Research Fellow Poster Celebration

May 16, 2018
Welcome to the 2018 Research Fellow Poster Celebration. Today’s program highlights the accomplishments of our very talented community of research fellows. MGH post doctoral research fellows are key members of the research endeavor at the hospital, and the Post Doctoral Division is proud to sponsor this day of recognition. Congratulations to all post doctoral research fellows who are sharing their research today.

PROGRAM AGENDA
May 16, 2018

9:00 am  
**Poster Session I**, *Bulfinch Tent*
- 9:00-9:30 am  even # posters presented
- 9:30-10:00 am  odd # posters presented

10:00 am  
**Welcome and Lectures**, *Thier Conference Room*
- Trends in Biomedical Science lecture  
  *Amy E. Keating, PhD, Professor, Biology and Biological Engineering, MIT*
- Research Career Development lecture  
  *Timothy P. Padera, PhD, Associate Professor, Radiation Oncology, HMS; Principal Investigator, Edwin L. Steele Laboratories for Tumor Biology*

11:30 am  
**Poster Awards Ceremony**, *Thier Conference Room*

12:00 pm  
**Poster Session II**, *Bulfinch Tent*
- 12:00-12:30 pm  odd # posters presented
- 12:30-1:00 pm  even # posters presented
ACKNOWLEDGEMENTS

REVIEW COMMITTEE

The 2018 Review Committee, made up of MGH faculty and research fellows, worked very hard to select the award-winning posters from among an excellent group of 81 abstract submissions. The committee reviewed the abstracts with authors/affiliations removed, according to criteria similar to those used in the review of NIH grants, and scored them based on three categories:

1. **Significance** (does the study address an important problem?)
2. **Approach** (are the conceptual framework and methods appropriate for the project?)
3. **Innovation** (does the project challenge existing paradigms or develop new methodologies?)

Following the initial abstract review, the top-ranked posters were reviewed to help select the award winners. We are extremely grateful to all members of the review committee for the thoughtful effort they put into reviewing each submission.

2018 Poster Celebration Review Committee members:

- **Nasim Maleki, PhD, Co-Chair**
- **Imran Rizvi, PhD, Co-Chair**
- **Ann Skoczenski, PhD, Co-Chair**
- **Jenn Walker, MA, Staff**
- Aranya Bagchi, MD
- Darrell Borger, PhD
- Xiqun Chen, MD, PhD
- Min-Kyung Choo, PhD*
- Jessica Collins, PhD
- Valentine Comaills, PhD*
- Hamdi Eryilmaz, PhD*
- Bryan Fuchs, PhD*
- Edmarie Guzman–Velez, PhD
- Oluwaseun Johnson Akeju, MD
- Kanakaraju Kaliannan, MD*
- Mohammed Khan, PhD
- Puja Kohli, MD
- Clotilde Lagier-Tourenne, MD, PhD
- Christian Lacks Lino Cardenas, PhD
- Cameron McAlpine, PhD*
- Anne Marie McCarthy, PhD
- Hamid Sabet, PhD
- Baehyun Shin, PhD*
- Michael Tolstorukov, PhD
- Alexandra Touroutoglou, PhD
- Marc Wein, MD, PhD
- Su Wu, PhD*

* denotes former poster celebration award winners
ABSTRACTS BY TOPIC

ANESTHESIA
Chronic Hypoxia Restores Hypoxic Pulmonary Vasoconstriction in a Mouse Model of Leigh Syndrome submitted by Grigorji Schleifer, MD #20
Increased HCN Channel Activity in the Gasserian Ganglion Contributes to Trigeminal Neuropathic Pain submitted by Weihua Ding, MD #15

BIOENGINEERING
Cell-specific drug delivery for the prevention of pulmonary fibrosis in vivo submitted by Md Nurunnabi, PhD #65
Development of a time-domain finite-element model of acoustic wave propagation within the cornea submitted by Behrouz Tavakol, PhD #52
Immunosolation and long-term function of human stem cell-derived² cells coencapsulated with CXCL12 in alginate in a murine model of type 1 diabetes submitted by David Alagpulinsa, PhD #69
Integrated Biosensor for Rapid and Point-Of-Care Sepsis Diagnosis submitted by Jouha Min, PhD #02
Integrated co-culture of hepatocytes and cholangiocytes as a 3D micro-organoid construct: toward engineering liver bile channel system submitted by Ehab O. A. Hafiz, MD, MSc #18
Integrated Magneto-Chemical Sensor For On-Site Food Allergen Detection submitted by Hsing-Ying Lin, PhD #55
Normothermic Machine Perfusion of Human Kidneys with no Oxygen Carrier submitted by Mohamed M. Aburawi, MD and Corey M. Eymard, MD #62
Radiomics of Coronary Artery Calcium in the Framingham Heart Study submitted by Parastou Eslami, PhD #77

