and IL-1β), as well as of the regulators of inflammatory responses (CD5L), in COVID-19 positive compared with convalescent patients. Similarly, circulating levels of inflammatory cytokines such IL-6, IL-8, CXCL10, and TNFα were significantly higher in plasma from COVID-19 positive than in convalescent patients (IL-6, IL-8, CXCL10, and TNFα). Furthermore, expression of ACE2 and TMPRSS2 in SARS-CoV-2 positive patients was elevated compared to convalescent patients (ACE2, and TMPRSS2. Our data show that macrophages in SARS-CoV-2 patients are neither clearly M1 nor M2 polarized. However, monocytes and macrophages from symptomatic COVID-19 patients appear to produce large amounts of pro-inflammatory cytokines, which contribute to liver inflammation and injury.

Poster Number 139

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Tackling Recalcitrant P. aeruginosa Infections in Critical Illness by Targeting the Pseudomonas aeruginosa Quorum Sensing Regulator MvfR (PqsR) with Novel Non-Ligand-Based Antagonists

INVESTIGATORS: V. K. Singh, M. Almpani, L. G. Rahme

Pseudomonas aeruginosa, presents a serious threat to critically ill and immunocompromised patients. P. aeruginosa infections are challenging to eradicate due to their high levels of antibiotic resistance and the development of Antibiotic Tolerant, Persister (AT/P) cells, and biofilms. One of the most attractive novel anti-microbial strategies is inactivation of quorum sensing (QS), a cell-cell communication signaling mechanism employed by bacteria to efficiently coordinate their behaviors. Our group discovered MvfR, the transcriptional regulator of one of the four interconnected P. aeruginosa QS systems that governs many virulence functions in this pathogen. The present work reports a novel family of highly efficient anti-MvfR non-ligand-based compounds, namely the N-Aryl Malonamides (NAMs). Our results indicate that NAMs inhibit P. aeruginosa virulence, as shown by the reduced pyocyanin production and expression of the MvfR-regulated operon, with a half maximal inhibitory concentration (IC50) at a nanomolar range. NAMs drastically prevent the formation of P. aeruginosa AT/P cells and biofilm, and they also inhibit pyocyanin production in several clinical P. aeruginosa isolates. In vivo, our compounds protect the host intestinal barrier function following P. aeruginosa infections and lower the levels of inflammatory cytokines (TNF-a and IL-6) in the small intestine of the mice. The present study shows the efficacy breadth of our novel family of non-ligand-based anti-MvfR agents in vitro and in vivo. Further research will determine whether the reported compounds could be effective antibiotic adjuncts against P. aeruginosa.

Poster Number 140

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Unique brain-specific antibody signatures in chronic HIV infection

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Background: The brain is an important HIV reservoir. Given its difficult accessibility to quantify viral burden, HIV-specific cerebrospinal fluid (CSF)-antibodies (rather than systemic antibodies) have been suggested as critical biomarkers of HIV-disease in the brain.

Methods: We applied a Systems Serology approach to thoroughly dissect the antibody profiles (Ig (sub)classes, Fcγ receptors [FcγR] binding capacity and antibody-mediated innate immunity functions) in the plasma and CSF of 20 chronically infected (11 ART-treated and 9 untreated) HIV+ individuals.

Results: High titers of HIV-specific antibodies were detected in both plasma and CSF. However, striking brain-specific antibody signatures were identified: 1) unlike plasma antibodies that were of all Ig classes, CSF showed predominantly IgG1, IgG3, and no IgM; 2) CSF-antibodies had lower capacity to activate innate immunity functions and bind FcγR; 3) CSF-antibodies showed a weak binding to the neonatal FcR (FcRN), which mediates the transport of circulating antibodies to and from the brain; 4) this low FcRN affinity was not observed for antibodies targeting Flu, HSV1, HSV2, CMV and EBV, suggesting a retention of HIV-specific antibodies (but not antibodies to other viruses) within the brain; 5) ART-treatment was associated with higher polyfunctionality of plasma-antibodies compared to CSF-antibodies, pointing to a reduced effect of ART in the brain.

Conclusions: These data suggest a unique compartmentalization of subpopulations of antibodies in the CNS during chronic HIV infection, either through selective antibody transfer from the periphery across the blood-brain barrier or by local production by B cells undergoing maturation to plasma cells under brain-specific selective pressure.

Poster Number 141

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Mosaic chromosomal alterations (mCAs) in leukocytes are a novel risk factor for diverse infections, including severe COVID-19

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Age is the dominant risk factor for infectious diseases, but the mechanisms linking the two are incompletely understood. Age-related mosaic chromosomal alterations (mCAs) detected from blood-derived DNA genotyping, are structural somatic variants associated with aberrant leukocyte cell counts, hematological malignancy, and mortality. Whether mCAs represent independent risk factors for infection is unknown.

Here we use genome-wide genotyping of blood DNA to show that mCAs predispose to diverse infectious diseases. We analyzed mCAs from 768,762 individuals without hematological cancer at DNA acquisition across five biobanks (Mass-General Brigham Biobank, Columbia University Biobank, UK Biobank, FinnGen, Biobank Japan). Expanded mCA (cell fraction >10%) prevalence approached 4% by 60 years of age. In particular, expanded autosomal mCAs were associated with diverse incident infections (HR 1.25; 95% CI 1.15-1.36; P=1.8x10^-7), including sepsis (HR 2.68; 95% CI 2.25 to 3.19; P=3.1x10^-28), pneumonia (HR 1.76; 95% CI 1.53-2.03; P=2.3x10^-15), digestive infections (HR 1.51; 95% CI 1.32-1.73; P=2.2x10^-9), genitourinary infections (HR 1.25; 95% CI 1.11-1.41; P=0.00037), and cardiac infections (HR 1.97; 95% CI 1.28-3.03; P=0.0021). Additionally, expanded autosomal mCAs were associated with coronavirus disease 2019 (COVID-19) hospitalization (OR 2.44; 95% CI 1.33 to 4.46; P=0.0038) in the UK Biobank and FinnGen. A genome-wide association study of expanded mCAs identified 63 significant loci, enriched at transcriptional regulatory sites for immune cells.

Our results link mCAs with impaired immunity and predisposition to infections. Furthermore, these findings may also have important implications for the ongoing COVID-19 pandemic, particularly in prioritizing individual preventive strategies and evaluating immunization responses.

Poster Number 142

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Assessment of Ankle Fractures using Deep Learning Algorithms and Convolutional Neural Network

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Background: Early and accurate detection of ankle fractures is crucial for reducing morbidities and disabilities in the patient while it also reduced the burden for the healthcare system. Deep learning (DL) methods as a subset of artificial intelligence have shown promising potentials in clinical image analysis and detection of abnormalities through creating convolutional neural networks (CNN). Given radiographs are the most accessible technique in different settings, we aimed to create DL algorithms for detecting ankle fractures, particularly occult fractures, using single-view and 3-views radiographs.

Methods: A novel DL method was used to create our CNN using radiographs obtained from 1050 patients with ankle fractures and the same number of individuals with otherwise healthy ankles. Out of 1050, 72 individuals were labeled as occult fractures as they were not detected in the primary radiographic assessment. Data stacks were created based on single-view and 3-view (anteroposterior, mortise, lateral) radiographs.

Results: Our CNN showed a better performance using 3-view images versus single-view based on greater values for accuracy, F-score, and area under the curve (AUC). The sensitivity and specificity in detection of ankle fractures using single-view images were 97.5% and 93.9% compared to 98.7% and 98.6 using 3-views, respectively. Using our first algorithm we missed 3 occult fractures while on the second try we missed only one case.

Conclusion: Here we showed the promising potential of deep learning algorithms applied to the current diagnostic radiography technique that can act as an assistant to the clinicians for diagnosing ankle fractures faster and more accurately.

Poster Number 143

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What is the Difference in Survival and Complication Profile Between Patients Undergoing Surgery for an Impending versus a Pathological Fracture in Metastatic Bone Disease?

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The purpose of this study was to assess differences between surgical treatment of impending versus pathological fractures in long bone metastases in (1) 90-day and 1-year survival and (2) 30-day postoperative complications, reoperations, intraoperative blood loss, anesthesia time, perioperative blood transfusion, and duration of hospitalization. Therefore, we retrospectively performed a propensity score matched cohort study of 1,064 patients including 462 impending pathological fractures and 602 pathological metastatic long bone fractures. After matching based on 22 explanatory variables, including primary tumor type and visceral metastases, 270 impending pathological fracture cases were matched with 270 pathological fracture cases.

We found that 90-day survival did not differ between both groups (HR, 1.09, 95% confidence interval (CI), 0.79-1.51, P=0.589), while 1-year survival was higher in the impending pathological fracture group (HR, 1.25, 95%CI, 1.00-1.56, P=0.047. Regarding the secondary outcomes, the impending fracture group underwent fewer reoperations (OR, 2.50, 95%CI, 1.92-7.86, P=0.049); had less intraoperative blood loss (P=0.03); had less blood transfusions (P=0.01); and had shorter anesthesia time (P=0.04), but no differences were found for 30-days postoperative complications, and hospitalization duration.

In this study we show that patients undergoing surgery for an impending pathological fracture had a lower 1-year mortality rate and better secondary outcomes as compared to surgery for a pathological fracture, while accounting for a large number of potential confounders. Patients with an impending pathological fracture seem to benefit from early surgery as developing a complete fracture seems to negatively influence survival, reoperation risk, duration of surgery, blood loss, and blood transfusions.

Poster Number 144

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Automated Volume Measurement of the Syndesmosis Using 3D Weightbearing CT

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Background: Recent studies have shown that weightbearing computed tomography (WBCT) that allows 3D volume measurement of the distal syndesmosis while under physiologic load brings about a higher accuracy in detection of syndesmotic instability, especially if subtle. The volume measurement method is complex, time-consuming, and has a noticeable interobserver bias. Here we aimed to automatize the measurement using computer-assisted methods to reduce the interobserver bias make the process faster and more applicable in practice.

Methods: We included 30 patients with syndesmotic instability who had undergone WBCT and were diagnosed intraoperatively. Thirty individuals with otherwise healthy ankles were allocated to the control group. The volume measurement up to 5cm proximal to the joint was used to assess syndesmotic stability based on the literature. An algorithm was developed using MATLAB software that could recognize and calculate the syndesmotic volume using WBCT images. The volume measurement method was used by two orthopaedic surgeons as well. The time spent by each method was measured. The values were compared using the t-test; the interobserver correlation coefficient (ICC) was calculated. $P<0.05$ was considered statistically significant.

Results: There was no significant difference regarding the demographic data of the two groups. The ICC between the clinicians was 75% while using the algorithm showed 97%. The mean time spent by the clinicians was 268.4±56.4 and by the algorithm was 2.9±0.3 seconds ($p<0.001$).

Conclusion: Developing a faster and more accurate method for 3D volume measurement of the syndesmosis renders this method more practically reliable and easier to use by the clinicians.

Poster Number 145

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Racial and ethnic differences in the transition from acute to chronic low back pain: a cohort study

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We examined whether the rate of transition from acute low back pain (aLBP) to high-impact chronic low back pain (cLBP) differs by race/ethnicity. We used data from the TARGET Trial, a longitudinal cohort study of adults with aLBP from four US-based geographic regions (Baltimore, Boston, Pittsburgh, Salt Lake City). At 6 months we identified the incidence of high-impact cLBP, using the NIH cLBP definition (back pain for six months, on at least half of days) and at least moderate disability using the Oswestry Disability Index (ODI, >30 points). We also evaluated PCP-prescribed medications for aLBP. From May 2016 to June 2018, we recruited 9088 adults with aLBP (81.3% White; 14.3% Black; 4.4% Hispanic). At baseline, Black and Hispanic participants, compared to White participants, were younger and more likely to be female, obese, on Medicaid, have worse disability on ODI, and have high-risk of persistent pain using the STarTback Tool (all p<0.0001). At 6 months, the proportion of Black and Hispanic participants with high-impact cLBP (30% and 25%, respectively) was higher compared to White participants (15%, p<0.0001). Adjusting for baseline differences, compared to White participants, Black participants were more likely to develop high-impact cLBP (aOR=1.56 95%CI: 1.16-2.08). Black and Hispanic participants were less likely than Whites to receive an opioid prescription (11%, 11%, and 20%, respectively, p<0.0001) and more likely to receive an NSAID prescription (38%, 41%, and 26%, respectively, p<0.0001). In a large national cohort of patients with aLBP, Black and Hispanic participants were more likely to develop high-impact cLBP.

Poster Number 146

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Increased bone mass, bone formation, and bone resorption caused by adult onset compound deletion of Sik2 and Sik3 in mice

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Osteoporosis is a serious public health concern in our rapidly aging population. By 2025, there will be 3 million osteoporotic fractures costing $25 billion annually. FDA-approved medications are insufficient to increase bone mass and reduce fracture risk.

Salt inducible kinases (SIK) control parathyroid hormone action in osteocytes downstream of cAMP and protein kinase A. Previous work by our lab demonstrated that in vivo combined deletion of Sik2 and Sik3 in osteocytes and osteoblasts using DMP1-Cre showed dramatic increase in bone mass, increased bone turnover, and gene expression changes similar to those seen with constitutive PTH signalling. These results suggest that SIK2/3 inhibitors may represent a new class of PTH-like small molecules. However, the organism-wide consequences of deleting these kinases in postnatal mice remains to be determined. Here, we addressed this problem by breeding Sik2/3 floxed mice to ubiquitin-CreERt2 animals to achieve ubiquitous deletion of these kinases in a tamoxifen-dependent manner. Combined Sik2/3 deletion in adult mice led to a dramatic increase in trabecular bone mass and growth plate expansion by micro-CT. Fasting serum analysis demonstrated accelerated bone turnover with elevated bone formation and bone resorption levels in Sik2/3 mutants versus tamoxifen-treated control mice. Also, SIK2/3 DKO mice showed significant higher number of osteoblasts and osteoclasts. Differences at the level of bone resorption exist between genetic Sik2/3 deletion and our previous results using pharmacologic SIK inhibitors, supporting the notion that Sik2/3 inhibitors may represent a promising new class of pharmacologic agents for osteoporosis treatment.

Poster Number 147

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The enemy from within: Chronically aberrant inflammation within the dopaminergic neurons could be the inciting factor of neurodegeneration

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Dopaminergic neuron (DN) degeneration is the hallmark of Parkinson's disease. The etiology and course of events remain puzzling. The role of inflammation, as exerted by microglia surrounding the DNs has long been studied. Yet, inflammation within the DNs has not received adequate attention.

Employing the RNA interference technology, we developed a Drosophila melanogaster model, in which Cactus, the fly homologue of the mammalian IkB, is knocked down (KD) selectively in the DNs. Fly survival, and a series of brain functions (geotaxis, phototaxis, smell chemotaxis) were assessed. Brain inflammation was determined by measuring the levels of brain antimicrobial peptides (AMPs) by RT-PCR. Brain DN staining was performed to determine changes in the DN number overtime. Proton nuclear resonance spectroscopy (1H NMR) of the fly heads was employed to identify metabolic alterations.

Cactus KD leads to increased AMPs in the brain. Cactus KD flies have a shorter lifespan and exert impaired brain functions overtime as compared to their control counterparts. Brain DN staining reveals a loss of DNs overtime, which correlates well with the impairment of the fly brain functions. The NMR studies showed a reduction of trehalose, choline, phosphocholine and NADPH in the brain of the Cactus KD flies on day 14 as compared to control flies of the same age, as well as in comparison to day-5 Cactus KD flies.

Our results show that dysregulation of the immune signaling inside the brain DNs, rather than in microglia, could be the inciting event that causes chronic inflammation leading to dopaminergic neurodegeneration.

Poster Number 148

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Neuroinflammatory and functional connectivity signatures in radicular and axial chronic low back pain

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We recently showed elevated levels of the 18kDa translocator protein (TSPO), a marker of neuroinflammation, in chronic low back pain patients (cLBP) compared to healthy controls(Loggia et al., 2015). Here, we test whether TSPO signal 1) can further subtype cLBP patients based on their clinical presentation, and 2) is associated with functional connectivity measures (because neuroinflammation may affect neuronal communication(Clark et al., 2015)).

Patients with axial (cLBPAX; n=26; 43.7±16.6 y.o.) or radicular cLBP (cLBPRAD; n=28; 48.3±13.2 y.o.) received an integrated PET/MRI scan with the TSPO ligand [11C]PBR28. TSPO signal was quantified using standardized uptake values normalized by whole-brain signal (SUVR).

Functional connectivity from primary somatosensory cortex (S1) (i.e., a region significant in the PET group comparison) was calculated from BOLD resting-state fMRI data. Connectivity and PET measures were compared across groups, correlated with and against each other and against the Fibromyalgia Screening Questionnaire (FSQ) scores, a measure of pain "centralization".

In S1, TSPO signal and functional connectivity to the thalamus were: 1) higher in cLBPRAD compared to cLBPAX; 2) positively correlated with each other and 3) positively correlated with FSQ scores.

Our data support the existence of different "neuroinflammatory signatures" in patients with different clinical presentation and that S1 neuroinflammatory signal is more pronounced in patients with higher pain "centralization". Further, because S1 TSPO signal was correlated to S1-thalamus connectivity, our data support an association between neuroinflammatory changes and changes in neuronal communication, possibly indicating that the observed alterations reflect "neurogenic neuroinflammation".

Poster Number 149

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Inter-brain directed connectivity in patient-clinician dyads during evoked pain: An EEG hyperscan study

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In chronic pain management and treatment, a positive patient-clinician relationship is associated with higher patient satisfaction and treatment efficacy. Here, we investigated synchronous brain activity in patient-clinician dyads during an experimentally controlled augmented (empathetic) compared to limited (business-like) clinical interaction context. We recorded EEG simultaneously (hyperscanning, 18 dyads, two 64 channels) from low back pain (cLBP) patients and acupuncturists during a task with treatment trials (acupuncture) and no-treatment trials in conjunction with evoked cuff pain. Brain sources were reconstructed using the algorithm eLORETA on 17 ROIs defined by our previous fMRI study (Ellingsen 2020) and directed statistical connectivity patterns were obtained by comparing Granger Causality-based estimates for treatment and no-treatment. Patients in the augmented group, compared to the limited group, rated therapeutic alliance and clinician warmth significantly higher. LBP intensity decreased after acupuncture treatment, irrespective of clinical context. EEG analysis suggested that in Theta band (previously linked with empathy for pain), inter-brain density within social mirroring and pain/sensorimotor brain regions was altered when acupuncturists treated evoked pain. Interestingly, the prevalent direction of the inter-brain connections is clinician-to-patient during no-treatment, and patient-to-clinician during treatment. Also, the density of patient-to-clinician connections increased during treatment trials irrespective of clinical context, more significantly for the augmented style group. Both behavioral and brain responses across the patient/clinician dyad reflect clinical interaction context. EEG hyperscanning is an ecologically valid approach to identify inter-brain networks whose density and directionality is altered by treatment and clinical context, highlighting new brain mechanisms linking therapeutic alliance and chronic pain therapy.

Poster Number 150

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Mechanisms of chloride influx in hypoxic-ischemic encephalopathy

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Hypoxic-ischemic (HI) injury, with an incidence of 2-6 of every 1,000 live births, is a type of brain injury in newborns caused by oxygen deprivation and limited blood flow. HI injury is strongly associated with later neurodevelopmental disabilities and is the leading cause of neonatal seizures: Approximately 50-60% of newborns with HI injury develop seizures in their first few days of life. These acute seizures adversely affect the developing brain causing later handicaps such as cerebral palsy and epilepsy. Current treatments with available first line agents such as phenobarbital, phenytoin, and/or benzodiazepines are only successful in controlling less than half of the neonatal seizure cases. Excitatory actions of GABA in developing neurons may underlie the inefficacy of these drugs. Inhibitory or excitatory GABAergic signaling in adult or developing central nervous system is controlled by the balance between cation-chloride transporters (i.e. KCC2 and NKCC1) activity. Developing neurons often have higher expression of NKCC1 and higher intracellular chloride concentration ([Cl-]i), which renders GABA activity excitatory.

Here we show that in an in vitro model of HI injury neuronal volume and [Cl-]i change in response to injury. We found that these changes were correlated with neuronal age and predicated by its cation-chloride transporter expression. After HI injury, immature neurons, with high NKCC1 expression, shrink; while mature neurons, with high KCC2 expression, swell. We confirmed these findings with pharmacological application of NKCC1 or KCC2 blockers and immunohistochemistry. Our results indicate that cation-chloride transporter activity might explain the inefficacy of anti-seizure medication in neonatal seizures.