CARDIOLOGY
Investigation of ECG-Gated Non-invasive Cardiac Arrhythmia Ablation Using Double Scattering Proton Beam submitted by Hyeri Lee, PhD #32
Novel near-infrared fluorescence agent for the in vivo detection of activated platelets submitted by Khanh Ha, PhD and Xiaoxin Zheng, MD, PhD #35
Radiomics of Coronary Artery Calcium in the Framingham Heart Study submitted by Parastou Eslami, PhD #77
Sudden Cardiac Death Among Persons Living with HIV with Heart Failure without an Implantable Cardioverter Defibrillator submitted by Raza Alvi, MD #76

CELL BIOLOGY/SIGNALING
Fluidic shear stress induces chemoresistance in Ovarian Cancer in a 3D microfluidic model submitted by Shubhankar Nath, PhD #58
Genetically encoded calcium biosensors reveal cell signaling dynamics during kidney glomerular morphogenesis submitted by Lydia Djenoune, PhD #44
Inner mitochondrial membrane protein Bcl-2 as a potential major player in autism and cancer  
submitted by Ya Wen, PhD  
#64

Mitochondrial permeability uncouples elevated autophagy and lifespan extension  
submitted by Ben Zhou, PhD  
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Role of GP73 in fibrosis submitted by Gunisha Arora, PhD  
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Sequential ALK Inhibitors Can Select for Lorlatinib-Resistant Compound ALK Mutations in ALK-Positive Lung Cancer submitted by Satoshi Yoda, MD, PhD  
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The role of peroxidasin in liver fibrosis development during NASH submitted by Mozhdeh Sojoodi, PhD  
#72

Therapeutic insight into Mucolipidosis IV via in vitro glia models submitted by Amanda Furness, PhD  
#29

**CHEMICAL BIOLOGY**

Novel near-infrared fluorescence agent for the in vivo detection of activated platelets  
submitted by Khan Ha, PhD and Xiaoxin Zheng, MD, PhD  
#36

**CLINICAL OUTCOMES**

Comparing mean continuous blood glucose monitoring data within individuals wearing a bi-hormonal bionic pancreas or using conventional care submitted by Jason Sloane, MD  
#74

Identifying Lupus Patients in Electronic Health Records: Application of Rule-Based Algorithms and Development and Validation of Machine Learning Algorithms submitted by April Jorge, MD  
#38

Microwave ablation of lower renal pole tumors with and without adjunctive pyeloperfusion submitted by Katayoun Samadi, MD  
#07

Noninvasive optical monitoring of cerebral perfusion to predict the outcome of mechanical thrombectomy -Towards personalized acute stroke treatment submitted by Parisa Farzam, PhD  
#75

Prenatal Exposure to Acid Suppressant Medications and Risk of Recurrent Wheeze at 3 years of Age in Children with a History of Severe Bronchiolitis submitted by Lacey Robinson, MD  
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The stable glucagon analog dasiglucagon is well-tolerated and as effective as recombinant human glucagon when delivered by the bionic pancreas in response to insulin excess submitted by Rabab Z. Jafri, MD  
#26

Traumatic Auditory and Vestibular Injury Following Head Injury: A New Take on an Old Diagnosis submitted by Renata Knoll, MD  
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**COGNITION**

Compassionate Hearts Protect Against Wandering Minds: Self-compassion Moderates the Effect of Mind-Wandering on Depression submitted by Jonathan Greenberg, PhD  
#05

Learning Not to Fear: Mindfulness Improves Retention of Fear Extinction submitted by Gunes Sevenc, PhD  
#50

Patient-clinician concordance in social mirroring circuitry underpins non-verbal communication and placebo analgesia in the context of pain treatment - a fMRI hyperscanning study submitted by Dan-Mikael Ellingsen, PhD  
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5 of 106
Tau PET imaging corresponds with neuropsychological performance to dissociate dorsal and ventral stream dysfunction in atypical Alzheimer's disease *submitted by Deepti Putcha, PhD*

**COMPUTATIONAL BIOLOGY**

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

**ENDOCRINOLOGY**

Characterization of the Chromatin Landscape of Human Parathyroids and Identification of Direct Targets of Glial Cells Missing Homolog 2 (GCM2) *submitted by Ian M. Li, PhD*

Comparing mean continuous blood glucose monitoring data within individuals wearing a bi-hormonal bionic pancreas or using conventional care *submitted by Jason Sloane, MD*

Dietary Fat Quality and Genetic Risk of Type 2 Diabetes *submitted by Jordi Merino, PhD*

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What Machines Can Read: Sex Identification from Hand and Wrist Radiographs *submitted by Sehyo Yune, MD, MPH, MBA*

**ENGINEERING/TECHNOLOGY/DEVICES**

Cell-specific drug delivery for the prevention of pulmonary fibrosis in vivo *submitted by Md Nurunnabi, PhD*

Cortical depth specific capillary blood flow homogenization facilitates resting state brain oxygen delivery *submitted by Baoqiang Li, PhD*

Development of a time-domain finite-element model of acoustic wave propagation within the cornea *submitted by Behrouz Tavakol, PhD*

EEG-Based Brain Age and Its Relation with Mortality *submitted by Luis De Carvalho Paixao, MD, MSc*

Integrated Biosensor for Rapid and Point-Of-Care Sepsis Diagnosis *submitted by Jouha Min, PhD*

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Excess of rare protein truncating variants in patients with amyotrophic lateral sclerosis submitted by Sali Farhan, PhD #60

GWAS in 446,118 European adults identifies 78 genetic loci for self-reported habitual sleep duration supported by accelerometer-derived estimates submitted by Hassan S. Dashti, PhD #08

Inner mitochondrial membrane protein Bcl-2 as a potential major player in autism and cancer submitted by Ya Wen, PhD #64

Mitochondrial permeability uncouples elevated autophagy and lifespan extension submitted by Ben Zhou, PhD #25

The meta-analysis of rare coding variants in the whole-exomes sequences of 25,000 cases and 50,000 controls implicates individual risk genes for schizophrenia submitted by Tarjinder Singh, PhD #71

**IMAGING**

Amyloid is associated with greater tau burden in clinically-normal females relative to males: Findings from two independent cohorts submitted by Rachel Buckley, PhD #21

Associations between cerebral blood flow and structural and functional brain imaging measures in individuals with neuropsychologically defined mild cognitive impairment submitted by Channie Kim, PhD #46

Cell-specific drug delivery for the prevention of pulmonary fibrosis in vivo submitted by Md Nurunnabi, PhD #65

Characterizing anhedonia in adolescents using diffusion MRI and a cluster-based approach submitted by Viviana Siless, PhD #51

Cortical depth specific capillary blood flow homogenization facilitates resting state brain oxygen delivery submitted by Baoqiang Li, PhD #36

Deep Learning Pseudo-CT Synthesis for Pelvis PET/MR Attenuation Correction submitted by Angel Torrado-Carvajal, PhD #81

Development of a time-domain finite-element model of acoustic wave propagation within the cornea submitted by Behrouz Tavakol, PhD #52

Genetically encoded calcium biosensors reveal cell signaling dynamics during kidney glomerular morphogenesis submitted by Lydia Djenoune, PhD #44

Global Quantification of Structural Brain Connectivity submitted by Aina Frau-Pascual, PhD #01

In trauma-exposed individuals, self-reported hyperarousal predicts resting-state functional connectivity in frontocortical and paralimbic regions submitted by Jeehye Seo, PhD #16

Integrated co-culture of hepatocytes and cholangiocytes as a 3D micro-organoid constructs: toward engineering liver bile channel system submitted by Ehab O. A. Hafiz, MD, MSc #18

Learning Not to Fear: Mindfulness Improves Retention of Fear Extinction submitted by Gunes Sevinc, PhD #50

Lymph node metastasis contributes to distant metastasis in cancer mouse models submitted by Ethel R. Pereira, PhD #45

Measurement of retinal capillary oxygenation in mice submitted by Ikbal Sencan, PhD #33

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**INFECTIOUS DISEASE/IMMUNOLOGY**

Bivalent Oral Cholera Vaccine Induces Memory B Cell Responses *submitted by Brie Falkard, PhD*

Defining Correlates of Humoral Immune Protection Against EBV *submitted by Christina B. Karsten, PhD*

Distinguishing immunomodulatory commensal viruses in healthy and disease intestine *submitted by Fatemeh Adiliaghdam, MD, MPH*

Exercise instructs hematopoietic progenitor cells and reduces chronic leukocytosis *submitted by Vanessa Frodermann, PhD*

Immune Profiling of Coxiella burnetii Vaccination and Infection by Mass Cytometry *submitted by Patrick Reeves, PhD and Susan Raju Paul, MBBS*

Integrated Biosensor for Rapid and Point-Of-Care Sepsis Diagnosis *submitted by Jouha Min, PhD*

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Targeting bacterial quorum sensing to improve intestinal barrier function following burn - site infection *submitted by Marianna Almpani, MD*
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Role of GP73 in fibrosis submitted by Gunisha Arora, PhD #49
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Neuropathology Correlates of Amyloid PET Imaging among Patients with Synucleinopathies submitted by Julia Shirvan, MD, DPhil #06
Targeting FUS protein to mitigate RNA processing alterations linked to ALS and frontotemporal dementia submitted by Fernande Freyermuth, PhD #48
Tau PET imaging corresponds with neuropsychological performance to dissociate dorsal and ventral stream dysfunction in atypical Alzheimer's disease submitted by Deepti Putcha, PhD #13
Transplantation of diverse miPSC-derived RGCs into mouse models of Glaucoma submitted by Julia Oswald, PhD #11