Poster Number 151

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Sex-specific lesion patterns of long-term functional outcome after stroke

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Introduction: Acute ischemic stroke (AIS) has a varying impact on men and women. For instance, women feature a higher AIS severity than men that cannot be fully explained by key clinical variables. Further, we observed distinct sex divergences in lesion topographies in a study of 555 AIS patients: only in women, AIS severity was particularly strongly affected by lesions in the left posterior circulation. The aim of the current study was to determine sex-specific lesion pattern effects on long-term functional outcome.

Methods: We relied on data of 822 AIS patients of the MRI-GENIE study (age: 64.7(15.0), 39% female, 27% poor outcome, mRS>2). AIS lesions were segmented from DWI-images, spatially normalized and parcellated (atlas-defined 109 (sub) cortical regions, 20 tracts). Subsequently, we employed data-driven matrix factorization to obtain quintessential ten lesion patterns. Poor functional outcome was modeled via Bayesian hierarchical regression, taking the ten sex-specific lesion patterns and the covariates age, sex, cardiovascular risk factors and total lesion volume as inputs.

Results: We derived ten anatomically plausible lesion patterns. Three out of these ten patterns substantially contributed to the explanation of poor outcome for both men and women (AUC=0.81). These lesion patterns primarily comprised bilateral subcortical grey matter regions and left-lateralized regions in proximity to the insula. Additionally, a lesion pattern of left posterior circulation regions had a substantially higher relevance in women compared to men.

Conclusions: We here present evidence that female-specific lesion pattern effects relating to left-hemispheric posterior circulation regions not only explain AIS severity, yet also impact long-term outcomes.

Poster Number 152

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A Computational Model of a Microcoil-Based Magnetic Cochlear Implant

INVESTIGATORS: S. Fried

Around 466 million people worldwide are affected by disabling hearing loss. Over 90% of cases are due to sensorineural hearing loss (SNHL), which typically occurs when cochlear hair cells are damaged from aging, excessive noise, or disease. To date, more than 700,000 patients have received electrode-based cochlear implants (eCIs) which have been demonstrated to improve speech comprehension in quiet environments. Their effectiveness has been limited, however, in part due to their poor spectral resolution (each electrode activates a broad range of frequencies). Our group is investigating methods to improve cochlear implant efficacy by using magnetic stimulation from small, implantable bent-wire micro-coils (mCIs) as an alternative to traditional eCIs.

Here, we have developed a novel biophysically realistic human cochlear model to investigate the relationship between mCI design and the response of auditory nerve fibers. We compared both the strength of induced electric fields and the spectral resolutions of different coil widths, shapes, and positioning within the cochlea (perimodiolar vs. lateral wall).

A larger micro-coil width was associated with stronger induced fields along the auditory nerve fibers. Rectangular and u-shaped coils had a higher spectral resolution in comparison to v-shaped coils. Perimodiolar implants generated stronger fields but exhibited poorer spectral resolution compared to lateral wall implants. Our results suggest that computational modeling can be used to estimate mCI design efficacy; a comparison across different design features will help to accelerate design optimization. The model also suggests that mCIs have high spectral resolution, raising the possibility that they may help improve clinical outcomes.

Poster Number 153

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Accelerating the Super-Resolution Multi-Scale Deep Convolutional Neural Network in the Treatment of Degenerative Spondylolisthesis

INVESTIGATORS: S. Fatima, J. H. Shin

Background: Automated image characterization is one of the most crucial components of a computer-aided diagnosis (CAD) system for degenerative spondylolisthesis (DS). In this paper, we propose and evaluate a convolutional neural network (CNN) designed for deciding the best management option for patients with DS.

Methods: The dataset used for training was made using the database from multiple academic tertiary care hospitals in the U.S. In the preprocessing step, all X-Rays with a slightly different pixel spacing were rescaled to match a specific spacing value (i.e., 0.4 mm).

Results: The proposed network consists of 6 convolutional layers with 2*2 kernels and LeakyReLU activations, followed by average pooling with size equal to the size of the final feature maps and four dense layers. The second last dense layer has 2 outputs, equivalent to the classes considered: conservative and surgical. The last dense layer has outputs, equivalent to the classes considered: decompression alone, decompression with posterior fusion and instrumentation, decompression with posterior fusion without instrumentation, combination of anterior and posterior fusion and minimally invasive procedure. To train and evaluate the CNN, we used a dataset of 22100 image patches derived by 850 X-Rays from different scanners and academic hospitals. To the best of our knowledge, this is the first deep CNN designed for the specific problem. The classification performance is now tested prospectively and validating the outcome among patients in a real-time.

Conclusions: CAD system will assist the spine surgeons across the world and increase the diagnostic and management accuracy.

Poster Number 154

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Functional dyspepsia shows altered post-meal resting-state functional brain connectivity between nucleus tractus solitarii and cortical networks

INVESTIGATORS: R. Sclocco, R. Staley, K. Han, A. Mendez, C. Nguyen, B. Kuo, V. Napadow

Functional dyspepsia (FD) patients experience upper gastrointestinal (GI) symptoms possibly due to gastric motor or sensory dysfunction. The nucleus tractus solitarii (NTS) receives and integrates afference from visceral organs including the GI tract and projects to regions involved in central autonomic regulation and pain processing. We hypothesize that altered NTS connectivity to higher brain regions in FD will be associated with motor and sensory dysfunction during a meal challenge.

FD (N=15) and healthy control (HC) (N=14) subjects consumed their maximum tolerable amount of a 470ml high-calorie pudding. Post meal, resting-state blood oxygen-level dependent (BOLD) functional MRI data were acquired (Siemens 3T Skyra). Average BOLD timeseries were extracted from a left NTS region and used to generate seed-to-voxel whole-brain functional connectivity maps. These maps were contrasted between groups (FEAT, FSL) and compared to canonical resting state networks using point-biserial correlations.

NTS connectivity maps were most correlated with the Default Mode Network (DMN) in both groups, yet significantly more so in HCs ($p = 0.048$). In contrast, the NTS network was more correlated with the FrontoParietal Network (FP) in FD patients compared to HCs ($p = 0.03$). Relative to HCs, FD patients demonstrated NTS functional connectivity decreased to posterior cingulate cortex, and increased to medial prefrontal cortex, superior frontal gyrus, bilateral inferior frontal gyri, and left anterior insula.

Our results reveal a shift in NTS functional connectivity from the self-referential processing DMN to the executive control processing FP network, potentially related to an altered cognitive processing of interoceptive (gastric) signaling in FD.

Poster Number 155

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Conductance-Based Structural Brain Connectivity in Aging and Dementia

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Structural brain connectivity has been shown to be sensitive to the changes that the brain undergoes during Alzheimer's disease (AD) progression. In this work, we used our recently proposed structural connectivity quantification measure derived from diffusion MRI, which accounts for both direct and indirect pathways, to quantify brain connectivity in dementia. We analyzed data from the second phase of the Alzheimer's Disease Neuroimaging Initiative (ADNI-2) and the third release in the Open Access Series of Imaging Studies (OASIS-3) datasets to derive relevant information for the study of the changes that the brain goes through in AD. We also compared these datasets to that of the Human Connectome Project (HCP), as a reference, and eventually validated externally on two cohorts of the European DTI Study in Dementia (EDSD) database. Our analysis shows expected trends of mean conductance with respect to age and cognitive scores, significant age prediction in aging data, and regional effects centered among sub-cortical regions, cingulate, and temporal cortices. Results indicate that our conductance measure has prediction potential, especially for age, that age and cognitive scores largely overlap, and that this measure could be used to study effects such as anti-correlation in structural connections.

The method used in this work is sensitive to direct and indirect pathways in deriving brain connectivity measures from diffusion MRI, and therefore provides information that many state-of-the-art methods do not account for. As a result, this technique may provide the research community with ways to detect subtle effects of healthy aging and AD.

Poster Number 156

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The patient-clinician relationship in a longitudinal fMRI hyper-scanning study of chronic pain patients: Neural and behavioral correlates

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Previous research suggests that a warm-empathic (Augmented) compared to a neutral business-like (Limited) patient-clinician relationship can improve clinical outcomes. In this longitudinal study, we applied fMRI hyperscanning including a video connection between two scanners and an evoked cuff pressure pain-treatment paradigm to determine whether behavioral, neural, and clinical variables associated with the therapeutic alliance influence clinical outcomes. Eleven women with fibromyalgia (mean age=38.55, SD=11.25), randomly assigned to either an Augmented (N=5) or a Limited (N=6) patient-clinician dyadic interaction style, were treated with electro-acupuncture for 6 biweekly sessions, and scanned simultaneously before and after the intervention. Clinical outcomes and the quality of the patient-clinician relationship were assessed (e.g. pain levels, therapeutic alliance, trust, warmth, competence). As rated by patients, pooled therapeutic alliance and clinicians' warmth in the Augmented group (mean_alliance=43.17, SD=2.84; mean_warmth=4.00, SD=0.0) were significantly higher than in the Limited group (mean_alliance=28.84, SD=9.97; mean_warmth=3.14, SD=0.72; t_alliance(9)=3.04, p=0.014; t_warmth(9)=2.65; p=0.027). Additionally, over all 6 acupuncture treatment sessions, most patients experienced post-treatment clinical pain relief ($p<0.001$). Brain imaging data analysis focused on social mirroring areas such as the temporo-parietal junction (TPJ), as we found stronger dynamic coupling in dyads with stronger therapeutic alliance in our previous hyperscan study (Ellingsen et al., 2020). Our preliminary results from this longitudinal patient-clinician interaction study highlight the influence that the therapeutic relationship can have on brain processing of nociceptive signaling and clinical outcomes. Moreover, we hope that our results will inform future chronic pain treatment approaches, especially how to harness the positive effects of the patient-clinician relationship.

Poster Number 157

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Improving representative research participation in the Alzheimer's Disease Neuroimaging Initiative study

INVESTIGATORS: J. M. Ison, J. L. Gonzalez, J. D. Jackson

Research participation among historically minoritized communities remains low despite various initiatives to increase representative engagement, recruitment, and enrollment. For the present study, researchers developed a multi-site pilot intervention within the Alzheimer's Disease Neuroimaging Initiative study to examine methods of increasing diverse and inclusive research participation, with diverse participants in this sector defined as non-White, Hispanic/Latinx, and/or non-female. Four sites across the country were selected to participate. Each site identified a target population and a specific barrier they perceived this population to face. Sites also developed novel interventions to address this barrier within their chosen population. Seventy-five percent of those recruited from the target populations across the four sites were subsequently enrolled. Moreover, compared to nonparticipating sites in the Alzheimer's Disease Neuroimaging Initiative, those in the present study had a greater increase in minoritized participant recruitment over the same time period. However, significant differences emerged among sites in converting interested volunteers to enrollment. Despite vigorous community engagement and partnerships across sites, there was no single mechanism for effective diverse and inclusive research participation uptake. In summary, institutions may leverage a number of individual strengths to thoughtfully partner with local minoritized communities to improve equitable research participation.

Poster Number 158

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Mental health is an integral component of the Patient Reported Outcome Measure of Ataxia.

INVESTIGATORS: C. F. Villarin, J. MacMore, S. Pierce, G. L'Italien, J. Schmahmann, National Ataxia Foundation

Background: Scales for the assessment of cerebellar ataxia are agnostic to patient input; and the impact of cerebellar disease on cognition and emotion has been overlooked by medical providers.

Objective: We tested the hypothesis that symptoms reported by patients with cerebellar ataxia would reflect mental health deficits underlying the cerebellar cognitive affective / Schmahmann syndrome.

Methods: A conceptual framework was developed by asking 147 patients with cerebellar ataxia to describe their difficulties in an open-ended survey. Responses were refined to create a 70-item questionnaire, the Patient-Reported Outcome Measure of Ataxia (PROM-Ataxia). This was validated in a cognitive debrief of 17 novel ataxia patients, subjected to test-retest reliability in 78 new patients, and validated against existing measures of ataxia severity and mental health in another 20 patients with genetic ataxias.

Results: Three domains emerged from the patient reports. Two domains - Physical (975 items), and Activities of Daily Living (523 items) reflected motor dysfunction. A third domain, accounting for 18.8% of reported items (347/1,845), comprised the Mental Health Domain. Mental Domain Section 1 included feelings of depression, irritability, anxiety, anhedonia, social isolation and embarrassment, and impaired emotional control and motivation. Mental Domain Section 2 included difficulties with word finding, recall, multitasking, new learning, decision making, comprehension and concentration. The Mental Health domains correlated with the Beck Anxiety Inventory (0.58) and Beck Depression Inventory (0.44; $p < 0.05$).

Conclusions: Cognition and affect are integral components of the PROM-Ataxia. Recognition of these aspects of cerebellar dysfunction has relevance for diagnosis and patient care.

Poster Number 159

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Testing the quality of MEG source estimates using iEEG

INVESTIGATORS: M. Jas, D. Chinnapen, C. Chu, M. Hamalainen

Non-invasive magneto-/electro-encephalography (MEG/EEG) measure signals from the brain as a linear superposition of brain activity from thousands of brain locations. Source localization is an established method for unmixing the source time courses from the sensor-level signals. Despite its popularity, the source localized signals have rarely been compared directly to intracranial measurements on the same task. In this work, we analyze source-localized MEG data from an auditory sensory-gating paradigm compared to intracranial EEG (iEEG) data on the same task. We outline a framework to test the quality of source-localized data obtained from different estimation procedures (e.g., minimum norm estimates vs dipole fitting) or devices (conventional MEG vs on-scalp optically pumped magnetometer based MEG) by comparing it with nearby iEEG sensor data. Our data indicates that while such a comparison is certainly feasible, it comes with several pitfalls and challenges. In the future, we will develop a forward model for iEEG to reduce the bias and allow for a more fair comparison.

Poster Number 160

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Blocking translation to rescue ALS/FTD phenotypes associated with C9ORF72 repeat expansion

INVESTIGATORS: X. Jiang, C. I. Aguilar, C. Lee, M. Canori, A. Ray-Soni, K. Jansen-West, F. Rigo, L. Petrucelli, F. Martin, C. Lagier-Tourenne

Expansion of G4C2 repeats in a non-coding region of the C9ORF72 gene is the most prevalent inherited form of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The expansion produces sense G4C2 and antisense C4G2 repeats-containing RNA foci and five repeat dipeptide proteins (DPRs) through abnormal repeat-associated non-AUG (RAN) translation. Our previous work demonstrated that, both in vitro and in cultured cells, the translation of G4C2 transcripts requires a near cognate CUG codon located upstream of the repeats. Importantly, the mutation of the CUG codon does not affect the formation of G4C2 RNA foci, providing us with a unique tool to compare the impact of C9ORF72 RNA versus DPRs toxicity on neurodegeneration. To investigate the effect of CUG start codon on RAN translation in vivo, we performed intracerebroventricular injections of adeno-associated virus expressing G4C2 repeats with either CUG or a mutated CCG start codon in mice. We found that the CCG mutation dramatically decreased RAN translation suppressing the accumulation of toxic DPRs proteins in mice expressing 66 repeats. Blocking RAN translation, without disturbing the production of C9ORF72 transcripts and accumulation of RNA foci, was enough to rescue ALS/FTD-related hyperactivity and motor deficits in aged mice. This work provides mechanistic insights on the contribution of DPRs to neuronal death and demonstrates that targeting the aberrant production of DPRs is a potential therapeutic strategy for C9ORF72 disease. Encouraged by these results, we are now testing the therapeutic potential of antisense oligonucleotides to block RAN translation in iPSC-derived neurons from C9ORF72 ALS/FTD patients.

Poster Number 161

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Spontaneous hyperactive astrocytic processes and concomitant hypoactive endfeet accompany amyloid plaques in the brain of awake APP/PS1 transgenic mice

INVESTIGATORS: P. Kelly, S. S. Hou, M. Sanchez-Mico, S. Whiteman, E. Hudry, M. Arbel-Ornath, S. M. Greenberg, B. J. Bacskai

Astrocytes are an abundant brain cell and have a complex cellular architecture with processes that interact spatially with neurons and the vasculature. Astrocytes exhibit tightly regulated spontaneous intra- and intercellular calcium activity that may underlie many important neuronal and vascular-related physiological roles, including functional hyperemia and the paravascular clearance of solutes from the brain. Here we classified and quantified the diverse hierarchy of spontaneous calcium events in astrocytes within the brain of awake APP/PS1 mice, compared to non-transgenic littermates. Some spontaneous intracellular calcium events remained localized within astrocytic processes, of which the greatest proportion occurred within fine processes (32/98; 33% of all events, 0.2-1.4 events/min) and vascular endfeet (28/98; 29% of all events, 0.2-1.2 events/min) and to a lesser extent within some primary processes (12/98; 12% of all events, 0.2-0.6 events/min) in 9 awake non-transgenic mice. However, within the brain of 10 awake APP/PS1 mice, astrocytic spontaneous localized calcium activity was more active within fine processes (42/111; 38% of all events, 0.2-1.8 events/min) and primary processes (25/111, 23% of all events, 0.2-0.8 events/min) but hypoactive within astrocytic endfeet (11/111; 10% of all events, 0.2-0.4 events/min) when compared to non-transgenic littermates. Different spontaneous calcium events involving the central somatic compartment occurred with greater amplitude in the brain of APP/PS1 mice compared to wild-type mice. These data demonstrate that the amyloid plaque environment within the brain of awake APP/PS1 mice can disrupt the complex and compartmentalized spontaneous intracellular calcium events within cortical astrocytes, with potential pathophysiological implications for synaptic and vascular health.

Poster Number 162

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Amblyopia's Impact on the Fine-Scale Functional Organization of Human Extrastriate Visual Cortex Revealed Through Ultrahigh Field MRI

INVESTIGATORS: B. Kennedy, S. Nasr

Amblyopia is a developmental disorder caused by disruption of symmetric binocular visual input early in life. Most amblyopic individuals suffer from impairments in stereopsis, spatial vision, and motion perception, especially in the central visual field. However, the underlying neural disorders (e.g. neural degeneration vs. re-organization) have remained mostly unknown. This is mainly a result of the small size of cortical columns involved in motion and stereopsis perception compared to the spatial resolution of fMRI techniques used in conventional human studies.

We used high-resolution fMRI (1 mm isotropic) to study the impact of strabismus and anisometropia (two major natural causes of amblyopia) on visual processing. We localized color-, stereo-, and motion-selective columns in areas V2, V3, and V3A in 11 amblyopes (7 strabismic and 4 anisometropic) and 14 controls.

Our findings indicated that the absence of proper binocular input in amblyopia leads to a decrease in the size of stereo-selective sites. Interestingly, this effect was accompanied by an increase in the size of motion- and color-selective sites across all visual areas. Despite this increase in the size of motion-selective sites, the level of motion-selective response, especially in higher speeds and lower coherency levels (around the center of visual field), was weaker in the amblyopes compared to the controls.

Consistent with the general idea of brain plasticity, these results suggest that the absence of proper binocular input in amblyopia leads to changes in the distribution (i.e. re-organization rather than degeneration) and sensitivity of cortical columns that encode motion, color, and stereopsis.

Poster Number 163

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Training non-human primates for visual prostheses

INVESTIGATORS: Y. Kfir, J. S. Pezaris

The Visual Prosthesis Lab at MGH is developing a device to restore sight to the blind. Current invasive devices target either directly the retina or the primary visual cortex, however the lateral geniculate nucleus (LGN), a mid-brain relay station functionally and physically between the two has significant advantages that make it an attractive target for vision restoration. The LGN is a compact and highly organized structure with simple and well-characterized receptive fields, separated Magno, Parvo, and Koniocellular Pathways, and is accessible using techniques from deep brain stimulation.

To demonstrate the efficacy of LGN stimulation, and to study how electrical stimulation is perceived, we are using non-human primates as a model system. We have Rhesus macaques perform specific behavioral tasks, from which we infer the properties of their visual perception. These trial-based tasks, such as recognition of shapes through simulations of artificial vision, require extensive training that, when done in the time-limited laboratory environment can take upwards of a year to bring the animal to criterion performance. Here, we develop a home-cage training system that will shift the main training effort from the laboratory to the monkey home-cage so as to provide ad-libitum access every day of the week. This system will allow the monkeys to train on self-initiated visual tasks and perform an order-of-magnitude more trials than is possible today, shrinking the training time from a year down to 1-2 months.