NEUROLOGY/NEUROSCIENCE
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Tau Induces Increased Formation and Dysfunction in Small Diameter Capillaries in Aged Tau P301L Mice submitted by Rachel Bennett, PhD

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Traumatic Auditory and Vestibular Injury Following Head Injury: A New Take on an Old Diagnosis submitted by Renata Knoll, MD

**OBSTETRICS/GYNECOLOGY**

Obesity and Maternal Mortality in the United States submitted by Mark Clapp, MD, MPH and Alexander Melamed, MD, MPH

#21

#59

#51

#36

#61

#66

#60

#64

#63

#33

#06

#75

#68

#31

#24

#57

#29

#78

#70
### Oncology/Cancer

- **Sialyl-Tn contributes to the pathology of ovarian cancer and is a potential therapeutic target** submitted by Linah Al-Alem, PhD and Andrew Baker, PhD

- **Chemoresistance in triple negative breast cancer is mediated via a therapeutically actionable YAP/RASAL2 pathway** submitted by Siang-Boon Koh, PhD

- **Combination of mesothelin-targeted immune-activating fusion protein and anti-PD-L1 augments antitumor immunity and prolongs survival in murine model of ovarian cancer** submitted by Xiying Qu, PhD

- **Fluidic shear stress induces chemoresistance in Ovarian Cancer in a 3D microfluidic model** submitted by Shubhankar Nath, PhD

- **Immunotherapy for malignant mesothelioma that combines a mesothelin-targeted immune-activating fusion protein and CXCL12/CXCR4 blockade** submitted by Qiang Yu, MD, PhD

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- **Pathologic Findings in Reduction Mammaplasty Procedures Identified by Natural Language Processing of Breast Pathology Reports: A Surrogate for the Population Incidence of Cancer and High Risk Lesions** submitted by Francisco Acevedo, MD, PhD

- **Proton therapy for brain tumors: how to measure where the protons stop** submitted by Fernando Hueso-González, PhD

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- **UV scintillating particles as a novel radiosensitizer to enhance cell killing after X-ray excitation** submitted by Matthias Müller, PhD

### Psychiatry

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Supercooling of Human Livers to Extend the Preservation Time for Transplantation submitted by Reinier De Vries, MD

Transplantation of diverse miPSC-derived RGCs into mouse models of Glaucoma submitted by Julia Oswald, PhD

**SURGERY**

A Pilot Study of Inpatient Satisfaction Rating of Surgical Resident Care submitted by Sophia K. McKinley, MD, EdM

Association of High Human Leukocyte Antigen -B and -C Expression Level with Prolonged Overall Survival in Colorectal Cancer submitted by Sean Lee, MBBS

Neuronal cell therapy to restore colorectal motility in a novel animal model of enteric neuropathy submitted by Sukhada Bhave, PhD

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“Distinguishing immunomodulatory commensal viruses in healthy and disease intestine”

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

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

We tested our approach on 100 subjects of the publicly available WashU-UMN Human Connectome Project data set. We performed tissue segmentation and cortex parcellation into ROIs using FreeSurfer and reconstructed the diffusion tensors from dMRI images using DSI Studio. For comparison with standard approaches, we ran streamline tractography using DTI and generalized q-sampling imaging (GQI). Then, we computed connectivity matrices according to various connectivity conventions: plain tract count, tract count normalized by the median length, considering tracts crossing the ROI or ending in the ROI, etc. The rs-fMRI data was first detrended, bandpass-filtered at 0.01-0.08Hz, and smoothed with a 6mm FWHM kernel. We stacked four sessions of rs-fMRI data, and computed the correlation matrix for the ROIs.

Results: We computed the Pearson correlation coefficient between the elements of the structural – with our global and the standard approaches – and functional connectivity matrices per subject, and then compared the distribution of these correlation values. In our experiments, functional connectivity is more strongly correlated with the proposed global structural connectivity than with all DSI Studio methods. A two-tailed paired t-test between these distributions revealed a statistic of t=42.2 and p=10⁻⁶⁵ in the DTI case, and t=44.2 and p=10⁻⁶⁷ in the GQI case, when considering the most favorable measure to DSI Studio.

Per our results, it seems that indirect connections indeed play a role in structure-function correlation. By considering all possible structural paths between a pair of regions, we seek to include pathways that contribute to functional connectivity, but we may also include unwanted pathways that do not make such a contribution. We expect orientational information from dMRI and the distance between faraway regions to reduce the impact of the latter.