Poster Number 164

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A novel genetic mutation in the WWOX gene leads to mitochondrial dysfunction in amyotrophic lateral sclerosis

INVESTIGATORS: S. E. Kim, T. Petrozziello, A. N. Mills, S. M. Farhan, K. Vakili, G. Sadri-Vakili

Understanding the pathogenic mechanisms leading to motor neuron degeneration in amyotrophic lateral sclerosis (ALS) is vital for the development of new therapies. Here, we describe for the first time a novel genetic variant in the WW domain-containing oxidoreductase (WWOX) gene, which plays a role in DNA damage repair, oxidative stress, and neuronal survival, that may contribute to ALS pathogenesis. Genetic analysis of the Project MinE data set revealed several rare variants in WWOX in 4,366 ALS patients that were completely absent in gnomAD. Among these variants, we identified a stop codon mutation 261E (WWOXSTOP261E) located in the mitochondrial binding region of the short-chain alcohol dehydrogenases (SDR) domain of WWOX. Given that the SDR domain of WWOX is involved in regulating the mitochondrial electron transport chain (mtETC) and mitochondrial dysfunction has been suggested as an early pathogenetic event in ALS, we assessed the mechanisms whereby WWOXSTOP261E could alter mitochondrial function. Our findings revealed a significant decrease in the levels of the ATP synthase subunit alpha of complex V (ATP5A) and the cytochrome c oxidase of complex IV (COX II) in ALS mCTX, consistent with previous findings. Additionally, co-immunoprecipitation experiments revealed that WWOX interacts with ATP5A in ALS. Lastly, treatment of SH-SY5Y cells with the human recombinant WWOXSTOP261E protein reduced cell viability, decreased ATP levels, and increased reactive oxygen species levels by inhibiting mitochondrial aconitase activity. Collectively, our findings suggest that alterations in WWOX signaling may contribute to mitochondrial dysfunction in ALS.

Poster Number 165

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Characterization of Pediatric Small-Fiber Neuropathy

INVESTIGATORS: M. C. Klein, D. C. Dredge, K. Farhad, H. M. Downs, M. M. Klein, W. S. David, A. L. Oaklander

Adult small-fiber neuropathy (SFN) was characterized in the mid-90's when development of PGP9.5-immunolabeled lower-leg skin-biopsies enabled neurite visualization. Pediatric SFN emerged later; most children remain undiagnosed and untreated. In SFN, ectopic firing/degeneration of small-diameter sensory/autonomic/trophic peripheral axons causes exertional intolerance, orthostatic hypotension, distal sensory symptoms, and gastrointestinal distress. We newly reported SFN-diagnostic skin biopsies in 53% of juvenile fibromyalgia, implying need for characterization to assist diagnosis.

Data comprises all MGH patients <18y at time of SFN-confirming skin-biopsy, and >47 age-matched healthy controls. Children/parents completed on-line adult-validated Small-fiber Symptom Surveys (SSS) and neurologists administered the Mass. General Neuropathy Exam Tool (MAGNET). We analyzed causality (medical diagnoses, blood tests, genomics) and other neuropathy testing (electrodiagnostics, composite autonomic function (AFT)) data.

Among 158 patients thus far, age averaged 14.3±3.4y (2.3-17.9y), 76% were female, 3.2% Hispanic, 91.1% Caucasian, 2.3% Asian, 1.9% Black, and 5.1% multiracial/other/unknown. Age at symptom onset averaged 9.7±5.1y. SSS scores (n=75) averaged 42.1/136±24.3 (moderate). Most prevalent symptoms were physical and/or mental fatigue, sleep difficulties, headaches, orthostatic dizziness/faintness, and restless legs. MAGNET exams were largely normal. No patients had diabetic/nutritional/metabolic causes, e.g. diabetes. Among screening blood tests, dysimmunity markers were most prevalent, with pathogenic/likely pathogenic genomic variants secondary. Variants were almost exclusively in neuropathy-related and neurologic-associated genes, suggesting specificity.

Most children with confirmed-SFN have normal neurologic exams, thus symptoms and objective tests appear more important for diagnosis. Blood testing should guide precision treatment. Our data can inform development of pediatric case definitions, SSS, MAGNET, AFT and screening recommendations, including for genomic testing.

Poster Number 166

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Intracortical depth profile of superior temporal BOLD responses to auditory and visual stimuli

INVESTIGATOR: K. Lankinen, S. P. Ahlfors, F. Mamashli, A. Blazejewska, T. Raij, J. R. Polimeni, J. Ahveninen

Electrophysiological studies in non-human primates have shown different laminar activation profiles in response to auditory vs. visual (crossmodal) stimuli in auditory and adjacent association cortices. The laminar profiles have indicated feedforward (FF) and feedback (FB) type influences. Resolving them in humans, however, has been challenging. Here, we studied cortical depth profiles of functional magnetic resonance imaging (fMRI) blood-oxygen level dependent (BOLD) signals in 13 healthy subjects using 1-mm isotropic resolution 3D echo-planar imaging at 7T. Subjects were presented with 300-ms auditory noise bursts, visual static checkerboard patterns, and their audiovisual combinations. In an oddball task, subjects were asked to detect occasional target stimuli. The fMRI data were resampled into 11 equally spaced surfaces within the gray matter, and intracortical depth-profiles of %-changes of the BOLD signal were determined in five anatomically defined regions of interest in auditory (Heschl's gyrus, HG; Heschl's sulcus, HS; planum temporale, PT; posterior superior temporal gyrus, pSTG) and polymodal (superior temporal sulcus, STS) regions. The depth-profiles were modeled with a second-degree polynomial fit. According to a linear mixed-effect model, the first and second-degree terms of the BOLD depth-profile were modulated differently for auditory vs. visual stimuli in the auditory cortices but not in STS. We demonstrate distinct laminar profiles for auditory and visual stimulation in auditory regions in humas, consistent with earlier neurophysiological work in non-human primates. Our results suggest that FF vs. FB type influences could be distinguished by using non-linear statistical modeling of laminar profiles of BOLD signals.

Poster Number 167

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In vitro ictogenesis is stochastic at the single neuron level

INVESTIGATORS: K. P. Lillis, K. J. Staley

Seizure initiation remains a fundamental, unanswered question in epilepsy research. Studying ictogenesis is challenging due to limitations in identify the site of initiation in vivo and acquiring large scale, cellular resolution data. Here, we used novel GCaMP7-based calcium imaging to dissect neuronal activity during spontaneous, recurrent seizures in hippocampal organotypic slice cultures. With this preparation, it is feasible to image the entire network, ensuring that the earliest pathological activity is captured (as no outside inputs exist). Chronic calcium imaging of the entire hippocampal network, with paired electrophysiology, revealed 3 patterns of seizure onset: low amplitude fast activity, sentinel spike, and spike burst + low amplitude fast activity onset. These patterns recapitulate common features of human seizure onset, including low voltage fast activity and spike discharges. Weeks-long imaging of seizure activity showed a characteristic evolution in onset type and a refinement of the seizure onset zone. We utilized our cellular level tracking to test if a discrete population of cells was repeatedly active at seizure onset. Although the evolution of hippocampal regional onset was fairly consistent, we found that neuronal onset sequences were highly variable seizure to seizure. Transition to seizure onset may therefore be driven by widespread state changes that allow for re-entrant seizure activity in non-stereotyped sequences seizure to seizure.

Poster Number 168

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Protein kinase C inhibition alleviates disruption of nucleocytoplasmic transport in FUS-mediated amyotrophic lateral sclerosis and frontotemporal dementia

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Amyotrophic lateral sclerosis (ALS) is a progressive and devastating neurodegenerative disorder, which causes motor neurons death and leads to fatal paralysis with respiratory failure within 2-5 years following symptom onset. Approximately 15% of patients concomitantly develop pathological features of frontotemporal dementia (FTD), the second most common early-onset dementia after Alzheimer's disease. Mutations in the Fused in Sarcoma (FUS) gene, encoding a nuclear ribonucleic acid (RNA) / deoxyribonucleic acid (DNA) binding protein, are responsible for both familial FTD and ALS cases with young-aged onset and rapid progression. Several aggregation-prone proteins associated with ALS, including TDP43 and C9ORF72 dipeptide repeat proteins, were reported to disrupt nucleocytoplasmic transport of proteins and RNA. However, the link between FUS mutations and disruption of the nucleocytoplasmic transport has not yet been evaluated in ALS/FTD patient cells. Here we show that FUS mislocalization and aggregation in the cytoplasm induces mislocalization of Lamin B1 and components of nuclear pore complexes (Nup62, Nup98, Nup153, ELYS) that co-aggregate with FUS. Lamin B1 staining shows distortion and invagination of the nuclear membrane, indicating nuclear morphology abnormality. Using small molecules screening and small interfering RNA (siRNA) knockdown we found that inhibiting protein kinase C (PKC) increases nuclear localization of FUS and attenuates nuclear membrane anomalies. Our results represent the first evidence that cytoplasmic accumulation of FUS triggers disruption of the nuclear pore complexes and the nuclear membrane integrity to cause abnormal nucleocytoplasmic transport defects. Genetic or pharmacological inhibition of PKC emerges as a promising therapeutic strategy for FUS-ALS/FTD.

Poster Number 169

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Disruption of nucleocytoplasmic transport in amyotrophic lateral sclerosis and frontotemporal dementia

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Altered RNA metabolism and the aberrant aggregation of pathogenic RNA-binding proteins (RBPs) have emerged as a central theme in neurodegenerative disease research including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Fused in sarcoma (FUS) and transactive response element DNA-binding protein of 43 kDa (TDP-43) are RBPs that play a major role in RNA metabolism and were found to be mutated in ALS/FTD. Abnormal localization of FUS or TDP-43 in the cytoplasm form aberrant aggregates with stress granules (SGs) in response to a variety of stressors. Here we show evidence of distorted nuclear membrane shape and sequestered nuclear pore proteins within SGs in induced pluripotent stem cell (iPSC)-derived motor neurons and cortical neurons from patients carrying mutant FUS or TDP-43 under stress. Under conditions of oxidative stress induced with sodium arsenite or hyperosmolar stress induced with sorbitol, FUS or TDP-43 protein readily relocates to SGs along with components of the nucleocytoplasmic transport machinery including Ran GTPase-activating protein 1 (RanGAP1) and components of the including nuclear pore complex Nup62. We have also developed a robust method based on imaging flow cytometry allowing quantification of multiple morphological and spatial properties in thousands of cells per condition. Using this method, abnormal cellular localization of the RBPs as well as alterations of the nuclear membrane and Lamin B1 localization was identified in patient fibroblasts and iPSC neurons. Overall, mutations in RBPs triggers disruption of the nuclear lamina, sequesters essential nuclear import and export factors and leads to DNA damage in ALS/FTD patient-derived cell models.

Poster Number 170

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Patient Reported Outcome Measure for Ataxia (PROM-Ataxia): Further development with test-retest reliability and external validation

INVESTIGATORS: J. MacMore, S. Pierce, M. Beiner, G. L'Italien, J. Schmahmann

Background: We developed and validated the PROM-Ataxia (70 items, 3 domains, 49 subdomains) derived from patient reports of symptoms. A semi-structured survey completed by ataxia patients provided the conceptual framework. This was internally validated using cognitive debrief by patient focus groups.

Objective: To assess PROM-Ataxia test-retest reliability; perform external validation of its different domains against published measures; and develop a short form of the PROM-Ataxia.

Methods: 1. The PROM-Ataxia will be completed three times by 140 ataxia patients and submitted anonymously. Data points captured are diagnosis and ataxia severity (stage 0-3). Time points are Days 1, 15 and 30. 2. For external validation, 20 SCA patients of different severities will complete the PROM-Ataxia and f-SARA, Friedreich's Ataxia Rating Scale Part 1 (functional) and Part 2 (ADL), Patient Impression of Function and ADL Scale (PIFAS), Neuro-QOL Upper Extremity and Lower Extremity Scales, and Beck Anxiety and Depression scales. 3. Factor analysis will facilitate generation of a short form of the PROM-Ataxia.

Results: Previous cognitive debrief showed that for the domains of physical limitations (PHYS), activities of daily living (ADL), and mental health (MEN) scale items were comprehensible, important and relevant – PHYS: 100% comprehensible, 93% important, 78% relevant; ADL: 99%, 88%, 85%; MEN: 100%, 94%, 78%. Responsiveness (correlation between total score and severity) was excellent. R values: PHYS: 0.67; ADL: 0.71; MEN: 0.51 (all $P<.05$). Internal consistency and reliability were high (Cronbach's Alpha). PHYS: 0.94; ADL: 0.93; and MEN: 0.88. The new data will be analyzed and presented at the session.

Poster Number 171

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

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Protein N-glycosylation is a protein post-translational modification carried out in the endoplasmic reticulum (ER) that is important for the folding, stability, and secretion of proteins. The assembly of the precursor glycan is carried out by a set of ALG enzymes. Mutations that disrupt the functions of these ALG enzymes cause a family of rare metabolic disorders called congenital disorders of glycosylation (CDGs). No CDG has yet been reported for either ALG10 or ALG10B (alpha-1,2-glucosyltransferases), two paralogous ALG enzyme coding genes that were created by an intrachromosomal duplication in Hominoidea 25 million years ago. Using UK Biobank data, a GWAS of human sleep disturbance revealed variants near both ALG10 and ALG10B, which affect multiple sleep and chronotype traits, consistent with their paralogy. Additionally, a patient who has an ultra-rare quadruple mutant genotype for ALG10 and ALG10B exhibits CDG-like symptoms. Utilizing Drosophila as a model system, here we show that Alg10 and the other Alg enzymes in the N-linked glycosylation pathway have a conserved role in sleep and seizures. Furthermore, using glycoproteomics we have identified targets of the N-glycosylation pathway with altered levels of glycosylation upon RNAi knockdown of Alg10. Functional analysis of these target proteins has uncovered novel Drosophila sleep and seizure genes, some of which have been previously been linked to sleep and seizure disorders in humans. In summary, combining human and Drosophila genetics, we have been able to gain a better understanding of the mechanisms that lead to CDG symptoms and uncover unexpected connections between sleep disorders and epilepsy.

Poster Number 172

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Development of Seizures in a Large Animal Model of Post-Traumatic Epilepsy

INVESTIGATORS: L. Martinez Ramirez, R. Raiyyani, G. Price, J. Zhao, A. Ding, A. Duhaime, K. Staley, B. Costine-Bartell, The CURE Team Approach to the Prevention and Treatment of Post-Traumatic Epilepsy Grant

Traumatic brain injury (TBI) can lead to post-traumatic epilepsy (PTE) in a proportion of patients, yet the pathophysiology of epileptogenesis of PTE remains unknown and is primarily studied in the lissencephalic, rodent brain. Here, we aim to determine the rate and time course of epileptogenesis in a large animal, gyrencephalic model. 13 male, Yucatan swine aged 5 months received bilateral cortical impacts using our previously characterized cortical impact model. A wireless EEG transmitter was implanted in the neck with six bio-potential leads connected to intra-cranial screws creating a 3-channel bipolar montage. EEG and synchronized video were recorded. NeuroScoreTM and LabChart ReaderTM were used to manually analyze overnight EEG with synchronized video using previously published seizure criteria. Two spontaneous seizures at least two weeks post-TBI was considered the initiation of PTE. In this interim analysis, 3 of the 9 injured subjects developed PTE at 2-8 months post-injury. To date, there is no evidence of PTE in shams. An increase in total IED frequency was observed before the onset of PTE in 2 subjects and was coincident with PTE onset in 1 subject. IED were not observed in injured subjects that did not develop PTE. The incidence of PTE in this large animal model is similar to human PTE. IED may predict the development of PTE. This large animal model of PTE may be useful for studying the pathophysiology and potential therapeutic approaches to this form of acquired epilepsy.

Poster Number 173

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A novel time-delayed correlation method can decompose frequency mismatch response without using subtraction

INVESTIGATORS: S. M. Stufflebeam, S. Khan, J. Ahveninen, M. Hämäläinen, Y. Goto, T. Maekawa, S. Tobimatsu, K. Kishida

Mismatch response (MMR) is thought to be a neurophysiological measure of auditory novelty detection that could serve as a translational biomarker of various neurological diseases. The MMR is derived by subtracting the event-related potential/field (ERP/ERF) elicited to "deviant" sounds that occur within a train of repetitive "standard" sounds. To avoid several problems associated with this subtraction procedure such as adding noise and repetition suppression effects, we propose a novel method, called weighted-BSST/k, which uses only the deviant response to derive MMR. Here, we hypothesized that weighted-BSST/k highlights periodic responses related to the deviance detection, and more sensitive than independent component analysis (ICA). To test this hypothesis, the validity and efficacy of weighted-BSST/k vs. ICA (infomax) were evaluated in 12 healthy adults using magnetoencephalography. The auditory stimuli at a constant rate of 2 Hz were presented. Frequency MMR with subtraction procedure were obtained at sensor level at 96–276 ms (MMR time range, based on the results of sensor-space cluster permutation) in bilateral temporal lobes. In the application of weighted-BSST/k, the deviant responses were put constant weight on the MMR time range. The ERF elicited by weighted deviant responses demonstrated one or a few dominant components representing MMR with high signal-to-noise ratio and similar topography to that of the sensor-space analysis. By contrast, infomax or weighted-infomax revealed many minor components of MMR. Our new approach may help the use of MMR in basic and clinical research, and it opens a new and potentially useful window into complex event-related brain data.

Poster Number 174

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Neuronal Seizure Burden versus Cell Death after Oxygen-Glucose Deprivation

INVESTIGATORS: M. A. McNally, K. P. Lillis, T. Balena, L. Lau, K. J. Staley

Rationale: Neonatal seizures in the setting of hypoxia-ischemia (HI) are common in newborns and are strongly associated with significant mortality and other poor outcomes. However, whether seizures are biomarkers or drivers of these poor outcomes is not known. The central hypothesis of this project is that neonatal seizures independently worsen brain injury after HI. The goals are to validate imaging methods for real-time seizure and cell death monitoring and to define the relationship between neuronal seizure burden and the probability of cell death.

Methods: Organotypic hippocampal slices prepared from neonatal mice expressing a neuronal red fluorescent protein and green calcium indicator (pAAV-hSyn1-mRuby2-GSG-P2A-GCaMP6s-WPRE-pA, Addgene) underwent oxygen-glucose deprivation (OGD, 20 min). Chronic imaging was performed with a high-resolution two-photon microscope. The Fiji plugin TrackMate was used to select and track neuronal ROIs. All analyses were performed in ImageJ.

Results: Seizures are consistently triggered in DIV4 hippocampal slices 1-3 hours after OGD. 31% of cells die within 24h and 40% of cells survive to 10 days (vs 90% in slices not exposed to OGD). Neurons that die after OGD are less active and have higher calcium burden interictally than neurons that survive after OGD.

Conclusions: Two-photon imaging of genetically encoded calcium indicators and constitutively expressed fluorophores permits continuous monitoring of seizures and cell death with cellular resolution in vitro. Neuronal ictal and interictal activity post-OGD may be related to the probability of delayed neuronal death.

Poster Number 175

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Simulating the effect of asynchronous phosphene stimulation on artificial vision

INVESTIGATORS: N. Meital-Kfir, J. S. Pezaris

Visual prostheses are aimed to restore sight to those who lost their vision due to disease or dysfunction of the eye. Prosthetic devices translate physical images into electrical signals which are then projected to a selected area along the visual pathway, bypassing the impaired site. Each electrical signal evokes the perception of a spot of light, called a phosphene. Multiple phosphenes can be elicited through multielectrode arrays to form images.

The quality of artificial percepts depends on the temporal and spatial characteristics of the electrical pulses and the phosphenes they generate. Here, we describe an experiment to explore the temporal relationships between individual phosphenes and the impact of the electrical pattern on object binding and perception, hypothesizing that object perception will be enhanced with synchronously presented phosphenes, and reduced with asynchronously presented ones.

Non-invasive, virtual reality technology will be used to simulate artificial vision in sighted subjects. We will assess the effect of temporal desynchronization of phosphene presentation on performance in the MNREAD reading task. Subjects will attempt to read simple sentences out loud at varying font sizes under varying levels of phosphene temporal noise and will be scored by reading accuracy and reading speed. We expect to find that reading performance is highly sensitive to desynchronization. The results of this study will be fundamental to the creation of a visual prosthesis by predicting performance under real-world post-implant usage and providing a potential tool to the prosthesis designer to enhance figure-ground separation.

Poster Number 176

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EEG Talk – A new (and fun) way to learn EEG

INVESTIGATORS: F. A. Nascimento, B. M. Westover

Neurologists should be fully capable of reading an EEG upon residency graduation per the ACGME Milestone Project. This becomes even more important because EEGs are often read by general neurologists in current American practice. Nonetheless, recurrent resident perception data has highlighted that neurology residents do not feel confident in reading EEGs independently. Given this education gap, we created a novel, free, online EEG educational method (EEG Talk) targeted at neurology residents and fellows.

EEG Talk consists of a series of 10-to-25-minute videos wherein the authors talk through a pre-selected EEG focusing on teaching points deemed to be high yield for an audience of neurology residents and fellows. Some of the episodes feature guest appearances from prestigious academic electroencephalographers. Videos are available online at the author's YouTube channel. The first video premiered on December 9, 2020; since then, weekly videos have been published (total of 7 videos). The following topics have been covered thus far: normal adult EEG, 3 Hz generalized spike-and-wave discharges, left temporal epileptiform discharges, electroclinical silence/inactivity, brief potentially ictal rhythmic discharges (BIRDs), temporal seizures, and triphasic waves. The above-mentioned channel has 163 followers, and each EEGTalk episode has up to 157 views, as of Jan 11, 2021. Additionally, these educational videos have been shared via Twitter with a positive response from the neurology community worldwide.

Improving EEG education at a national level requires novel methods of EEG teaching. We believe that this format has potential for disseminating EEG knowledge in a rigorous but entertaining fashion.

Poster Number 177

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CRISPR/CAS12a mediated miR-21 gene editing in mouse glioma cells

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Glioblastomas are the most common and lethal primary tumors of the central nervous system. They are characterized by their heterogeneity, which makes treatment challenging. To further elucidate on the part of miR-21 in glioma progression and to examine the role of miR-21 in the communication between glioma and cells in its environment, the establishment of a miR-21 KO cell line would be valuable. Although many studies could successfully inhibit miRNA expression by the use of different methodologies, such as antisense inhibitors and sponges, the effectiveness has not been robust partly due to technical difficulties mainly caused by the short length of miRNAs. In this line we have chosen a different approach to create a miR-21 KO cell line in the context of glioma. Presented results demonstrate the use of the CRISPR technology to establish a miR-21 KO in the mouse glioma cell line GL261. Future studies could apply this methodology to target different microRNAs or different disease models for efficient KO.

Poster Number 178

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Modulating TDP-43-dependent loss of STMN2 in amyotrophic lateral sclerosis and frontotemporal dementia

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are related, but clinically distinct, devastating neurodegenerative diseases for which no disease modifying treatments are available. The majority of all ALS (97%) and FTD (45%) cases are associated with the dysfunction of the RNA-binding protein, TDP-43. Recent work from our group and others has identified aberrant splicing (with inclusion of an abnormal, cryptic exon 2a), premature polyadenylation and reduced levels of the neuronal growth-associated protein stathmin-2 (STMN2) in response to loss of nuclear TDP-43 in ALS affected neurons and ALS/FTD patient tissue. Stathmin-2 is a direct binding partner of tubulin dimers, essential for neurite outgrowth and axonal regeneration. Altered splicing and premature polyadenylation-mediated reduction in STMN2-encoding mRNA is therefore a hallmark of FTD/ALS that links reduced nuclear TDP-43 function to enhanced neuronal vulnerability and establishes STMN2 modulation as a therapeutic target applicable to the vast majority of all ALS and FTD patients. Here, we therefore sought to identify small molecule compounds and genes capable of increasing STMN2 expression despite TDP-43 deficiency. Using CRISPR-Cas9 edited neuronal cell lines bearing homozygous TDP-43 mutations and C-terminal STMN2-luciferase, we identify that small molecule inhibition of both DLK1 and JNK efficiently increases STMN2 expression in a dose-dependent manner via mediation of the axonal degeneration pathway. These findings provide a compelling argument for the high-throughput testing of large libraries of small molecule compounds - both experimental and FDA-approved – as well as genetic modulators, to alleviate STMN2 reduction in ALS/FTD.

Poster Number 179

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Systematic Review of Human Postmortem Immunohistochemical Studies Unveils the Complexity of Astrocyte Reaction in Alzheimer's Disease

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Background: Astrocytes undergo morphological and functional changes in central nervous system diseases, collectively termed "astrocyte reaction" or "reactive astrogliosis." While reactive astrocytes have traditionally been characterized by increased immunoreactivity for the cytoskeletal intermediate filament glial fibrillary acidic protein (GFAP), transcriptomics investigations such as single nuclei RNA-sequencing have begun to decipher the complexity and heterogeneity of astrocyte reaction beyond cytoskeletal remodeling. We hypothesized that a systematic review of human postmortem immunohistochemical studies coupled with bioinformatics analyses would unravel this complexity in Alzheimer's disease (AD).

Methods: We conducted a systematic review following PRISMA guidelines (search strategy "Alzheimer's disease" AND "astrocytes" in PubMed, WoS-SCI, and APA PsycInfo; prespecified eligibility criteria for article selection). We applied bioinformatics tools on the resulting protein set (e.g., pathway enrichment analysis [PEA], transcription factor enrichment analysis [TFEA], protein-protein interaction [PPI] functional network), followed by hypergeometric enrichment tests against published human brain/CSF proteomic and astrocyte transcriptomic datasets.

Results: Total 302 included articles rendered 196 proteins. PEA implicated cytokine signaling, extracellular matrix, lipoprotein metabolism, trophic factors, response to ROS, and protein degradation, among other functional changes. TFEA suggested CTCF and ESR1 as potential drivers of these changes, while IL-6, TNF-alpha, and MAPK1/3/8 were top hub proteins in our highly connected PPI functional network. Cross-validation with published human multiomics datasets demonstrated statistically significant enrichment ($p<1E-11$).

Conclusions: Our systematic review provides clues about the complexity of astrocyte reaction in AD, revealing altered immune response, extracellular matrix, lipid metabolism, trophic factors, oxidative stress, and proteostasis. These findings could inform ongoing biomarker discovery efforts.

Poster Number 180

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Injury alters brain extracellular matrix and local ion concentrations

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Background: The polarity of the response of activated GABA receptors (EGABA) is dependent upon the chloride concentrations on both sides of the neuronal membrane. Neurons are surrounded by variably sulfated glycosaminoglycans (sGAGs) that can displace extracellular chloride ([Cl-]o), effectively lowering [Cl-]o and altering the polarity of GABA receptor signaling. These sGAGs can be hydrolyzed by active matrix metalloproteinases (MMPs) released by tissue injury, which may contribute to early seizures after brain injury.

Methods: Using 2-photon Fluorescence Lifetime IMaging (FLIM) of a custom chloride-sensitive fluorophore constrained to the extracellular space, we studied how injury affects the extracellular matrix and thus [Cl-]o in acute and organotypic cultures of hippocampal slices.

Results: The steady state [Cl-]o between neurons in acute and organotypic hippocampal slice cultures was only half of the CSF chloride, consistent with the hypothesized displacement by sGAGs. We therefore tested whether [Cl-]o would change when the sulfate moieties of the matrix are released by endogenous MMPs after brain injury. We found a strong dependence of [Cl-]o vs distance from injury, and inhibition of MMPs reduced the injury effects on Clo. Thus [Cl-]o is displaced by sGAGs, and damage to the extracellular matrix following brain injury alters Clo which impacts neuronal volume and GABA signaling. These findings have immediate implications for the treatment of cytotoxic edema and seizures after acute brain injury.

Poster Number 181

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Reducing tau and DRP1 mitigates mitochondrial fragmentation in amyotrophic lateral sclerosis

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Accumulating evidence suggests that mitochondrial dysfunction is an early pathogenetic event in amyotrophic lateral sclerosis (ALS). Accordingly, alterations in mitochondrial function have been described in both ALS patient samples and animal and cellular models of the disease. Interestingly, studies in Alzheimer's disease (AD) have linked alterations in mitochondrial function to interactions between hyperphosphorylated tau and dynamin-related protein 1 (DRP1), the GTPase involved in mitochondrial fission. Therefore, we sought to verify whether DRP1 and tau play a role in mitochondrial fragmentation and dysfunction in ALS. Our findings revealed a significant increase in DRP1 and its active phosphorylated isoform at S616 (pDRP1-S616) in synaptoneurosomes (SNs) derived from ALS post-mortem motor cortex (mCTX). Similarly, hyperphosphorylated tau at S396 (pTau-S396) was mis-localized to synapses and interacted with DRP1, suggesting that tau hyperphosphorylation may contribute to mitochondrial dysfunction in ALS. In addition, the treatment of human neuroblastoma SH-SY5Y cells with ALS SNs, enriched in both pTau-S396 and DRP1, significantly reduced mitochondrial length and volume and resulted in altered mitochondrial networks. Importantly, knocking down DRP1 using siRNA as well as reducing pTau-S396 levels with the specific tau degrader, QC-01-175, significantly mitigated the morphological alterations induced by ALS SNs. Collectively, our results suggest that increases in DRP1 and pTau-S396 may cause mitochondrial fragmentation in ALS, thus leading to an unfavorable energetic state. Importantly, targeting this molecular pathway may provide a novel therapeutic strategy to reverse bioenergetic deficits in ALS.

Poster Number 182

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Ex-vivo whole human brain high b-value diffusion MRI at 550 micron isotropic resolution using a 3T Connectom scanner

INVESTIGATORS: G. Ramos-Llordén, C. Maffei, Q. Tian, B. Bilgic, J. Augustinack, T. Witzel, A. Scholz, B. Keil, A. Yendiki, S. Huang

We demonstrate, for the first time, ultra-high spatial resolution, high b-value diffusion Magnetic Resonance Imaging in an ex-vivo whole human brain specimen at 550 micrometer of spatial resolution and using b-values up to 80,000 s/mm2 on a 3T Connectom scanner.

Ex vivo diffusion MRI is an indispensable research tool for probing and validating the multiscale nature of human brain circuitry with enormous impact in both neuroscience research and clinical applications.

Here we show that the high spatial resolution achieved allows us to obtain exquisite diffusion contrast and delineate fine structures at an unprecedented level of detail, as the first step toward validation of structural connectivity and microstructure in the whole human brain. We present DTI and DKI maps of the whole brain, resolve fiber orientations in the primary somatosensory, auditory, and visual cortex, and illustrate tractography in the claustrum and hippocampus, revealing their fine circuitry in exquisite detail.

Whole-brain ex-vivo diffusion MRI at high b-values and ultra-high spatial resolution will open the door to richer analyses of human brain anatomy and circuitry, offering the capability to map mesoscale connectional anatomy and microstructure with unprecedented data quality. In addition to furthering our understanding of human neuroanatomy, these data will be invaluable for validating biophysical models and methods for mapping connectional anatomy from in vivo diffusion MRI.

Poster Number 183

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Measuring Ambulation, Motor, and Behavioral Outcomes (MAMBO) with post-stroke fluoxetine in Tanzania: A Phase II Clinical Trial

INVESTIGATORS: D. R. Rice, K. Okeng'o, E. Massawe, N. Mworia, S. Ismail, A. C. Vogel, B. Kapina, N. Mukyanuzi, D. Buma, J. Gluckstein, M. Wasserman, F. Chiwanga, S. E. Fasoli, F. J. Mateen

Background: Fluoxetine may improve post-stroke motor recovery, but results from phase III trials, conducted in mostly Western populations, are mixed.

Methods: Adults within 14 days of first-ever acute ischemic stroke with new motor deficits were enrolled 11/2019–10/2020 at Muhimbili National Hospital, Tanzania. Participants underwent clinical assessments, neuroimaging, and laboratory testing. Daily fluoxetine 20mg was given for 90 days with follow-up assessments every 30 days. The cumulative incidence of adverse events was calculated and categorized as serious or non-serious.

Results: Of 34 patients (11 female, mean age 52.2 years, mean 3.3 days since symptom onset, hypertension (74%), diabetes (18%), and cigarette smoking (18%)), the median NIH Stroke Scale score at baseline was 10.5 and median Fugl-Meyer motor score (FMMS) was 28.5. 31/34 participants (91%) survived to 90 days. Deaths were due to gastrointestinal illness with low serum sodium, previously unrecognized gastric cancer with hemorrhage, and respiratory failure from COVID-19 (average fluoxetine administration before death: 42 days). The cumulative incidence of adverse events was 0.29 including 7 serious and 3 non-serious adverse events. The average serum sodium level at 90 days was 139 mmol/L (range 133–146) and ALT was 28 U/L (range 10–134). Fluoxetine adherence was 96%. At 90 days, median modified Rankin Scale score was 2, FMMS was 65.5, PHQ-9 score was 3.5 (minimal depression).

Conclusions: Fluoxetine administration for 90 days post-stroke in Tanzania was generally safe with excellent adherence, but serious comorbid post-stroke events occurred. Feasibility to perform post-stroke clinical trials of emerging therapies in this setting was established.

Poster Number 184

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Withdrawal of Life-Sustaining Therapy in Patients with Traumatic Brain Injury in Massachusetts General Hospital Intensive Care Units

INVESTIGATORS: W. R. Sanders, N. A. Wright, B. L. Edlow, Y. G. Bodien

This study aims to characterize Massachusetts General Hospital patients with acute severe traumatic brain injury (sTBI) who die after withdrawal of life-sustaining therapy (WLST). Despite the potential for patients with sTBI to make a meaningful recovery, decisions regarding WLST are made early in the acute course, when prognostic uncertainty is highest. Furthermore, there are no established guidelines to inform decision-making regarding treatment withdrawal after TBI. We abstracted clinical data from a REDCap (Research Electronic Data Capture) database that includes 963 patients with TBI admitted to MGH ICUs between April 2016 and January 2021. We conducted an analysis of the first 100 patients in the database and determined the time from TBI to death and whether death followed a change in goals of care to WLST. Eighty-three of 100 patients survived acute hospitalization after TBI. WLST was documented in 15 of the 17 patients who died in the ICU. The median [Interquartile Range] age of survivors was 49 years [27.0 – 63.0] as compared to 79 years [58.0 – 85.0] for those who died. The median time from injury to death was 4 days [2.0 – 8.0]. Our findings suggest that most patients survive acute hospitalization after sTBI. Despite prior studies suggesting that recovery after sTBI is possible, in our sample WLST was the most common cause of death and typically occurred in the first week post-TBI. A more nuanced understanding of the potential for recovery after sTBI and factors influencing WLST is needed.

Poster Number 185

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Novel modulators of Arc function for the development of pro-cognitive therapeutics in neuropsychiatric disorders

INVESTIGATORS: S. Santarriaga, K. Gerlovin, S. Reis, J. Lalonde, S. J. Haggarty, R. Karmacharya

There is convergent genetic evidence for the involvement of multiple components of the Arc (Activity-regulated cytoskeleton-associated protein; Arg3.1) protein complex in glutamatergic dysregulation in neuropsychiatric disorders. Functional studies of the consequence of loss of Arc on synaptic plasticity and memory consolidation in rodent systems further suggest that deficits in Arc may contribute to the pathophysiology of cognitive impairments in neuropsychiatric disorders. Conversely, overexpression of Arc has been shown to increase dendritic spine density and to restore plasticity in the adult cortex in animal studies. We conducted a high-content, image-based screen of a library of approved drugs and known bioactive small molecules in mouse cortical neurons to identify potentiators of neuronal activity-dependent induction of Arc protein. Based on our results, we performed a preliminary structure-activity relationship assay and identified specific chemical moieties that modulate Arc levels in human cortical neurons differentiated from induced pluripotent stem cells (iPSCs) of schizophrenia subjects and healthy subjects. Our studies will lead to the development of novel chemical probes to enable dissection of Arc biology in synaptic function and to undertake pre-clinical testing of our hypotheses related to the role of Arc modulation to ameliorate cognitive deficits in neuropsychiatric disorders.

Poster Number 186

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Correlating extracellular spike shape and neuronal responses in the lateral geniculate nucleus of awake macaques

INVESTIGATORS: S. H. Sun, J. S. Pezaris

Have we identified all cell types in the lateral geniculate nucleus of the thalamus (LGN)? Unlike the exhaustive determination of the retina, key populations in LGN may have been missed through sampling bias. Of retinal cells projecting to LGN, 80% carry signals associated with classic thalamic responses. We want to identify the missing 20% through rigorous analysis of LGN recordings in awake monkeys.

Extracellular spikes (or action potentials) are instrumental in studying neuronal responses. Spikes are dominated by brief negative-voltage excursions when measured with traditional metal electrodes, caused by the action of sodium and potassium channels. Spike waveforms have been used to classify the visual pathway cells into either regular-spiking or fast-spiking units, commonly associated with excitatory and inhibitory neurons, respectively. With the development of dense multi-electrode arrays and sophisticated spike-sorting algorithms we can now sample the brain, and in particular the LGN, with detailed identification of spike waveforms and reduced sampling bias to reveal previously overlooked signals.

Using these tools, we characterise the full range of neuronal responses in LGN. We find classes of spike waveforms that are not regularly reported in the literature, such as ones that are positive-dominant unlike the traditional negative-dominant forms. We correlate these spike classes against the response characteristics of corresponding cells to visual mapping stimuli to identify relationships between spike shape and neuronal class (magnocellular, parvocellular, koniocellular or, importantly, other). The ongoing effort will answer the question of what spike shapes and visual responses characterise the missing 20% of LGN cell types.

Poster Number 187

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Elucidating hippocampal circuit mechanisms underlying fear generalization

INVESTIGATORS: H. Twarkowski, V. Steininger, M. Kim, A. Sahay

Memories lose details over time and become more generalized. Hippocampal-cortical communication is thought to determine the extent to which memories generalize over time, but the underlying circuit mechanisms are poorly understood. Addressing this gap is critical to develop new therapeutic strategies for psychiatric disorders such as post-traumatic stress disorder (PTSD) that are characterized by overgeneralization of fear. We identified a molecular brake of GABAergic inhibition in the hippocampus, Ablim3, in dictating recruitment of feed-forward inhibition (FFI) onto CA3 and governing time-dependent memory generalization. This motivated investigation of how FFI in DG-CA3 affects neuronal ensemble dynamics in hippocampal-prefrontal cortical networks over time. Here, we performed single-photon calcium imaging in hippocampal CA1 and the anterior cingulate cortex (ACC) of awake, behaving mice - with increased FFI in DG-CA3 - to longitudinally track neuronal ensembles during encoding and recall of contextual fear memory in the fear conditioned and neutral contexts at recent and remote timepoints. Consistent with prior theory, we directly observed time-dependent refinement of context-associated ensembles in ACC over time. Importantly, increasing FFI in DG-CA3 potentiated this refinement and we observed a decrease in the overlap between ensembles of the fear-conditioned and neutral contexts in CA1 and ACC at remote timepoints. Our findings provide direct evidence for time-dependent evolution of memory ensembles and identify FFI in DG-CA3 as a neural substrate for memory consolidation in hippocampal-cortical networks. Targeting FFI by downregulation of Ablim3 in the hippocampus may represent a new strategy to reduce overgeneralization of fear memories.

Poster Number 188

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Analysis of serum-derived extracellular RNA to monitor treatment response to dacomitinib in adult patients with EGFR amplified recurrent glioblastoma

INVESTIGATORS: A. Yekula, R. R. Kitchen, S. K. Chakrabortty, T. Batchelor, A. Chi, B. S. Carter, X. O. Breakefield, J. Skog, L. Balaj

Background: Liquid biopsy for the detection and monitoring of brain tumors is of significant clinical interest. The ability to non-invasively profile tumors can avoid a risky biopsy and opens avenues for testing novel therapies by accurately stratifying patients to receive the right therapy. Here, we provide evidence of EV RNA-based diagnosis, patient stratification, and assessment of response to therapy in the setting of a clinical trial evaluating the efficacy of dacomitinib, an EGFR tyrosine kinase inhibitor in patients with recurrent, EGFR amplified GBM(NCT01112527).

Results: Firstly, our longRNASeq allowed the detection of thousands of mRNA, lincRNAs and antisense RNAs enabling the study of a wider repertoire of potential RNA based biomarkers.