Conclusion: We have proposed a new approach to measure the global structural connectivity, which allows to account for indirect brain connections that would not otherwise be considered by standard techniques. Using this methodology, we observed structural connectivity measures that are significantly more correlated with functional connectivity than by using more standard approaches. This supports the hypothesis on the role of indirect connections in the relationship between functional and structural connectivity. Our approach could also be used in a more general-purpose connectivity analysis, as an alternative to classic tractography algorithms. Moreover, it can enable the computation of voxel-wise and thus parcellation-independent connectivity.

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Integrated Biosensor for Rapid and Point-Of-Care Sepsis Diagnosis

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Purpose: Sepsis is an often fatal condition that arises when the immune response to an infection causes widespread systemic organ injury. A critical unmet need in combating sepsis is the lack of accurate early biomarkers that produce actionable results in busy clinical settings. Here, we report the development of a point-of-care platform for rapid sepsis detection. Termed IBS (integrated biosensor for sepsis), our approach leverages i) the newly-found pathophysiological role of cytokine interleukin-3 (IL-3) in early sepsis, and ii) a hybrid magneto-electrochemical sensor for IL-3 detection.

Materials & Methods: Assay. The IBS assay starts by extracting the target protein IL-3 through immunomagnetic enrichment. The captured protein is then subsequently labeled with detection antibodies and oxidizing enzyme (horseradish peroxidase, HRP). Finally, the beads are mixed with chromogenic electron mediators (3,3′,5,5′-tetramethylbenzidine, TMB); the enzyme catalyzes the oxidation of TMB and the reduction of H2O2. The oxidized TMB is then reduced by receiving electrons from the electrode, which generates electrical current as an analytical read out. Using magnetic beads allowed for direct and fast extraction of IL-3 from blood samples. Signal can also be amplified by concentrating magnetic beads underneath the electrodes. Clinical study. We applied IBS to detect IL-3 in clinical samples. Plasma or serum samples were collected from patients with symptoms of systemic infection, inflammation, or both. We obtained 23 samples from septic patients and another 39 from non-septic patients.

Results: The magneto-electrochemical sensing strategy developed here enables POC diagnostics: i) magnetic beads provides large surface area (~230 mm²) for target capture and an easy way to perform assay steps; ii) the analytical signal is read out by compact, rugged electronics. Applying the IBS prototype, we achieved rapid (<1 hr), highly sensitive (<10 pg/mL) IL-3 detection in human plasma samples. Whole blood can also be used without preprocessing, which would better position the assay into most clinical workflows. The pilot clinical study supported the potential of IL-3 as a surrogate biomarker of sepsis: the sensitivity was 91.3%, and the specificity 82.4%. In comparison, procalcitonin (PCT) has been shown to have accuracies below 80%. Because IBS is fast and consumes small amount of samples (100 µL), it can be readily adopted to track temporal changes of biomarkers. This capacity would aid in not only the timely diagnosis of acute septic shock, but also reliable prognostication assessment of the risk. Also, using the small amount of samples would be especially advantageous for detection of sepsis in newborns/infants as blood samples from preterm infants (with a blood volume of <50 mL) are limited in volume.

Conclusion: The developed platform produces test results within 1 hour from native blood samples, and detects IL-3 at a sensitivity of <10 pg/mL; this performance is 5-times faster and 10-times more sensitive than a current gold standard, enzyme-linked immunoabsorbent assay. Using clinical samples, we show that high plasma IL-3 levels are associated with high organ failure rate and thus greater risk of mortality, confirming the potential of IL-3 as an early diagnostic biomarker. Compact and fast, the IBS platform can be readily integrated into clinical workflows, enabling timely diagnosis and proactive treatment of sepsis.

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Pathologic Findings in Reduction Mammoplasty Procedures Identified by Natural Language Processing of Breast Pathology Reports: A Surrogate for the Population Incidence of Cancer and High Risk Lesions

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**Purpose:** Breast reduction surgery removes a random sample of breast tissue in otherwise asymptomatic women and thus provides a method to evaluate the background incidence of breast pathology in this population. Our goal was to identify the rate of atypical breast lesions and of cancers in women of various ages in the largest mammoplasty cohort reported to date.

**Materials & Methods:** With Institutional Review Board (IRB) approval, pathologic reports from patients undergoing bilateral mammoplasty procedures in 5 institutions were analyzed using Natural Language Processing, verified by human review. Patients with a previous history of cancer were excluded.

**Results:** A total of 5121 patients were included. The median age was 41 years (range 13-85). The median age was higher in patients with any incidental finding when compared to patients with normal pathologic report (51 vs 39, p=0.0001). Pathological findings were detected in 7.1% of procedures (table 1). Benign high-risk lesions (LCIS and atypical hyperplasia) were found in 319 patients (6.2%). Invasive carcinoma and DCIS was detected in 19 (0.4%) and 25 (0.5%) patients, respectively. The rate of atypias and cancers increased with age (table 2).