Secondly, we observed a differential expression profile is serum EV RNA of GBM patients and healthy controls. Combining our findings with TCGA data and literature screening, we generated a 25 gene signature representative of critical pathways in several hallmarks of cancer.

Thirdly, we observed a differential expression profile is serum EV RNA of responders to dacomitinib compared to non-responders in pre-treatment serum. Specifically, the EV mRNAs ZNF35 and LAMTOR2 distinguish responders from non-responders (p-adjusted = 2.6E-8 and 2.4E-6, respectively) allowing potential patient stratification. EV mRNA DNMT3A is significantly enriched (p-adjusted = 1.8E-4) in post-treatment serum of responders compared to non-responders to dacomitinib allowing potential monitoring of response to therapy.

Conclusion: This study represents the first longitudinal profiling of the EV-RNA in a cohort of genomically selected GBM patients demonstrating a potential of liquid biopsy in patient stratification and monitoring.

Poster Number 189

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Not all unconscious states are equal: Differences in response to electrical stimulation during sleep and under general anesthesia in humans

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Even though being asleep or under general anesthesia are considered states of unconsciousness, they are separable physiological conditions. Under the assumption that the brain's response to perturbations is different during these states, we used multi-region single-pulse direct electrical stimulation (SPES) to probe the human brain. In twelve patients with semi-chronically implanted depth-electrodes we performed SPES while simultaneously recording intracranial EEG during wake, sleep (N=8), or propofol induced anesthesia (N=9).

Here we show a differential response during sleep or under anesthesia in contrast to a conscious state. The percentage of responsive brain channels was significantly smaller under anesthesia than during wake (Wilcoxon test $p<0.01$; Nch=44 channels). When dividing per region, this difference held when stimulation occurred in anterior (frontal and anterior cingulate cortex) ($p<0.01$; Nch=24), but not in temporal/posterior channels. In contrast, wake vs. sleep were statistically different only in temporal/posterior regions ($p=0.04$; Nch=13). Even after removing channels clinically considered epileptogenic, these comparisons prevailed. Complexity (PCI) was smaller during anesthesia ($p<0.01$; median PCI=52.3) compared to wake OR (PCI=107.3) and during sleep ($p<0.01$; PCI=45.8) compared to wake EMU (PCI=86.4).

The brain's distinct response to stimulation during different states not only improves our understanding of the mechanisms of unconsciousness, but has also therapeutic implications for disorders of consciousness, and could inform loss of consciousness during general anesthesia.

Funding: This work was supported in part by the Tiny Blue Dot foundation, NIH grant K24-NS088568, and NIH grant R01-NS062092. RZ was supported in part by the MGH ECOR Fund for Medical Discovery Postdoctoral Fellowship Award.

Poster Number 190

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Pressure Injury Development, Mitigation, and Outcomes of Patients Proned for ARDS Due to COVID-19

INVESTIGATORS: V. Capasso, C. Snydeman, K. Miguel, X. Wang, M. Crocker, Z. Chornoby, M. Vangel, M. A. Walsh, J. Murphy, S. Qualls

Prone positioning improves ventilation in patients with Acute Respiratory Distress Syndrome (ARDS) due to Covid-19. Potential complications include pressure injuries (PI). The purpose of the was to describe trends in PI development in adult critical care (CC) patients proned by the Proning Team (4/9/2020-6/8/2020) for severe ARDS due to COVID-19 and examine effectiveness of products and strategies to mitigate PIs. A retrospective chart review was conducted. Demographic data were analyzed using descriptive statistics. Differences between groups with and without PIs were analyzed using t-tests for continuous data, X2 and Fisher's exact tests for categorical data, and Cox regression analysis for survival analysis. Among 147 patients, significant PI risk factors included male gender ($p=.019$), BMI>40 ($p=.02$), Braden score<12 ($p=.019$), and vasopressor therapy ($p=0.02$). Significantly fewer facial PIs occurred with taping ETT vs. securing with commercial ETT holder ($p<.0001$). Maximum prone duration/session>32 hours ($P=0.03$) was significant risk factor for anterior PIs, which dropped 71% after introduction of new pressure redistribution products (PRP). D-Dimer>3200 ug/mL ($P=0.04$) was significant risk factor for sacro-coccygeal PIs while supine. The mortality rate was 34%; significant risk factors included age>60 years ($P=0.004$), SOFA score>11 ($P=0.003$), and comorbid congestive heart failure ($P=0.02$). This study illustrates extrinsic (e.g. prolonged immobility) and intrinsic risk factors (e.g. body weight, vasopressors) for PI development. Significantly fewer facial PIs occurred when ETT secured with tape vs. commercial tube holder. PRP may vary in effectiveness to prevent PIs. Standardized methods for testing PRPs are needed to inform product selection and customized patient care.

Poster Number 191

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Social Determinates of Health Relate to Sweetener Knowledge and Consumption in People with HIV

INVESTIGATORS: E. Kileel, K. A. Dickins, H. Zheng, S. Looby, K. V. Fitch

Increased added sweetener consumption is associated with adverse metabolic outcomes including cardiovascular disease (CVD) in people with HIV (PWH). Improved understanding of social determinants of health (SDoH) that may influence knowledge and consumption of added sweeteners may inform effective and sustainable nutrition interventions that are feasible in diverse social, financial and physical environments of PWH. This study evaluated knowledge and consumption of added sweeteners in a US cohort of PWH and explored associations of these variables with SDoH.

An online survey was conducted for this cross-sectional study. Demographic data and information pertaining to SDoH were collected, as well as assessment of added sweetener knowledge and consumption. Sweeteners included natural sugars and artificial sweeteners added to food and drinks. Scores were generated for sweetener knowledge (range 0-11) and sweetener consumption (range 0-26), a higher score indicated greater knowledge and greater consumption. Multivariable analyses assessed relationships between explanatory variables, sweetener knowledge and sweetener consumption.

Nine-hundred PWH from 46 US-states responded to the survey. Total Sweetener Knowledge Score was lower and Total Sweetener Consumption Score was higher in female versus male participants ($p<0.0001$). In multivariable analyses, female sex, race, and less than a high school diploma/GED associated with lower Total Sweetener Knowledge Score ($R2=0.17$, $p<0.0001$); race, age, BMI, income, reduced access to fresh fruits/vegetables, and low Sweetener Knowledge significantly associated with high Total Sweetener Consumption ($R2=0.21$, $p<0.0001$).

Findings highlight the need for nutritional interventions to be inclusive of added sweetener education and tailored to meet the social needs of PWH.

Poster Number 192

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Understanding Nurses Confidence Teaching Pediatric Tracheostomy Care

INVESTIGATORS: W. Hardiman

Introduction: Children with chronic medical conditions impacting their ability to breathe independently will undergo a tracheostomy. Caregivers need to learn complex care to safely care for their child at home Bedside nurses provide the teaching to families and need the knowledge, skills and confidence to provide the discharge education

Methods: A targeted sample of 21 staff nurses participated in a discharge teaching simulation before and after a single intervention consisting of a didactic presentation on basic tracheostomy care, emergency management, discharge education, and adult learning and teach-back principals. They evaluated themselves using the Student Satisfaction and Self-Confidence in Learning instrument. The nurses playing the role of surrogate caregiver completed the Teach-back Observation tool. The project manager used a discharge checklist to evaluate the nurse's skill in teaching discharge tracheostomy skills.

Results: Nurse participants rated a 44% increase in confidence levels, which was statistically significant $p = <.0001$. Surrogate caregivers rated an 47.6% increase in the use of teach-back 47.6% which was statically significant $p = .0016$.

Conclusions: All staff improved their confidence in knowledge, skills, and teaching basic and emergency tracheostomy care and use of teach-back. This indicates tracheostomy teaching should be a part of nursing orientation. Ideally, the project's intervention will impact the care and education that parents receive given the increase in nurses' confidence in knowledge and skills in tracheostomy care.

Poster Number 193

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Strategies for Sustaining Research Participant Recruitment and Safe Study Implementation for Single- and Multi-Site Studies During the COVID-19 Pandemic

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Due to the COVID-19 pandemic, clinical research practices at academic medical centers were challenged to establish timely, systematic approaches to safely maintain participant contact and procedures for research studies. This research unit-based process improvement initiative was executed to create innovative outreach, virtual communication, and regulatory strategies to sustain and optimize participant recruitment, engagement, safety, and ongoing operations for single and multi-site studies. The research nurse practitioners convened with the Unit Director, clinical investigators, research coordinators, and staff assistant to discuss study-related challenges encountered during COVID-19. Best practices for participant recruitment and protocol implementation were shared, a review of published literature and online resources for contemporary research implementation strategies occurred. Outcomes: (1). Establishment of the "Participant Engagement and Recruitment Committee" that created a logo representative of the mission of the unit, a Facebook page, Twitter account, and opportunities for community partnerships for sharing information about our research studies, investigative teams, and resources; (2). Single-Site Study Strategies: Implementation of a COVID-19 Contingency Plan with frequent participant check-ins, virtual visits/e-consenting, pre-visit COVID screening, options for remote specimen collection, and mailing of study drug; (3). Clinical Coordinating Center for a Global Multi-Site Clinical Study Strategies: Enhanced site-support with participant engagement; development of innovative data collection and tracking logs for use across multiple sites to track remote data collection, adherence to protocol integrity, and monitoring of study visits when in-person encounters were not possible. Collectively, these three initiatives promoted teamwork within the research unit, and introduced augmented processes for study participant recruitment, retention, and safety.

Poster Number 194

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Bridging Childhood Experiences and Adult Development: Boston Early Adversity and Mortality Study

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Background: Longitudinal studies of aging often begin in midlife. Although they provide rich data on health trajectories in adulthood, the reliance on retrospective data on early conditions and a lack of "birth-to-death" data on intervening processes have limited progress in studying pathways linking early experiences to later-life health. The Boston Early Adversity and Mortality Study (BEAMS) aims to overcome these shortcomings by using administrative record linkage methods to augment three nearly complete longitudinal aging studies with prospective data on the cohorts' early life conditions.

Methods: BEAMS comprises the all-male VA Normative Aging (n= 2280), Grant (n=456), and Glueck (n=268) Studies (74%-94% deceased). It extends coverage to siblings (including women) such that our combined sample is representative of the early 20th century Northeastern U.S. population. BEAMS acquires prospective information on early-life socioeconomic (e.g., neighborhood poverty) and environmental (e.g., lead exposure) conditions, and later-life health through administrative record linkage. This multi-step, human review process involves connecting individual identifiers to records in databases such as the 1900-40 U.S. Census, vital statistics, military, and Medicare records.

Conclusions: Through record linkage, BEAMS will create a cradle-to-grave dataset with prospective data on early-life exposures and lifespan health data. Including siblings will enable within-family comparisons. Our approach of a coordinated analysis across studies will enhance the replicability and robustness of our results, and our methodology can be adopted by other longitudinal aging studies to advance knowledge about pathways linking early-life conditions to lifespan health outcomes.

Poster Number 195

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Mechanisms of Cognitive Behavioral Therapy Effects on Symptoms of Body Dysmorphic Disorder: A Network Intervention Analysis

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Body dysmorphic disorder (BDD) is severe and undertreated. BDD is also common and has higher rates of suicide than most, if not all other, psychiatric conditions. Therefore, improving treatment for BDD is a public health priority. Although cognitive behavioral therapy (CBT) tailored to BDD has empirical support, how this and other interventions work is only minimally understood. Consequently, many patients do not receive optimal treatment or recover.

We examined a large trial comparing CBT to supportive psychotherapy. Network intervention analyses utilizing mixed graphical models were conducted to explore treatment-induced changes in specific symptoms and the larger disorder system across time. We show that the treatments impacted outcomes via different targets. For example, CBT directly strengthened efforts to challenge problematic thoughts and ritualistic behaviors at the intended points in treatment. Indirect effects showed these shifts in effort leading to downstream successful reductions in such phenomena. Supportive psychotherapy instead best addressed poor insight (delusionality), a prominent feature of BDD and challenging barrier to treatment response.

Results shed light on two very different psychotherapies' change mechanisms. Understanding how changes unfold could improve treatment expectations, credibility, and adherence. This breakdown could also aid in refining or reorganizing modular treatments to better fit patient needs given greater confidence in which treatment components influence which factors. For example, selected strategies might reflect the level of insight, pervasiveness of rituals, or the respective role of core symptoms in maintaining others within a patient. Overall, this line of work supports efforts towards more precision or personalized medicine.

Poster Number 196

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Association of Choroid Plexus Enlargement with Oligo-amenorrhea in Adolescents with Restrictive Eating Disorders

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Infrequent menstruation from reduced estrogen is associated with weight loss and malnutrition seen in restrictive eating disorders (R-EDs) such as anorexia nervosa (AN) and atypical-AN. Animal models demonstrate that reduced estrogen results in permeable brain barriers, increasing neuroinflammation. Non-invasive imaging of the main structure maintaining barrier homeostasis, the choroid plexus (CP), allows us to explore the role of estrogen on brain barriers homeostasis in R-EDs. We aimed to improve our understanding of the relations between CP structure and estrogen in a cohort of 71 postmenarchal females (R-EDs = 46; HC = 25). Using T1 weighted scans, we hypothesized that the CP would be larger in patients with R-EDs compared to matched HC's and that increased CP volume would be associated with less estrogen in R-EDs.

As hypothesized, left CP volume was significantly larger in R-EDs($M = 564.9$, $SD = 164.2$) compared with HC($M = 443.6$, $SD = 115.9$) ($f=9.9$, $p< 0.001$). In individuals with R-EDs, left CP volume was negatively associated with endogenous estrogen exposure($R = -0.43$, $p =0.002$). There were no significant relationships between estrogen exposure and the CP in HC.

In the largest study of CP volume in R-EDs, our results support our hypothesis that CP enlargement exists in R-EDs and is related to decreases in estrogen. Disruptions in estrogen in adolescents with R-EDs may increase brain-barrier permeability and should be further explored using more direct measures such as CSF. Longitudinal studies administering estrogen will be important in determining whether estrogen normalizes CP volume and in turn, R-ED psychopathology.

Poster Number 197

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Resiliency is a Protective Factor for Self-Rated Health among Sexual Minority Women

INVESTIGATORS: J. D. Foley, J. Morrison, A. W. Batchelder

Resiliency, or the process of effectively coping with stressful life events, may protect against the harmful effects minority-related stressors have on health. This study evaluated whether higher levels of resiliency attenuated the negative associations of perceived discrimination and stigma consciousness (i.e., extent to which individuals anticipate being stereotyped by others) on self-rated health in a sample of cisgender women that identified as a non-heterosexual identity. Participants responded to either online (e.g., Facebook) or hard copy advertisements (i.e., flyers) to complete an online screener, and if eligible, an online survey. The battery included the Connor-Davidson Resilience Scale, items from the Experiences of Discrimination Scales, and the Lesbian Stigma-Consciousness Scale. Participants also rated their overall health on a Likert scale ranging from "poor" to "excellent." Overall, 191 women (mean age=29.3, SD=6.9; 85% White) completed the survey. After controlling for demographics (i.e., age, race and education), perceived discrimination (b =-.02, SE=.008, t=-2.07, p=.04) and stigma consciousness (b=-.02, SE=.006, t=-2.69, p=.008) were each significantly and negatively associated with self-rated health. Resiliency was significantly and positively associated with self-rated health (b = .02, SE=.004, t=4.66, p<.001). Neither the interaction of perceived discrimination (p=.51) or stigma consciousness (p=.41) by resiliency were associated with self-rated health. Thus, resiliency was significantly associated with better health; however, it did attenuate negative associations with perceived discrimination or stigma consciousness. Expanding access to psychological interventions that include an emphasis on coping with minority-related stressors is an innovative area to improve health outcomes in sexual minority populations.

Poster Number 198

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Neurocognitive Dimensional Constructs and Noninvasive Neuromodulation Therapy in Pediatric and Adult Obsessive Compulsive Disorder- A Review

INVESTIGATORS: S. Homayoun , D. A. Geller, J. A. Camprodon

Current evidence-based treatments for obsessive compulsive disorder (OCD) do not always lead to adequate symptom response; therefore, the search continues for more effective and better-tolerated treatments. One such promising avenue is the expanding field of noninvasive neuromodulation, e.g., Transcranial Magnetic Stimulation (TMS) and transcranial Direct Current Stimulation (tDCS). While TMS was recently cleared by the U.S. Federal Drug Administration as safe and effective for the treatment of adult OCD, much progress is needed to further improve outcomes, increase access, and in particular, tailor and expand indications for youth afflicted by OCD.

Rational treatment development in neuropsychiatry has found success from dissecting clinical syndromes into core dimensions to define pathophysiological mechanisms and identify biomarkers. Specific to OCD, maladaptive processes have been described in three critical neurocognitive dimensions: a) fear extinction, b) response inhibition, and c) cognitive flexibility (particularly in the context of balancing goal-orientated verses habit-based behaviors).

To assess the current extent of translational research in the area of noninvasive neuromodulation of neurocognitive dimensional constructs of pathological relevance in OCD, a systematic PubMed search was conducted in November 2020.

Here we show that no published articles were found which used noninvasive neuromodulation in the investigation of fear extinction, inhibitory control or goal-orientated verses habit-based behavior in children, or adults with OCD.

In a field that has so far been unable to reach consensus on optimal parameters for noninvasive neuromodulation in the treatment of OCD, we recommend that research employing this mechanistically-informed approach for this condition is needed, especially in pediatric populations.

Poster Number 199

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Evaluating the mental health impacts of the COVID-19 pandemic: Perceived risk of COVID-19 infection and childhood trauma predict adult depressive symptoms in urban South Africa

INVESTIGATORS: A. W. Kim, T. Nyengerai, E. Mendenhall

Introduction: South Africa's national lockdown introduced serious threats to public mental health in a society where one in three individuals develops a psychiatric disorder during their life. We aimed to evaluate the mental health impacts of the COVID-19 pandemic using a mixed-methods design.

Methods: This longitudinal study drew from a preexisting sample of 957 adults living in Soweto, a major township near Johannesburg. Psychological assessments were administered across two waves between August 2019 and March 2020 and during the first 6 weeks of the lockdown (late March–early May 2020). Interviews on COVID-19 experiences were administered in the second wave. Multiple regression models examined relationships between perceived COVID-19 risk and depression.

Results: Full data on perceived COVID-19 risk, depression, and covariates were available in 221 adults. In total, 14.5% of adults were at risk for depression. Higher perceived COVID-19 risk predicted greater depressive symptoms ($p < 0.001$), particularly among adults with histories of childhood trauma, though this effect was marginally significant ($p = 0.063$). Adults were about two times more likely to experience significant depressive symptoms for every one unit increase in perceived COVID-19 risk ($p = 0.021$; 95% CI 1.10–3.39). Qualitative data identified potent experiences of anxiety, financial insecurity, fear of infection, and rumination.

Conclusions: Higher perceived risk of COVID-19 infection is associated with greater depressive symptoms during the first 6 weeks of quarantine. High rates of severe mental illness and low availability of mental healthcare amidst COVID-19 emphasize the need for immediate and accessible psychological resources.

Poster Number 200

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Smoking trajectories from in adolescence to early adulthood as a longitudinal predictor of mental health in later adulthood

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Significance: Although the association between trajectories of tobacco smoking and adverse physical health has been well documented, little is known about how smoking trajectories in the developmentally critical period predict subsequent mental health.

Methods: Data were drawn from the National Longitudinal Survey of Youth 1997 in which a representative cohort of Americans born between 1980 and 1984 was interviewed from 1997 to 2017 (N=8,570). Group-based multi-trajectory modeling was performed to identify smoking trajectories over 10 years from adolescence to young adulthood. We then used linear regression models to estimate the relationship between the smoking trajectories and mental health measured by the 5 items of the Mental Health Inventory in later adulthood. In addition, we conducted a causal mediation analysis to test the mediating role of alcohol and marijuana use in such relationships.

Results: Late-onset moderate smokers (β=-1.95, p=.021), late-onset accelerated smokers (β=-2.53, p=.005), early-onset heavy smokers (β=-3.72, p<.001), and early-onset moderate smokers (β=-2.66, p=.004) showed poorer mental health in later adulthood than stable abstainers, even after controlling for baseline mental health condition and covariates. The mental health score of quitters was not significantly different from that of stable abstainers. Alcohol and marijuana use mediated significant percentages of the association between each smoking trajectory and mental health (late-onset moderate smokers, 30.4%; late-onset accelerated smokers, 28.1%; early-onset heavy smokers, 16.7%; early-onset moderate smokers, 32.7%).

Conclusions: Continued smoking, especially early-onset and heavy smoking, from adolescence to young adulthood may have a long-lasting negative impact on mental health, and quitting may mitigate such impact.

Poster Number 201

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Life stress and prospective associations with depression and anxiety in preadolescent children: A six-year multi-wave study

INVESTIGATORS: R. Y. Levin, R. T. Liu, Longitudinal Studies on Child Abuse and Neglect (LONGSCAN)

Background : The relationship between life stress and depression and anxiety is well characterized in adolescents and adults. Further, research has shown that adolescents and adults with a history of childhood maltreatment are more likely to develop depression and anxiety after being exposed to stress than those without this history. However, the processes underlying risk for depression and anxiety in maltreated preadolescent children are unclear. The current study sought to identify these processes in at-risk preadolescents.

Methods: This study analyzed data from the Longitudinal Studies of Child Abuse and Neglect and evaluated interpersonal and non-interpersonal life stress as predictors of depression and anxiety specifically, and internalizing symptoms more generally in a sample of children vulnerable or exposed to maltreatment (n = 1,049). Participants were assessed repeatedly over a six-year period of early-to-mid childhood.

Results: Interpersonal life stress prospectively predicted greater depression and anxiety, but not general internalizing symptoms after emotional and behavioral problems, as well as child's sex, family income and baseline maternal depressive symptoms, were covaried. Non-interpersonal life stress was not prospectively predictive of depression and anxiety or general internalizing symptoms.

Limitations: The study was unable to identify specific types of interpersonal stress most relevant to risk for depression and anxiety in preadolescent children.

Conclusions: These findings lend support for the importance of interpersonal stress when screening for risk for depression and anxiety among preadolescent children vulnerable or exposed to maltreatment. Early intervention to decrease the occurrence and impact of these stressors could have long-lasting impacts on this vulnerable population.

Poster Number 202

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A prospective study of time-dependent exposures to adversity and DNA methylation in childhood and adolescence

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Background: Childhood adversity is a potent risk factor for depression, with recent evidence suggesting that it may confer even higher risk during sensitive periods when physiological systems are more responsive to external influences. Although the mechanisms underlying these effects remain unknown, prior studies suggest that DNA methylation may capture these time-dependent effects of childhood adversity. However, it remains unknown whether epigenetic alterations persist into adolescence and how the timing of adversity might influence their developmental trajectories. As such, we examined the relationship between the timing of early-life adversity and genome-wide DNA methylation during childhood and adolescence using data from the ALSPAC cohort (n=1,018).

Methods: We assessed the relationship between seven types of adversity (measured 5-8 times between ages 0-12) and DNA methylation at ages 7 and 17. Specifically, we analyzed the link between adversity in very early (age 0-2), early (3-5), middle (6-8), or late (9-12) childhood and DNA methylation at age 17, as well DNA methylation trajectories from age 7 and 17.

Results: Adversity occurring during early childhood was associated with differences in age 17 DNA methylation at 19 loci (FDR<0.05). By contrast, very early childhood adversity was associated with the change in DNA methylation from age 7 to 17 at 3 loci.

Conclusions: Our results suggest that adversity between ages 0-5 may have greater impact on epigenetic patterns across development than later ages. These findings provide insight into the sensitive periods that shape lifelong vulnerability to depression, which may ultimately improve our ability to predict and prevent depression.

Poster Number 203

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Neural Abnormalities in Trichotillomania and Skin-Picking Disorder During Learning

INVESTIGATORS: M. Migó, A. T. Peters, T. Chou, T. Peris, E. Ricketts, J. Grant, E. Cavic, N. Keuthen, J. Piacentini, D. D. Dougherty, T. Deckersbach

Background: Impulse-control disorders, such as Trichotillomania (TTM) and skin-picking disorder (SPD), are associated with reduced flexibility and increased internally focused attention, which may negatively impact learning and flexible accommodation of new information. Using a Bayesian Learning Model, we evaluated the neural basis of learning and accommodation in individuals with TTM and SPD.

Methods: Participants were 26 healthy controls (HC) and 127 individuals with TTM and SPD recruited from three sites (age 18 – 57, 16% male). During fMRI, participants completed a shape-button associative learning and reversal task. Bilateral anatomical ROIs were defined, a-priori, in the basal ganglia, with exploratory analyses in the hippocampus, dorsolateral prefrontal cortex (dlPFC), and dorsal anterior cingulate cortex (dACC). An independent samples t-test comparing the groups in the Initial Learning-Reversal contrast was conducted in SPM8, with site as a covariate. Significant results achieved a false discovery rate correction of $p < 0.05$ with a minimum cluster size (k) of 16 voxels in the basal ganglia, and 15 voxels in the dlPFC and dACC.

Results: Relative to HC, patients demonstrated reduced activation during initial learning than reversal learning in the right basal ganglia (BA 48). Similarly, patients demonstrated reduced activation in several clusters in the dlPFC (BA 6/46 bilaterally, left BA 8) and dACC (left BA 24/32, right cingulate sulcus).

Conclusions: Individuals with TTM and SPD exhibit altered activation within the basal ganglia, dlPFC, and dACC during associative learning compared to controls, reflecting reduced frontal-subcortical activation during initial learning.

Poster Number 204

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Autonomic, Endocrine and Inflammation Profiles in Functional Neurological Disorder: A Systematic Review and Meta-Analysis

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Background: Functional neurological disorder (FND) is a disorder at the intersection of neurology and psychiatry. To date, promising yet inconsistently identified neural circuit profiles were observed in patients with FND, suggesting important gaps remain in our systems-level neurobiological understanding. As such, other important physiological variables including autonomic, endocrine and inflammation findings need to be contextualized for a complete mechanistic picture.

Objective: Here, we performed a systematic review and meta-analysis of available case-control and cohort studies in FND.

Methods: PubMed, PsycINFO and Embase databases were searched from January 1, 1900 to September 1, 2020 for studies that investigated autonomic, endocrine and/or inflammation markers in patients with FND.

Results: Sixty-six of 2,056 screened records were included in the review representing 1,699 patients, with data from 23 articles used in meta-analyses. Findings show that children/adolescents with FND vs. healthy controls (HCs) have increased resting heart rate; and a tendency towards reduced resting heart rate variability in patients with FND across the lifespan vs. HCs. In adults, peri-ictal heart rate differentiated FND from individuals with epileptic seizures. Other autonomic and endocrine profiles in patients with FND were heterogeneous, with several studies highlighting the importance of individual differences. Inflammation research in FND remains in its early stages.

Conclusion: Moving forward, there is a need to use larger sample sizes to consider the complex interplay between functional neurological symptoms and behavioral, psychological, autonomic, endocrine, inflammation, neuroimaging and epigenetic/genetic data. More research is needed to determine whether FND is mechanistically (and etiologically) similar or distinct across phenotypes.

Poster Number 205

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Feature-Level Analysis of a Smoking Cessation Smartphone App Using a Positive Psychology Approach

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Background: Many smokers seek out smartphone apps to help them quit smoking, with recent estimates of 780,000 app downloads per month. Additional data is needed to better understand how app-specific content and app interface parameters influence user engagement and smoking outcomes.

Objective: Using data from a pilot trial of a positive psychology based smoking cessation app, we (1) describe how nondaily smokers used the app, and (2) tested if app usage during the prescribed treatment period predicted smoking abstinence (self-reported 30-day point prevalence).

Methods: In an open pilot study, nondaily smokers (n=100) were asked to use the "Smiling Instead of Smoking" (SiS) app for a prescribed period of 7 weeks. The app prompted users to complete daily positive psychology exercises and behavioral challenges designed to engage smokers with the app's elective smoking cessation tools.

Results: Participants interacted with positive psychology content on more days than with smoking-related content ($p<0.0001$). Ad libitum use of tools was low, but completion of assigned tasks was relatively high. Days of app use significantly predicted smoking abstinence at 6 weeks ($p=0.0019$) and 6 months post quit ($p=0.0133$). Completion of assigned content was related to smoking abstinence ($ps<0.01$) with higher R-squared values for smoking content (r-squared=0.14) than happiness content (r-squared=0.10) at end-of-treatment, but similar values at 6-month follow-up (r-squared=0.06 for both).

Conclusion: Higher app use was predictive of subsequent smoking abstinence, both short-term and long-term. Our findings further suggest that prescriptive clarity in assigning smartphone app interactions may support treatment adherence and effectiveness.

Poster Number 206

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Outcomes of Patients Undergoing Surgery with Perioperative SARS-CoV-2 Infection in the USA: A Multicenter Cohort Study

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Background: The US continues to be the most affected country by the COVID-19 pandemic. We aimed to report the 30-day outcomes of patients with perioperative SARS-CoV-2 infection undergoing surgery in the US.

Methods: As part of the COVIDSurg multicenter study, between Jan 1 and June 30, 2020, all patients aged ≥17 years with perioperative SARS-CoV-2 infection (confirmed within 7 days before or 30 days after surgery) in 70 hospitals across 27 states were included. The primary outcomes were mortality and pulmonary complications (defined as pneumonia, acute respiratory distress syndrome, or unexpected postoperative ventilation). Multivariable analysis (adjusting for patient and procedure characteristics) was performed to identify predictors of mortality.

Results: A total of 1,581 patients were included. More than half the patients were males (822 [52.0%]) and ≥50-year-old (835 [52.8%]). Most procedures were emergent (1,261 [79.8%]), and abdominal (538 [34.1%]). At 30-days postoperatively, 174 patients (11.0%) died, and 622 (39.5%) developed pulmonary complications. Independent predictors of mortality were age ≥70 years (OR [95% CI] 2.46 [1.65-3.69], $p<0.001$), male sex (2.26 [1.53-3.35], $p<0.001$), American Society of Anesthesiologists (ASA) grades 3-5 (3.08 [1.60-5.95], $p=0.001$), malignant disease (2.97 [1.58-5.57], $p=0.001$), emergent surgery (2.44 [1.31-4.54], $p=0.005$), pre-operative white cell count ≥11 K/uL (2.16 [1.48-3.14], $p<0.001$), respiratory comorbidities (COPD/asthma) (2.08 [1.30-3.32], $p=0.002$), and higher Revised Cardiac Risk Index (1.20 [1.02-1.41], $p=0.025$).

Conclusions: Patients with perioperative SARS-CoV-2 infection have a significantly high risk for postoperative mortality and pulmonary complications, especially elderly males. Postponing elective surgery and adopting non-operative management, when reasonable, should be considered during the pandemic.

Poster Number 207

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Passing the Scalpel: Defining and Preparing for Surgeon Transitions from Clinical Practice

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Background: One-third of all practicing surgeons are currently older than 55, but there are limited institutional or societal guides to support these surgeons as they prepare to eventually reduce or stop clinical work. The aim of this pilot study was to explore why surgeons retire, define the major barriers, and identify potential resources that may help surgeons prepare for this transition.

Methods: We used a convenience sampling strategy of non-clinically active surgeons affiliated with our institution. Semi-structured interviews were conducted, and each transcript was inductively coded by two researchers. Emergent themes were identified via a grounded theory approach.

Results: We interviewed 14 surgeons. Influencers on the decision to retire were categorized under "pulling" and "pushing" forces. Pushing are negative forces that contribute to the decision of retirement (deteriorated health, change in practice conditions, organizational change); pulling are positive features that pull towards retirement (desire to spend more time with family). Major barriers for a successful transition from clinical practice included: unclear timing of retirement, overly self-reliance, and strong association between self-identity and profession. Resources they identified that could be helpful in preparing surgeons for this transition included assistance in early career financial planning, defining personalized goals and skillsets, and community guidance such as mentorship. The ideal timing of presenting these resources was variable, but the majority felt that it should start with early financial planning.

Conclusion: There are numerous barriers for surgeons to effectively transition from clinical practice that could be solved with dedicated education research efforts.

Poster Number 208

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Aggregating Long-Term Context for Learning Laparoscopic and Robot-Assisted Surgical Workflows

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Analyzing surgical workflow is crucial for surgical assistance robots to understand surgeries. With the understanding of the complete surgical workflow, the robots are able to assist the surgeons in intra-operative events, such as by giving a warning when the surgeon is entering specific keys or high-risk phases. Deep learning techniques have recently been widely applied to recognizing surgical workflows. Many of the existing temporal neural network models are limited in their capability to handle long-term dependencies in the data, instead, relying upon the strong performance of the underlying per-frame visual models.

We propose a new temporal network structure that leverages task-specific network representation to collect long-term sufficient statistics that are propagated by a sufficient statistics model (SSM). We implement our approach within a long-short-term-memory (LSTM) backbone for the task of surgical phase recognition and explore several choices for propagated statistics. We demonstrate superior results over existing and novel state-of-the-art segmentation techniques on two laparoscopic cholecystectomy datasets: the publicly available Cholec80 dataset and MGH100, a novel dataset with more challenging and clinically meaningful segment labels.

Poster Number 209

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Long-term reconstructive surgery of the burned hand: 16-year experience at a major burn center

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Background: Excision and grafting of deeper acute hand burns preserves long-term hand function. Little information exists on long-term reconstructive and revision operations after acute grafting. Limited quantitative data is available on early predictors of this outcome.

Methods: A retrospective review was conducted using medical records of patients admitted with acute burns to a regional burn center from 1999-2015 who subsequently underwent acute burn excision and grafting. Information collected included demographics, burn size and etiology, anatomical involvement, and operative details. Regression analysis assessed for demographic and clinical predictors for future contracture release with grafts and/or local tissue rearrangement surgery.

Results: A total of 704 hands in 532 adults (71% male, median age 40 years, average burn size 14.9% Total Body Surface Area, i.e. TBSA) met study criteria. Ninety-eight patients underwent at least one reconstructive surgery (122 burned hands). Multivariable logistic regression analysis showed that female gender, white race, and burn size ≥21% TBSA were positively associated with contracture release with graft. Female gender and burn size (5-10% and ≥21% TBSA) had a positive association with local tissue rearrangement.

Conclusions: Approximately 1 in 6 acutely grafted hands underwent at least one reconstructive surgery of clinically significant contractures. Female gender and burn size were positive predictors of both categories of reconstructive surgery while white race was a positive predictor of release and graft. Predictors of reconstructive surgeries may help risk-stratify those who will need longitudinal follow-up and potentially target interventions to mitigate contracture formation and future surgery.

Poster Number 210

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Artificial Intelligence- Enabled Evaluation of Pain Sketches to Predict Outcomes in Headache Surgery

INVESTIGATORS: L. Gfrerer, W. G. Austen, Jr.

Background: Recent evidence has shown that patient drawings of pain can predict poor outcomes in trigger site deactivation surgery for migraine headache. Given that interpretation of pain drawings requires some clinical experience, the authors developed a machine learning framework capable of automatically interpreting pain drawings to predict surgical outcomes. This platform will allow clinicians with less clinical experience, neurologists, primary care practitioners, and even patients to better understand candidacy for headache surgery and seek evaluation by certified headache surgeons.

Methods: A random forest machine learning algorithm was trained on 131 pain drawings provided prospectively by headache surgery patients prior to undergoing trigger site deactivation surgery. Twenty- four features were used to describe the anatomic distribution of pain on each drawing for interpretation by the machine learning algorithm. Surgical outcome was measured by calculating percent improvement in Migraine Headache Index at least 12 months after surgery. Artificial intelligence (AI) predictions were compared with surgical outcome to evaluate performance.

Results: Evaluation of the data test set demonstrated that the algorithm was consistently more accurate (94%) than trained clinical evaluators. Our algorithm weighted diffuse pain, facial pain, and pain at the vertex as strong predictors of poor surgical outcome.

Conclusion: This study indicates that structured algorithmic analysis is able to correlate pain patterns drawn by patients to MHI percent improvement with good accuracy (94%). Further studies on larger datasets and inclusion of other significant clinical screening variables are required to improve outcome predictions in headache surgery and apply this tool to clinical practice.

Poster Number 211

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Early Enteral Nutrition in Critically Ill Patients Receiving Vasopressor Support

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Background: Hemodynamic instability and vasoactive medications have detrimental effects on GI physiology that could undermine the beneficial effects of early enteral nutrition (EEN). The aim of this study was to compare in-hospital outcomes for early vs. late enteral nutrition (LEN) in mechanically ventilated patients receiving vasopressor support.

Methods: The national eICU collaborative database was used as the data source. Adult patients who received vasopressors (norepinephrine, epinephrine, dopamine, phenylephrine or vasopressin) and mechanical ventilation within 24hrs of admission and for ≥2 days were included. Patients with an admission diagnosis that could constitute a contraindication for EEN (e.g. GI perforation, immediate post-operative) were excluded. EEN and LEN were defined as tube feeding within 24hours and between 24hours-1week(NPO during first 24hrs) of admission. Propensity score matching was performed to derive two comparable cohorts.

Results: A total of 1002 patients were included (EEN=501 & LEN=501). Median time to EN was 19 vs. 57 hours from admission in the EEN and LEN groups. There was no significant difference in mortality or hospital length of stay (LoS) between two nutrition strategies. EEN was associated with shorter ICU LoS and also lower incidence of AKI, hemodialysis requirement and electrolyte abnormalities ($p<.05$). However, EEN was also associated with a two-fold higher incidence of hospital acquired pneumonia ($p=.010$).

Conclusion: Findings of this study suggest that, the benefit of EEN in critically ill patients receiving vasopressor support is equivocal given the lack of improvement in survival and complex association with in-hospital outcomes including a higher incidence of hospital acquired pneumonia.

Poster Number 212

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Chitosan-based Lifefoam improves survival in lethal non-compressible abdominal bleeding

INVESTIGATORS: L. Naar, A. Dorken Gallastegi, M. Dowling, H. Mashbari, B. Wallace, B. Bankhead-Kendall, J. Beagle, J. Burke Pallotta, K. Breen, G. C. Velmahos, M. J. Duggan, D. R. King

Introduction: In military combat settings, non-compressible closed cavity exsanguination is the leading cause of potentially survivable deaths, with no effective treatment available at point of injury. We hypothesized that hm-chitosan may be used as a locally injectable haemostatic agent for the treatment of a lethal, closed-abdomen, non-compressible, high-grade hepato-portal injury in a swine model.

Methods: A closed-cavity, grade V hepato-portal injury was created in all animals resulting in massive non-coagulopathic, non-compressible bleeding. Animals received either fluid resuscitation alone (control, n=8) or fluid resuscitation plus intraperitoneal hm-chitosan agent through an umbilical port (experimental, n=18). The experiment was terminated at 180 minutes or death (defined as ETCO2<9 or mean arterial pressure [MAP]<15mmHg), whichever came first.

Results: All animals had profound hypotension and experienced a near-arrest from hypovolemic shock (mean MAP of 24mmHg at 10 minutes). Mean survival time was higher than 150 minutes in the experimental arm vs. 27 minutes in the control arm (p-value<0.001). Three-hour survival was 72% in the experimental group and 0% in the control group (p-value=0.002). Hm-chitosan stabilized rising lactate, preventing acute lethal acidosis. MAP improved drastically after deployment of the hm-chitosan and was preserved at 60mmHg throughout the three hours. Mean fluid resuscitation (LRS) was higher in the experimental arm (7.3±1.5L vs. 4.4±1.1L, p-value<0.001). Post-mortem examination was performed in all animals and the hepatoportal injuries were anatomically similar.

Conclusions: Here we show that intraperitoneal administration of hm-chitosan for massive, non-compressible abdominal bleeding improves survival in a lethal, closed-cavity swine model. Chronic safety and toxicity studies are required.

Poster Number 213

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A machine learning approach to predicting progression on active surveillance for prostate cancer

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Introduction: To date, studies that have developed models to predict progression on AS for prostate cancer have invariably used traditional statistical approaches. We evaluated whether a machine learning approach could improve prediction of progression on AS.

Methods: We performed a retrospective institutional cohort study of 790 very-low or low-risk prostate cancer patients managed with AS. The sample was split into a training and test set (ratio 80%/20%). In the training set, we developed a traditional logistic regression classifier (LRC), and alternate machine learning classifiers (MLCs) (support vector machine, random forest, and a full connected artificial neural network) to predict grade progression. Features considered for inclusion were clinical and biopsy characteristics measured at diagnosis, as well as time between diagnostic biopsy and last biopsy, and number of biopsies on surveillance. We used backward elimination to select features for the multivariable LRC. For the MLCs, all features were included in model development. We tuned the hyperparameters of the MLCs. Model performance was evaluated in the test set. The primary performance metric was the F1 score.