**Conclusion:** The rate of abnormal findings in asymptomatic patients undergoing mammoplasty was 7.1%, and showed increasing incidence with older age. As only a random sample of breast tissue was removed at operation, our findings likely underestimate the incidence of these findings in normal women.

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UV scintillating particles as a novel radiosensitizer to enhance cell killing after X-ray excitation

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**Purpose:** Radiation therapy is the gold standard treatment for inoperable malignant tumors. To increase the efficiency of radiation therapy, we propose combining traditional X-ray treatment with tumor-localized UVC emitting LuPO₄:Pr³⁺ nanoparticles. Using the combined treatment, X-rays are converted by nanoparticles into UVC radiation inside the tumor. UVC damages the DNA directly via an oxygen-independent mechanism which could improve treatment efficacy within hypoxic tumor areas. The purpose of this work was to demonstrate feasibility of the combined treatment via in vitro experiments.

**Materials & Methods:** The effect of generated UVC emission under X-ray irradiation was tested on human foreskin fibroblasts (HFF1). Cells were incubated with the nanoparticles and irradiated subsequently with an X-ray dose of 2 Gy. Viability of the cells was measured 48 h and surviving fraction 14 days after combined treatment using the MTT and colony formation assays. UV specific DNA damages were investigated by using an immunofluorescence staining assay for cyclopyrimidine dimers.

**Results:** The MTT assay as well as the colony formation assay illustrated a decrease of cell viability following the combined treatment. While the MTT-assay revealed a minor effect, a significant decrease in the surviving fraction of about 95 % was demonstrated by the colony formation assay. The results of the immunofluorescence staining assay for cyclopyrimidine dimers (CPDs) were compared to UVC irradiation of 60 J × m⁻². The data show an increase of CPDs of about 55 % for the combined treatment with nanoparticles and X-rays.

**Conclusion:** The data of the colony formation assay revealed that the combined treatment with a concentration of 2.5 mg × ml⁻¹ nanoparticles and X-ray irradiation with 2 Gy results in a reduction of surviving fraction, which is equivalent to an X-ray dose of 3 Gy alone. The amount of CPDs after the combined treatment with 2.5 mg × ml⁻¹ LuPO₄:Pr³⁺ and 4 Gy suggests that the generated amount of CPDs is similar to an exposure with UVC radiation of about 15 J × m⁻². Combining LuPO₄:Pr³⁺ with ionizing radiation results in a local increase of the dose, which can be useful for increased tumor control or lowering the radiation dose for sparing the surrounding normal tissue. Further experiments will be performed to evaluate if the oxygen independent mechanism of the UVC radiation can be used to increase cell inactivation under hypoxic conditions.

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Compassionate Hearts Protect Against Wandering Minds: Self-compassion Moderates the Effect of Mind-Wandering on Depression

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Purpose: People spend almost half of their waking hours mind-wandering rather than focused on their current activity or surroundings. Prior research has indicated that “a wandering mind and an unhappy mind”, with mind-wandering significantly exacerbating depressive symptoms. In contrast, self-compassion is linked to decreased depressive symptoms and increased well-being. Mindfulness Based Cognitive Therapy (MBCT) specifically trains individuals to be more compassionate and pay attention to their immediate experiences, and has been shown to reduce depressive symptoms. Currently very little is known about whether and how self-compassion and mind-wandering interact with one another over the course of treatment for depression and the potential impact of this interaction on longitudinal change in depressive symptoms. Such an interaction may better inform clinicians about potential mechanisms of depressive improvement and help optimize treatment programs for depression. We hypothesized that MBCT would effectively reduce mind-wandering and depressive symptoms, and improve self-compassion compared to a control group receiving treatment as usual. Moreover, we hypothesized that self-compassion would mediate the relationship between mind-wandering and depressive symptoms.

Materials & Methods: Forty participants with mild to severe depression were assigned to an eight week MBCT program or treatment as usual. Levels of self-compassion, mind-wandering and depressive symptoms were assessed before and after the program.

Results: At baseline, mind-wandering was associated with higher depressive symptoms only among individuals with low self-compassion. Self-compassion additionally predicted depressive improvement. MBCT increased self-compassion and reduced mind-wandering compared to the control group. Overall, longitudinal changes in self-compassion produced a moderation effect similar to the one at baseline so that increases in mind-wandering were associated with increases in depressive symptoms only among those who decreased in self-compassion.

Conclusion: Results provide the first evidence that self-compassion can protect against the deleterious effects of mind-wandering among depressed participants, both at baseline and longitudinally. Findings also suggest that self-compassion is an effective predictor of depressive improvement. Finally, MBCT is effective not only at reducing depressive symptoms, but also at targeting protective and risk factors associated with depression.