Results: With a median follow-up of 6.3 years, 234 developed grade-progression. In descending order, the F1 scores were: support vector machine 0.600 (95% CI 0.593–0.605), artificial neural network 0.507 (95% CI 0.500–0.511), random forest 0.413 (95% CI 0.400–0.418), traditional LRC 0.182 (95% CI 0.151–0.185). All MLCs had a significantly higher F1 score than the traditional LRC (all $p<0.001$).

Conclusions: Alternative MLCs significantly outperformed a traditional LRC in predicting progression on AS for prostate cancer.

Poster Number 214

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Viability of discarded human livers during normothermic machine perfusion is associated with activation of repair mechanisms

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Background: Up to 30% of patients with end-stage liver disease awaiting liver transplantation die prior to transplant due to a shortage of available organs. Normothermic machine perfusion (NMP) combined with adjunct therapies may be able to rehabilitate marginal or discarded livers, thereby increasing the supply of organs for transplant.

Methods: Six discarded whole human livers, rejected by local transplant centers, were obtained from donation after circulatory death (DCD). Livers underwent 12 hours of NMP with oxygenated blood. Serial tissue and plasma samples were collected. Clinical viability criteria were used to determine if livers were theoretically transplantable (viable) or not (nonviable). Transcriptome sequencing was performed on biopsies taken at 0 (pre-), 3, and 6hr of perfusion. The significance cutoff for differential gene expression was set to a Benjamini-Hochberg false-discovery rate < 0.05.

Results: Three of six livers met viability criteria for transplant. The vast majority of significant differentially expressed genes were upregulated during perfusion. Viable livers demonstrated robust activation of innate immune responses, such as Toll-like receptor, IL-10, and IL-6 canonical pathways compared to nonviable livers. Gene expression and Western immunoblot analysis demonstrated significant expression of proteins involved in autophagy and the unfolded protein response in viable compared to nonviable livers (Fig. 1).

Conclusion: Here we show discarded human livers that met viability criteria during NMP demonstrated robust activation of autophagy in response to ischemia-reperfusion injury. Therapeutic targeting of this pathway during NMP may improve liver viability and graft utilization for transplantation.

Poster Number 215

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The influence of day-to-day variability in fibromyalgia pain on clinical outcomes following cognitive behavioral therapy

INVESTIGATORS: M. Barton-Zuckerman, A. Grahl, A. Lazaridou, M. Paschali, J. Lee, M. Berry, L. Isaro, R. Edwards, V. Napadow

Previous studies suggest that greater day-to-day variability in clinical pain predicts better outcomes following therapy. Specifically, patients reporting greater fluctuations in pain at baseline experienced greater placebo effects and were more likely to be considered responders in drug trials. In our longitudinal neuroimaging study, female fibromyalgia (FM) patients used a daily diary to report pain severity for seven days before and after an 8-week intervention of cognitive behavioral therapy (CBT) or education control (N=46, nCBT=30, nEDU=16). Patients also completed an evoked pressure pain paradigm (left leg, medium intensity) during fMRI scans before and after treatment (Siemens 3T, TR/TE=1250/33ms, flip angle=65°, voxel size 2mm3 isotropic, SMS MB acc. factor 5, 75 slices; 284 volumes). Clinical outcomes included the Pain Catastrophizing Scale (PCS), a specific target of CBT. We found that greater day-to-day clinical pain fluctuations at baseline were associated with reduced hyperalgesia (i.e. lower evoked pressure pain ratings, $r = -0.39$, $p = 0.007$). Furthermore, greater day-to-day clinical pain fluctuations were also associated with greater post-therapy improvement in pain catastrophizing for the CBT group ($r = -0.38$, $p = 0.043$). These results highlight the importance of daily diaries for chronic pain characterization and suggest that greater fluctuations in pain may reflect a more malleable pain experience leading to optimistic expectations and better clinical outcomes for a novel pain therapy. Future fMRI analyses will assess the brain correlates supporting this phenomenon.

Poster Number 216

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Enhanced activity of B7-H3 Chimeric Antigen Receptor (CAR) T cells against triple negative breast cancer (TNBC) induced by radiation

INVESTIGATORS: G. Cattaneo, L. Maggs, F. Kontos, S. Ferrone

Background: Triple negative breast cancer (TNBC) is a highly aggressive tumor. Since current therapies have limited efficacy, we have developed a chimeric antigen receptor (CAR) T cell-based combinatorial immunotherapy. B7-H3 has been selected as target antigen: it has a restricted distribution in normal tissues and a high expression on differentiated TNBC cells and cancer initiating cells (CICs). Application of CAR T cells against solid tumors is challenging, due to the immunosuppressive microenvironment, downregulation or loss of target antigen and other escape mechanisms. To enhance the anti-tumor activity of B7-H3 CAR T cells we combined them with radiation, since it modulates the expression of molecules relevant to CAR T cells activity.

Methods: TNBC cell lines SUM159 and MDA-MB-231 were treated with radiation. B7-H3 and pro/anti-apoptotic molecules expression was determined by flow cytometry and Western Blot, respectively. TNBC cells elimination by B7-H3 CAR T cells was assessed with co-culture experiments.

Results: Radiation induced an upregulation of B7-H3 expression on TNBC cells, as determined by a significant increase of mean fluorescence intensity. Radiation also induced a downregulation of the anti-apoptotic protein Bcl-xl. These changes led to an increased susceptibility of TNBC to B7-H3 CAR T cells as indicated by the significantly higher elimination of tumor cells in coculture experiments at 1:4 effector/target ratio.

Conclusions: These results show that radiation of TNBC can significantly increase the anti-tumor activity of B7-H3 CAR T cells. These results provide the rationale for including radiation in the design of B7-H3 CAR T.

Poster Number 217

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A novel exon specific U1 snRNA therapeutic strategy to prevent retinal degeneration in familial dysautonomia

INVESTIGATORS: A. K. Chekuri, E. Morini, E. M. Logan, A. J. Krauson, M. Salani, G. Romano, F. Riccardi, F. Pagani, L. H. Vandenberghe, S. A. Slaugenhaupt

Familial dysautonomia (FD) is an autosomal recessive neurodegenerative disorder caused by a splice mutation in the gene encoding Elongator complex protein 1 (ELP1, also known as IKBKAP). A T-to-C base change in the 5' splice site of ELP1 exon 20 results in exon 20 skipping with tissue specific reduction of ELP1 protein predominantly in the nervous system. In addition to complex neurological phenotype, FD patients also exhibit progressive retinal degeneration severely affecting their quality of life. To test novel splicing-targeted therapeutic approaches, we developed a phenotypic mouse model of FD, TgFD9; Ikbkap□20/flox which exhibits most of clinical features of the disease while displaying the same tissue specific mis-splicing observed in patients. Here, we report an thorough characterization of the retinas of our FD mouse using SD-OCT and immunohistochemical assays during disease progression. Our findings showed a significant decrease in the thickness of the retinal nerve fiber layer (RNFL) and the ganglion cell layer (GCL) starting from 3 months of age, demonstrating that our mouse model correctly recapitulates the retinal degeneration observed in patients. To correct ELP1 splicing defect and rescue retinal degeneration, we have designed a novel splice targeted therapy using modified version of the spliceosomal U1 snRNAs (ExSpeU1s) that bind to intronic sequences downstream of the mutant 5' splice site. We have analyzed the efficiency of splicing correction of the ExSpeU1s through in vivo delivery using adeno associated vectors (AAV). Our preliminary data demonstrate the valuable therapeutic potential of ExSpeU1 RNA delivery to treat retinal degeneration in FD.

Poster Number 218

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Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants

INVESTIGATORS: K. A. Christie, R. T. Walton, M. N. Whittaker, B. P. Kleinstiver

The use of CRISPR-Cas nucleases to manipulate DNA in a site-specific manner holds immense potential for the study and treatment of genetic disease. Despite their promise, naturally occurring CRISPR nucleases cannot freely target within a given genome. They are restricted by the requirement to recognize a short DNA sequence called a protospacer adjacent motif (PAM), which in the case of commonly used SpCas9 is NGG. Here we engineer new enzymes that completely expand targeting range by relaxing PAM preference beyond the canonical NGG, maximizing the potential of CRISPR nucleases for understanding of the complexity of the genome.

Initially, we engineered a highly active variant, named SpG, which can target NGN PAMs. To enable even broader targeting, we then utilized SpG for further engineering, leading to the generation of a novel SpCas9 variant named SpRY (for targeting NRN>NYN PAMs). We found that SpRY can efficiently target sites with NGN and NAN PAMs (collectively NRN), and also has detectable but reduced activity against sites harboring NCN and NTN PAMs (collectively NYN). Thus, when considering both DNA strands, in principle SpRY enables modification of any genomic sequence.

As a proof-of-concept of the expanded targeting range of these variants we generated previously inaccessible single nucleotide variants associated with protecting individuals against complex genetic disease. Collectively these results demonstrate that SpG and SpRY enable targeting of many sequences previously inaccessible to SpCas9 and other Cas9 orthologs. Enabling high-resolution targeting for a suite of genome-editing applications to facilitate the study and treatment of genetic disease.

Poster Number 219

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AAV-based Gene Therapy for Pediatric Neurodevelopmental Disease

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Mucolipidosis IV (MLIV) is a rare lysosomal storage disorder characterized by debilitating neuromotor and cognitive deficits in addition to vision loss in the second decade of life. It is caused by loss-of-function mutations in the MCOLN1 gene which encodes a lysosomal transient receptor potential channel mucolipin 1 (TRPML1). There is no existing therapy or treatment for this disease. We report that AAV-mediated gene transfer of the human MCOLN1 gene rescues motor dysfunction and brain pathology in the Mcoln1-/- MLIV mouse model. Treatment of symptomatic mice with AAV-PHP.B, a vector designed to transduce CNS from systemic flow in mice, lead to sustained expression of the human MCOLN1 transgene in the brain and resulted in full and long-term restoration of motor function and significant delay of paralysis. To show translational potential, we designed self-complimentary AAV9-MCOLN1 vectors. We then demonstrated that CNS-directed (intracerebroventricular) administration of this vector in post-natal day 1 mice reduced lysosomal storage load and significantly improved myelination in the MLIV mouse brain. Mcoln1-/- mice treated with AAV9 showed no motor deficits compared to untreated mice. Based on our data and general advancements in the gene therapy field we propose scAAV9-mediated, CSF-targeted MCOLN1 gene transfer as a therapeutic strategy for MLIV.

Poster Number 220

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Treatment of interstitial cystitis with dehydrated and micronized human amnion and chorion membranes reduces inflammation, pain, and urinary pathology

INVESTIGATORS: M. Jensen, A. Schults, W. Jia, S. Oottamasathien

Bladder inflammation underlies multiple debilitating chronic conditions in the lower genitourinary tract including interstitial cystitis/painful bladder syndrome (IC/PBS). IC/PBS causes an estimated economic loss of $21.1 billion dollars annually due to increased medical expenses and decreased work productivity. Current therapies use analgesics to provide temporary relief or attempt to reinforce the bladder's mucus coating. However, these therapies take months to provide any effect and fail to provide lasting relief for the majority of patients. A regenerative approach using growth factors derived from analogous tissues could ameliorate underlying inflammation, pain, and urinary urgency. Using an LL-37-induced model of IC/PBS in mice, we tested dehydrated human amnion and chorion membranes (dHACM), which contain a mix of growth factors, immunomodulators, and anti-inflammatory cytokines, as a regenerative bladder coating. Intravesical administration of 4 mg/ml micronized dHACM into the bladder via a liquid carrier decreased myeloperoxidase concentration, an inflammatory marker in the bladder, by 94% ($P \leq 0.05$) and reduced pain response to suprapubic mechanical stimulation by 55% compared to sham controls ($P \leq 0.05$). Additionally, dHACM treatment decreased urinary pathology in only 24 hours after treatment as measured using behavioral voided spot assays($P \leq 0.05$). Treatment of IC/PBS in a mouse model with dHACM dramatically reduced the key pathophysiological features of IC/PBS including inflammation, pain, and urinary pathology. Regenerative medical therapy, based upon the use of autologous tissues, offers a promising avenue to rapidly translate new treatment strategies that alleviate the burdens of chronic bladder disease for patients and their families.

Poster Number 221

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Therapeutic effects of recombinant heparin-binding domain protein in a mouse model of diabetic retinopathy

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Purpose: Diabetic retinopathy (DR) is characterized by increased numbers of leukocytes attachment (leukostasis) and vascular hyperpermeability (VP) of the retinal vessels. Previous experimental evidence supports that the heparin-binding VEGF165 isoform, but not the VEGF121 isoform that lacks the heparin-binding domain (HBD), is responsible for inducing retinal leukostasis. Preliminary data showed that the recombinant HBD (rHBD) inhibited VEGF165-induced leukostasis and pathological angiogenesis in an oxygen-induced retinopathy model. Thus, we hypothesize that rHBD also has potential therapeutic effects in a mouse model of DR.

Methods: Streptozotocin (STZ) injection of 6-8 weeks old C57BL/6 male mice were used to induce diabetes. Retinal VP was measured with the Fluorotron Master Ocular Fluorophotometer and retinal leukostasis was quantified by FITC-concanavalin A perfusion assay with retinal flat-mount and fluorescence microscopy. The mice were injected with rHBD or vehicle control intravitreally 6 months after induction of diabetes. Statistical analysis of data was performed using t-test and one-way ANOVA.

Results: Retinal VP was significantly increased in diabetic mice compared to non-diabetic mice. A single intravitreal injection of rHBD significantly reduced retinal VP by about 20% in diabetic mice compared to vehicle injected control. Diabetic mice received vehicle treatment had increased leukostasis compared to non-diabetic mice, while rHBD injection significantly reduced leukostasis compared to vehicle injected control, and to the levels comparable to that of the non-diabetic mice.

Conclusion: These data demonstrated that intraocular injection of rHBD significantly suppress retinal vascular hyperpermeability and leukostasis in a mouse model of DR, therefore rHBD could be an efficacious therapeutic for DR.

Poster Number 222

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Baseline posterior cingulate cortical responses to pain catastrophizing predict cognitive behavioral therapy outcomes in fibromyalgia

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We previously that the posterior cingulate cortex (PCC) encodes pain catastrophizing in fibromyalgia (FM) patients. We also showed distinct roles for different PCC subregions; ventral PCC (vPCC, which supports self-referential cognition) activity was correlated with patient-rated relevance of catastrophizing thoughts, while dorsal PCC (dPCC, associated with sensorimotor processing) activity was associated with FM pain severity. Catastrophizing-linked PCC activity may be an important phenotypic factor that shapes long-term fibromyalgia outcomes; it is not clear, however, if PCC activation can also predict individual differences in clinical outcomes for psychological therapies targeting pain catastrophizing. In this neural mechanism-focused clinical trial, we replicated previous findings linking vPCC activation with patient-reported catastrophizing and dPCC activation with fibromyalgia pain severity. We extend these findings by evaluating the predictive capacity of baseline PCC activation for FM treatment outcomes. Here we show that greater baseline PCC activation during pain catastrophizing prompts was predictive of reduced benefit (e.g., less post-therapy reduction in catastrophizing and pain severity). This implies that baseline brain activity to pain catastrophizing may serve as an important predictive biomarker that could: 1) identify a subgroup of FM patients prone to catastrophizing, 2) offer insight into the neural mechanisms by which cognitive and emotional processes influence pain, 3) provide a brain target for neuro-modulatory treatments, and 4) (eventually) help to guide treatment recommendations in order to optimize individual patient outcomes.

Poster Number 223

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A Novel Therapeutic Approach for Familial Dysautonomia

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Familial Dysautonomia (FD) is a neurodegenerative disorder caused by a splice mutation in the Elongator complex protein 1 (ELP1) gene. This mutation leads to skipping of exon 20 with tissue specific reduction of ELP1 protein. FD is a complex neurological disorder characterized by severe gait ataxia and retinal degeneration. There is currently no effective treatment to restore ELP1 protein in FD patients and the disease is ultimately fatal. After the compound kinetin was identified to correct ELP1 splicing defect, we worked on optimizing its potency and efficacy to generate novel splicing modulator compounds (SMCs) that can be used in patients. We recently developed a novel compound, PTC-258, which restores correct ELP1 splicing and rescues several disease hallmarks in a phenotypic mouse model of FD. PTC258 was administered postnatally in chow to the TgFD9; Elp1□20/flox mouse. Treatment increased full-length ELP1 transcript in a dose-dependent manner and, importantly, led to a two-fold increase in functional ELP1 protein in the brain. To evaluate the effect of the treatment on disease phenotypes, we monitored weight, spinal abnormalities, motor coordination, and retinal degeneration in treated versus untreated FD mice. The treatment was tolerated and improved the survival of FD pups in a dose-dependent manner. Importantly our findings demonstrate that by targeting the disease-specific splicing mutation and increasing exon inclusion it is possible to reverse gait ataxia and to improve retinal degeneration, the two main hallmarks of the disease, providing hope to the FD community as a potential therapeutic approach.

Poster Number 224

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Enhancement by HDACi of the susceptibility of solid tumor cells to recognition and elimination by B7-H3 CAR T cells

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Background: Clinical results generated by tumor antigen (TA)-specific chimeric antigen receptor (CAR) T cell-based immunotherapy against solid tumors have not been highly encouraging. This is at least in part because of the multiple escape mechanisms utilized by cancer cells to avoid recognition and destruction by CAR-T cells. Histone deacetylase inhibitors (HDACi) restore histone acetylation resulting in epigenetic changes within tumor cells that may counteract these escape mechanisms and make them more susceptible to recognition and elimination by CAR-T cells.

Methods: Chondrosarcoma and TNBC cells were treated with the HDACi SAHA (vorinostat). IFN-gamma ELISA and flow cytometry analysis of residual tumor cells were used to assess B7-H3-specific CAR-T cell recognition and antitumor activity in vitro. Mice orthotopically grafted with chondrosarcoma or TNBC cells were treated with SAHA and CAR-T cells.

Results: Tumor cells pre-treated with SAHA were recognized and eliminated more effectively by B7-H3.CAR-T cells compared to cells that were not treated with HDACi. Pretreatment with SAHA significantly prolonged the overall survival of mice orthotopically grafted with chondrosarcoma or TNBC cells that subsequently received B7-H3.CAR-T cell therapy. B7-H3 was upregulated in a dose- and time-dependent manner after SAHA treatment in terms of mean fluorescent intensity in vitro and when assessed by immunohistochemical analysis in vivo.

Conclusion: The results presented suggest that the changes mediated by HDACi in cancer cells enhance the antitumor activity of CAR-T cells both in vitro and in vivo and justify the translation of this combinatorial therapy to a clinical setting.

Poster Number 225

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Safety and Feasibility of Injectable Slurry for Fat-Selective Treatment of Obstructive Sleep Apnea

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Background: Obstructive sleep apnea (OSA) is a sleep disorder associated with significant morbidity and mortality. Accumulation of fat in the base of the tongue is highly associated with OSA. We developed an injectable ice slurry, as a selective and minimally-invasive fat removal technique. In this study we investigate safety and feasibility of slurry injection for selective destruction of OSA-associated fat in the base of the tongue.

Methods: We developed for-human-use prototype device to produce sterile and injectable ice-slurry. Four Yorkshire swine were injected with slurry at the base of the tongue using standard syringe and needle. Animals were observed for 2 months, after which tongue was harvested for histologic analysis by blinded pathologist.

Results: No difficulty in breathing, feeding or any sign of infection was observed post-injection. Tongue tissue at 2 months post-treatment showed no scarring, necrosis or nonspecific damage. Histological analysis of biopsy samples from the base of the tongue did not show damage to the surrounding muscle, nerve or vessels (necrosis score of 0±0). Histologic evidence of selective fat loss along with new collagen deposition was observed.

Conclusion: Here we show that injection of slurry is feasible and safe for selective targeting of fat tissue at the base of the tongue in preclinical swine model. Results of this study will serve as data for obtaining Investigational device exemption approval from the Food and Drug Administration for anticipated first in human studies in mid 2021. We hope this will become a minimally-invasive and novel therapy for OSA.

Poster Number 226

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Impact of androgen receptor splice variant expression on outcomes of post-prostatectomy therapy

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Radiotherapy with or without androgen deprivation therapy (ADT) plays a key role in the salvage treatment of prostate cancer recurrent following prostatectomy. However, not all patients benefit from salvage therapy, and there is an unmet need for biomarkers to distinguish responders from non-responders. Prostate cancer growth depends on androgen receptor (AR) signaling, and expression of AR splice variants (ARV) that activate AR in an androgen-independent fashion is associated with ADT resistance. Recent in vitro data suggest that ARVs also mediate DNA repair after irradiation, leading us to hypothesize that ARVs may serve as biomarkers of resistance to salvage therapy in post-prostatectomy patients. Here we show that ARV expression is associated with treatment resistance in a cohort of prostate cancer patients treated with salvage radiotherapy with ADT. We retrospectively identified 46 prostate cancer patients treated with post-prostatectomy salvage radiotherapy with ADT at MGH from 1995 to 2012. Formalin-fixed paraffin-embedded prostatectomy samples were subjected to ultra-deep targeted RNA-seq of all AR exon/intron regions. We comprehensively interrogated the landscape of ARVs, including 23 splice junction sites related to 22 AR variants. Expression of the variant AR-V7 was associated with earlier recurrence after salvage therapy (median biochemical failure-free survival: 8.85 vs 73.4 months, P=0.0002). Expression of AR-V4, a variant not previously associated with clinical significance, was also associated with earlier recurrence after salvage therapy (median biochemical failure-free survival: 15.7 vs 73.4 months, P=0.0171). In conclusion, comprehensive evaluation of ARVs revealed potential biomarkers of resistance to salvage therapy for recurrent prostate cancer after prostatectomy.

Poster Number 227

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Personalized Ex Vivo Testing of Therapies for Bladder Cancer Via Organoid Models

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Although radical, life-altering surgery is often recommended for muscle-invasive bladder cancer, bladder-preserving trimodality therapy, consisting of transurethral resection of bladder tumor (TURBT) followed by radiotherapy with concurrent chemotherapy, can be an effective alternative treatment approach. Ongoing clinical trials are testing whether the addition of immunotherapy to chemoradiation will further improve outcomes after trimodality therapy. However, not all bladder cancers respond to chemoradiation, and it remains unknown how best to select appropriate patients for trimodality therapy. Here we show the potential of patient-derived 3D organoid models to serve as a useful platform for the personalized ex vivo testing of therapies for bladder cancer. We utilized an air-liquid interface method to establish bladder cancer organoid cultures from tumors excised from patients undergoing TURBT for bladder cancer. Unlike previously described bladder cancer culture systems, these organoid models preserve the tumor immune microenvironment architecture including functional tumor-infiltrating lymphocytes, thus enabling the evaluation of response to a range of therapies including immune checkpoint inhibitors in combination with radiation and chemotherapy agents. In an initial series of 12 successfully established bladder cancer organoid models, we demonstrate preservation of the histologic appearance of the tumor parenchyma, stroma, and immune cells. We show that these patient-derived organoid models are amenable to ex vivo testing of sensitivity to radiation therapy alone and in combination with chemotherapy (cisplatin, 5-FU, gemcitabine, paclitaxel), and/or immune checkpoint inhibitors (nivolumab). This proof-of-concept study demonstrates the potential of personalized organoid models to guide therapeutic decisions in the management of patients with bladder cancer.

Poster Number 228

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Somatic CAG expansion in Huntington's disease is dependent on the MLH3 endonuclease domain, which can be excluded via splice redirection

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Somatic expansion of the CAG repeat tract that causes Huntington's disease (HD) is thought to contribute to the rate of disease pathogenesis. Therefore, factors influencing repeat expansion are potential therapeutic targets. Genes in the DNA mismatch repair pathway are critical drivers of somatic expansion in HD mouse models. Here, we have tested, using genetic and pharmacological approaches, the role of the endonuclease domain of the mismatch repair protein MLH3 in somatic CAG expansion in HD mice and patient cells. A point mutation in the MLH3 endonuclease domain completely eliminated CAG expansion in the brain and peripheral tissues of a HD knock-in mouse model (HttQ111). To test whether the MLH3 endonuclease could be manipulated pharmacologically, we delivered splice switching oligonucleotides in mice to redirect Mlh3 splicing to exclude the endonuclease domain. Splice redirection to an isoform lacking the endonuclease domain was associated with reduced CAG expansion. Finally, CAG expansion in HD patient-derived primary fibroblasts was also significantly reduced by redirecting MLH3 splicing to the endogenous endonuclease domain-lacking isoform. These data indicate the potential of targeting the MLH3 endonuclease domain to slow somatic CAG repeat expansion in HD, a therapeutic strategy that may be applicable across multiple repeat expansion disorders.

Poster Number 229

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Synergistic antibacterial action of antibiotic-analgesic combinations against Staphylococcal biofilms

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Periprosthetic joint infection (PJI) is a devastating complication associated with total joint replacement. Staphylococcus species remains the predominant causative microorganism implicated in PJI. Antibiotic resistance coupled with the biofilm-forming capability of the bacteria confers additional drug resistance which warrants a multimodal therapeutic strategy to manage the burden of infection. Local administration of antibiotics and analgesic combinations is common during the treatment of PJI. Previously we showed that several commonly used analgesics when used in combination with gentamicin, yield pronounced synergistic antibacterial effects against planktonic Staphylococcal bacteria. However, the effect of the synergistic activity against Staphylococcal biofilms has not been determined. Here we show that concurrent use of ketorolac with gentamicin yielded pronounced antibacterial synergy against Staphylococcal biofilms. Gentamicin susceptible and resistant strains of Staphylococcus aureus and Staphylococcus epidermidis demonstrated synergy [FIC index<0.5] for gentamicin sulfate-ketorolac tromethamine combination. Interestingly, both species exhibited distinctly different biofilm dynamics over time and minimum biofilm eradication concentration (MBEC) values for gentamicin was directly proportional to the biofilm maturity of the Staphylococcal strains. Furthermore, ketorolac when used in combination with gentamicin was able to significantly increase the anti-biofilm activity of gentamicin. Taken together, these results could serve as a foundation to stratify the risk of Staphylococcal biofilms and to test multimodal therapeutic strategies against microbial biofilms that pose a significant threat to PJI patients. Our findings have a potential to decrease post-arthroplasty mortality and morbidity thereby reducing the burden on the healthcare system.

Poster Number 230

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Radiation sensitizes tumor cells to B7-H3-specific IL-15 TriKE based immunotherapy

INVESTIGATORS: Y. Sekigami, L. Maggs, F. Kontos, D. A. Vallera, S. Ferrone, C. Ferrone

Background: Tri-specific killer engagers (TriKEs) in combination with natural killer (NK) cells are a novel strategy for antigen-specific immunotherapy. We present a B7-H3/IL-15 TriKE which targets NK cells to tumor cells expressing B7-H3. B7-H3 is a tumor antigen (TA) highly expressed on many tumor types and minimally expressed on normal tissues. NK cell/TriKEs are able to completely eliminate tumor cells in vitro, but not in vivo. To enhance the susceptibility of cancer cells to antibody-mediated destruction, we have utilized radiation to upregulate B7-H3 expression and increase their susceptibility to apoptosis. We tested whether radiation-induced changes could render squamous cell carcinoma of the head and neck (SCCHN) more sensitive to elimination by B7-H3/IL-15 TriKEs.

Methods: SCCHN cell line PCI-13 was irradiated at varying doses (0-12Gy) and cultured for 48 hours. B7-H3 expression was analyzed by flow cytometric analysis. Irradiated PCI-13 cells were co-cultured with NK cells at an E:T of 5:1 for 4 hours either alone, with IL-15 or B7-H3/IL-15 TriKEs. Degranulation of NK cells was assessed by surface expression of CD107a.

Results: Radiation of PCI-13 cells significantly upregulated B7-H3 expression in a dose-dependent manner. This effect is also time-dependent as B7-H3 expression levels returned to normal after 5-6 days. Cells that were irradiated with 12Gy were significantly more sensitive to B7-H3/IL-15 TriKE-mediated tumor cell elimination.

Conclusions: Radiation induced increased susceptibility of B7-H3-expressing cancer cells to the activity of NK cell/TriKEs, suggesting a strong rationale for including radiation to sensitize tumor cells to NK cell/TriKE-based immunotherapies in the clinical setting.

Poster Number 231

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Treatment of Benign Nervous System Tumors Using Attenuated Bacteria

INVESTIGATORS: G. J. Brenner

Schwannomas are slow-growing, benign neoplasms that develop throughout the body including along the spinal cord and within the cranium. These tumors cause pain, sensory/motor dysfunction, and death through compression of peripheral nerves, the spinal cord, and/or the brain. The great suffering and debility associated with schwannomas, in conjunction with the paucity of therapeutic options makes their treatment a major unmet medical need. Current treatment of benign schwannoma usually involves surgery and/or radiation therapy with the limitations of the risk of neurologic damage and low efficacy. In the present study, we report for the first time the preclinical application of intra tumoral attenuated bacteria as an immunotherapy for a benign neoplasm. The significance of this therapeutic option is that it is less invasive and safer than surgical resection. Our data show that this strategy decreases the volume of the injected tumor through direct cytotoxic and anti-angiogenic effects, and more importantly induces a systemic immune response that targets distal tumors and a memory response that prevents further development of new lesions. These data support further development of attenuated bacteria "armed" with potentially therapeutic genes, and to test their combination with immune check point inhibition treatment to further enhance the efficacy and thus the translational potential.

Poster Number 232

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Neurokinin-1 Receptor Antagonism Ameliorates Ocular Pain and Immune Responses in Dry Eye Disease

INVESTIGATORS: A. Naderi, S. Wang, T. Blanco, A. Yung, J. Yin, T. Dohlman, Y. Chen, S. Chauhan, R. Dana

Ocular pain is a common symptom of dry eye disease (DED). Our previous work has demonstrated increased levels of the neuropeptide substance P (SP) in DED. SP preferentially activates the neurokinin 1 receptor (NK1R) to mediate an inflammatory response. However, the direct effects of SP in ocular pain in DED are unknown. The purpose of this study was to determine the contributions of SP to ocular pain and inflammation in DED through antagonism of NK1R. Here we show that antagonism of NK1R simultaneously reduces ocular pain and suppresses inflammation in a murine model of DED, suggesting SP blockade as a new therapeutic strategy in the management of DED.

To assess ocular pain, DED was induced in C57BL/6 female mice by housing them in a dry chamber for 14 days and eye wiping test was performed to evaluate pain on days 0, 2, 4, 7, 10, and 14. L-733,060 (1μg/μl), an NK1R antagonist, was administered topically twice per day from day 0 to day 14. Eye wiping was significantly increased in the DED group compared to the normal group ($P < 0.001$). Application of L-733,060 led to significantly decreased eye wiping at day 4 and 14. Cornea and draining lymph nodes were collected on day 14 to assess SP expression level and MHC-IIhighCD11b+cells frequencies. Corneal SP expression levels were 25% lower in the L-733,060 group compared to the untreated DED group ($P = 0.051$). Additionally, the L-733,060 group showed significantly fewer MHC-IIhighCD11b+cells compared to the untreated DED group ($P = 0.001$).

Poster Number 233

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Senolytic therapy selectively eliminates senescent cancer-associated fibroblasts in skin

INVESTIGATORS: Y. Zhou, T. Kuehl, D. Lagares

Targeting Cancer associated fibroblasts (CAFs) is emerging as a novel therapeutic strategy to improve immunotherapy in desmoplastic tumors. We have recently showed that ABT-263, which targets the pro-survival protein BCL-XL, attenuates skin fibrosis by targeting myofibroblasts for apoptosis. Here, we explore the therapeutic potential of targeting CAFs for apoptosis in squamous cell carcinomas (SCCs). We first isolated dermal CAFs and cancer cells from patients with SCC along with normal fibroblasts from the adjacent healthy dermal tissue from the same patient. We determined that CAFs showed a senescent phenotype characterized by upregulation of p16 and were sensitive to ABT-263. However, ABT-263 did also promote apoptosis of cancer cells, suggesting BCL-XL dependency of tumor cells as well. To identify cell-specific strategies to target CAFs for apoptosis without affecting tumor cells, we applied BH3 profiling, which uses pro-apoptotic BH3 peptides to assess survival mechanisms in CAFs vs tumor cells, and can predict the efficacy of senolytic drugs to induce apoptosis. Our results show that CAFs are more sensitive to BIM/BID peptides which can directly activate BAX/BAK to promote apoptosis, while tumor cells are more sensitive to PUMA and HRK peptides, indicating that tumor cells rely on BCL-XL for survival. We further demonstrate that BAX agonist, a small molecule which has the same function as BIM/BID peptides, specifically targets CAFs for apoptosis without affecting tumor cell survival. Western blot analysis confirmed BAX upregulation in CAFs, further supporting our findings. Our studies suggest that BAX-agonist has potent and selective senolytic activities over senescent CAFs.

Alphabetical Listing of all Poster Presenters

Last Name	First Name	Poster Number
Abohashem	Shady	52
Acun	Aylin	1
Adiliaghdam	Fatemeh	115
Adkins-Jackson	Paris	99
Ahmadi Azar	Dara	2
Alagpulinsa	David	116
Almpani	Marianna	147
Alser	Osaid	206
Alshelh	Zeynab	148
Amatullah	Hajera	117
Anteby	Roi	207
Antic	Mina	194
Anzolin	Alessandra	149
Aryee	Emmanuel	100
Ashkani-Esfahani	Soheil	142
Ashraf	Shoaib	128
Bahari	Fatemeh	150
Bai	Yan	57
Ban	Yutong	208
Barton-Zuckerman	Maya	215
Bernstein	Emily	195
Bonkhoff	Anna	151
Bouley	Stephanie	92
Breithaupt	Lauren	196
Bukhari	Syed	29
Buta	Martin	209
Cannon	Taylor	12
Capasso	Virginia	190
Carroll	Shannon	76
Cattaneo	Giulia	216
Chand	Sidharth	129
Chartier	Christian	210

Last Name	First Name	Poster Number
Chekuri	Anil	217
Cheon	Se Yun	118
Choi	Jongmun	13
Chovel Sella	Aluma	81
Christie	Kathleen	218
Chu	Sarah	193
Colling	Caitlin	82
Copeland	Jessica	101
Correia	Dora	30
Cromer	Sara	83
Das	Alvin	14
Datta	Chandreyee	31
Datye	Aditya	152
De Silva	Pushpamali	119
DeRosa	Samantha	219
Detmer	Felicitas	15
Donahue	Nora	32
Dorken Gallastegi	Ander	211
Douglas	Vivian Paraskevi	130
Dubash	Taronish	33
Dugdale	Caitlin	131
Dunn	Samantha	34
Eaton	Amity	58
Ebrahimian	Shadi	102
Eyme	Katharina Marieke	35
Fatima	Nida	153
Ferguson	Scott	36
Fisher	Harrison	154
Fitch	Kathleen	191
Fitzmaurice	Kieran	132
Flynn	Elizabeth	3
Foley	Jacklyn	197

Alphabetical Listing of all Poster Presenters

Last Name	First Name	Poster Number
Fraschilla	Isabella	120
Frau-Pascual	Aina	155
Fuchs	Christiane	77
Gao	Boya	37
Garcia	Gian-Gabriel	68
Garg	Itika	16
Gerlovin	Kaia	59
Ghaednia	Hamid	4
Gomes dos Santos	Igor	121
Goodchild-Michelman	Isabella	69
Gorrepati	Pavane	103
Grahl	Arvina	156
Groot	Olivier	38
Guennoun	Ranya	39
Gupta	Rahul	93
Gustafsson	Karin	78
Halls	Justin	94
Hanford	Lindsay	17
Haque	Farah	60
Hardiman	Wendy	192
Harrison	Jon	70
Harshman	Stephanie	85
Hazhir Karzar	Nazanin	86
Hoebel	Katharina	18
Homayoun	Saffron	198
Hornstein	Shira	80
Inoue	Koetsu	40
Ioakeim Ioannidou	Myrsini	41
Ison	Juliana	157
Jacoby	Brigitte	158
Jarolimova	Jana	133
Jas	Mainak	159
Jaskiewicz	Justyna	134
Jensen	Mark	220
Jeon	Soomin	71
Ji	Zhenyu	221
Jiang	Xin	160
Kelly	Patricia	161
Kennedy	Bryan	162
Kfir	Yoav	163
Kfoury	Youmna	61
Kikuchi	Hiroto	42
Kim	Doyun	19
Kim	Jae Jung	135
Kim	Spencer	164
Kim	Andrew	199
Kimball	Allison	87
Klein	Madeleine	165
Lankinen	Kaisu	166
Lans	Amanda	143
Lau	Lauren	167
Lee	Younga	95
Lee	Boram	200
Lee	Jeungchan	222
Lei	Pinji	43
Levin	Rachel	201
Li	Jian	20
Li	Nan	168
Lim	Su Min	169
Liu	Jiyuan	136
Logan	Emily	223
Lubberts	Bart	144
Lupon	Elise	5
Lussier	Alexandre	202
Lynch	Jason	6
MacMore	Jason	170
Maggs	Luke	224
Mandigo	Torrey	171

Alphabetical Listing of all Poster Presenters

Last Name	First Name	Poster Number
Marcondes	Felippe	104
Martinez Ramirez	Luis	172
Matheson	Rudy	122
Matsubara	Teppei	173
Matzko	Michelle	137
Mazina	Varvara	44
McCormick	Natalie	105
McCreery	Chloe	123
McDowell	Alex	106
McNally	Melanie	174
Meinsohn	Marie	79
Meital-Kfir	Noya	175
Mezue	Kenechukwu	53
Michelakos	Theodoros	45
Migó	Marta	203
Moradi Tuchayi	Sara	225
Morales	Manuel	54
Morrison	Tierney	107
Muhammed	Maged	88
Naar	Leon	212
Nandy	Sreyankar	21
Nascimento	Fabio	176
Nayan	Madhur	213
Nieland	Lisa	177
Njauw	Ching-Ni Jenny	46
Nolan	Matthew	178
Noori	Ayush	179
Normoyle	Kieran	180
Onorati	Angelique	62
Otani	Keisuke	226
Otani	Yukako	227
Paredes-Echeverri	Sara	204
Paruchuri	Kaavya	55
Perlman	Or	22

Last Name	First Name	Poster Number
Petrozziello	Tiziana	181
Posada	Jessica	23
Qi	Meifang	72
Rabe	Daniel	7
Raef	Haya	8
Raghu	Vineet	108
Raigani	Siavash	214
Ramos Llorden	Gabriel	182
Rayle	Maya	73
Rice	Dylan	183
Roseen	Eric	145
Rosenberg	Chen	124
Roy	Jennie	228
Ruan	Yunfeng	96
Ruscic	Katarina	109
Ryu	Sang Baek	9
Saad	Mohammad	24
Saha	Amrita	125
Sailer	Clara O	89
Salloum	Shadi	138
Samuelsson	John	25
Sanders	William	184
Sanjari Moghaddam	Ali	47
Santarriaga	Stephanie	185
Santoso	Kate	84
Satinsky	Emily	110
Sekar	Amita	229
Sekigami	Yurie	230
Selberg	Alexandra M.	56
Selen	Daryl	90
Serifis	Nikolaos	126
Sevilla-Gonzalez	Magdalena	111
Shao	Manlin	231
Shu	Daisy	63

Alphabetical Listing of all Poster Presenters

Last Name	First Name	Poster Number
Siegel	Kaitlyn	205
Simoneau	Antoine	48
Singh	Praveer	74
Singh	Vijay	139
Sirbulescu	Ruxandra	75
Slepneva	Natalya	26
Spatola	Marianna	140
Stafford	Celia	112
Stalnaker	Katherine	64
Stamou	Maria	91
Subudhi	Sonu	113
Sun	Shi Hai	186
Taketani	Yukako	232
Tan	Yuting	65
Tang	Cheng-Chia	146
Tao	Zhipeng	66
Twarkowski	Hannah	187
Valentine	Kathrene	49
Vyas	Maulik	127
Weiss	Max	114
Westerman	Kenneth	97
Wu	Xiaotian	27
Xiao	Yao	50
Yang	Haibo	67
Yekula	Anudeep	188
Yu	Hojeong	10
Zekavat	Seyedeh (Maryam)	141
Zelmann	Rina	189
Zhou	Chaochao	28
Zhou	Shu Feng	51
Zhou	Yan	233
Zhu	Jinjin	98
Zwang	Theodore	11

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