Implications of these findings include (1) the use of self-compassion measures to predict clinical prognosis and chances of recovery in depression; (2) the moderating role of self-compassion suggests a novel potential mechanism of depressive improvement, and (3) adopting a self-compassionate attitude may be better emphasized in therapeutic programs as a means to alleviate depressive symptoms and guard patients against the deleterious effects of their mind’s increased predisposition to wander.

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Neuropathology Correlates of Amyloid PET Imaging among Patients with Synucleinopathies
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Purpose: We sought to investigate the neuropathological correlates of antemortem amyloid neuroimaging in patients with dementia with Lewy bodies (DLB) and Parkinson’s Disease (PD). Although DLB and PD are both synucleinopathies, characterized histopathologically by Lewy body deposition, the majority of these patients additionally show post-mortem changes of amyloid and tau, features characteristic of Alzheimer’s disease (AD). This co-pathology is clinically relevant because its severity correlates with a shorter latency to the onset of clinical dementia and with a higher risk of mortality in both DLB and PD. The imaging modality focused on amyloid, whereas the pathology assessment included amyloid, Lewy body and tau burden.

Materials & Methods: PET-neuropathological correlations were performed on 18 subjects recruited from Massachusetts General Hospital’s Movement and Memory Disorder Units into a longitudinal study approved by the IRB of Partners Healthcare, Inc. Clinical diagnoses of DLB and PD were made using DLB Consortium and UK Brain Bank criteria, respectively. Clinical diagnoses at last study visit were 10 DLB and 8 PD (1 cognitively normal, 3 with mild cognitive impairment, and 4 with dementia). All subjects underwent PET imaging with the amyloid radioligand [11C]Pittsburgh compound B (PiB), along with annual neurological and cognitive testing. At death, subjects underwent neuropathological evaluation for burden of Lewy bodies (Braak-LB), neuritic amyloid plaques (C-amyloid), beta-amyloid plaques (Thal-amyloid), and neurofibrillary tangles of tau (B-tau). Neuropathology correlates of PiB retention in the precuneus region were investigated through standard regression models that controlled for interval between imaging and autopsy (range 0.2-7.0 years) using R Software.

Results: Significant correlations were observed between PiB retention in the precuneus region and the pathology measures of B-tau (p=7.8E-4) and Braak-LB (p=0.03). Correlations were weaker with C-amyloid (p=0.06) and Thal-amyloid (p=0.17). DLB disease status was associated with greater Braak-LB score (p=1.8E-3) but did not differ from PD disease status with respect to age at imaging, age at death, or other neuropathology measures.

Conclusion: Antemortem PiB PET retention correlates with neuropathological changes seen at autopsy in patients with DLB and PD. This correlation is strongest for B-tau, a histopathologic hallmark of AD. Next is Braak-LB, the histopathologic hallmark of these synucleinopathies. C-amyloid and Thal-amyloid measures appear to be rather weak indicators of regional fibrillar amyloid burden, as measured with PiB PET. The detection of neuropathological species through in-vivo neuroimaging could influence clinical management of patients with these common neurodegenerative diseases, particularly with regard to prognostication.

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Microwave ablation of lower renal pole tumors with and without adjunctive pyeloperfusion
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Purpose: To compare primary and secondary efficacy, complication rate and technical success in Computed Tomography (CT) guided Microwave Ablation (MWA) of lower pole renal tumors with and without pyeloperfusion as a modality to protect ureter from untargeted thermal injury.

Materials & Methods: Retrospective review of records was undertaken to identify patients with RCC who were treated with MWA with adjunctive pyeloperfusion; we also recruited similar number of patients with RCC who only underwent MWA without pyeloperfusion to compare the outcomes. The choice of pyeloperfusion was made on case by case basis by interventional radiologist considering the risk of thermal injury to the ureter. The distance between the tumor and ureter and tumor size was measured on axial imaging. Location of the tumors was categorized based on Gervaise classification system into three groups of exophytic, parenchymal and mixed. Pyeloperfusion was performed in 9 patients in this series. Pyeloperfusion was performed by a urologist after placement of ureteral stent and instillation of diluted contrast into the ureter. Afterward MWA of the tumors were performed under CT guidance. Hydromedication was performed to displace other at risk structures. Creatinine was measured before and after the procedure as an index of renal function. A CT scan was performed at the end of the procedure and at 1, 3, 6 months intervals post procedure to identify the presence of residual disease and complications.

Results: 18 biopsy proven RCC in 18 patients were treated with 20 sessions of MWA. Mean age of the patients was 69±2.4. The average follow-up time for this study was 180 days. Average distance between ureter and the tumors in axial CT views was 20.8 (±2.9) mm. Primary efficacy was achieved in 88 percent of pyeloperfused patients and 100 percent of none pyeloperfused patients. Two pyeloperfused patients required secondary procedure and full secondary efficacy was achieved for both. There was only one grade two urologic complication which occurred in a patient who underwent pyeloperfusion, we assume the failure in protecting urinary tract was pyeloperfusion malfunction which we noticed after ablative procedure that pyeloperfusion set was leaking and the amount of infused fluid was not sufficient to protect the ureter. Level of creatinine was not significantly different after the procedure for either group (p-value 0.4). In this study, technical success was 100% .Tumor location, polarity and size were not identified to be independently predictive of primary success in MWA. Procedure duration was significantly longer in patients with pyeloperfusion (p-value 0.004).

Conclusion: In this study MWA of lower pole renal tumor was successfully performed using pyeloperfusion as a protective measure from unintended thermal injury to ureter. Considering all the benefits that MWA can offer compared to other ablative methods, developing a safer technique for this method would be of great value.

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GWAS in 446,118 European adults identifies 78 genetic loci for self-reported habitual sleep duration supported by accelerometer-derived estimates

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Purpose: Sleep disturbances have negative consequences on health, yet, molecular mechanisms regulating sleep and underlying the link to diseases remain poorly understood. Genome-wide association studies (GWAS) for sleep duration have thus far identified two GWA signals associated with sleep duration.

Materials & Methods: We performed GWAS in 446,118 participants of European ancestry from the UK Biobank using BOLT-LMM adjusted linear mixed models. In a subgroup \( n=85,499 \) with up to 7-day accelerometry, we tested associations between the GWAS signals and 8 activity-monitor derived sleep estimates using a weighted genetic risk score using the GTX package in R. For replication purposes, we conducted a lookup of lead self-reported sleep duration signals in self-reported sleep duration GWAS results from adult (CHARGE) and childhood/adolescent (EAGLE). Gene-based analyses were performed using Pascal and MAGMA gene-set enrichment analyses. Tissue enrichment analysis was conducted using FUMA for 53 tissue types. Genome-wide genetic correlation analysis of LD Score Regression (LDSC) using LDHub was conducted for sleep duration with 224 published GWAS. Mendelian randomization analyses were carried out using MR-Base, using the inverse variance weighted approach for significantly correlated traits.

Results: We discover 78 loci for self-reported sleep duration that further impact accelerometer-derived estimates of sleep duration, daytime inactivity duration, sleep efficiency and number of sleep bouts. Individual signals exert an average effect of 1.04 [95% CI: 1.00-1.07] minutes per allele, with the largest effect at the \( PAX8 \) locus. The 5% of participants carrying the most of the 78 sleep duration-increasing alleles self-reported 22.2 [22.1-22.3] minutes longer sleep duration compared to the 5% carrying the fewest. Genome-wide SNP-based heritability was estimated at 9.8 (0.001)%.

Conclusion: The present findings expand our understanding of the genetic architecture of sleep and the shared genetics links with disease traits.

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A Pilot Study of Inpatient Satisfaction Rating of Surgical Resident Care

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Purpose: Patient satisfaction is an increasingly important quality metric and resident physicians have a significant contribution to inpatient patient care. However, current institutional quality measures do not typically include patient experiences with resident physicians. This study explores inpatient satisfaction with surgical resident care.

Materials & Methods: Patients recovering from major abdominal surgery were surveyed regarding their satisfaction with and attitudes toward surgical resident care on postoperative days 2-4. Eight items were adapted from the Surgical Consumer Assessment of Healthcare Providers and Systems Survey (S-CAHPS). Patients positively identified residents from photos prior to providing ratings.

Results: 102 of 112 approached patients participated (91%, mean age=62.9, 51.6% male). Patients positively identified both seniors and interns 88% of the time. Thirteen seniors and 19 interns were rated, with 1-14 evaluations per resident. Overall quality of care ratings for seniors and interns were 9.35 and 9.09 respectively (1-10 scale, 10 = “best possible care”). Sixty-three percent of senior resident evaluations and 60% of intern evaluations received a score of 10. Item top-box scores ranged from 59.5% (“During your stay in the hospital, did this intern doctor discuss the outcome of the surgery with you?”) to 97.7% (“During your stay in the hospital, did this senior resident show respect for what you had to say?”). Across the entire adapted S-CAHPS instrument, 35% percent of senior resident and 38% of intern evaluations received top-box scores for all items. There was 25% concordance between senior and intern perfect evaluations, indicating that patients likely evaluate seniors and interns independently. Over 95% of patients reported strong or moderate agreement with the statements “I feel it is important to help in the education of future surgeons” and “I am comfortable having resident/intern doctors involved in my care.

Conclusion: Surgical inpatients harbor a positive attitude towards participating in surgical resident education and typically can recognize their resident physicians to provide individual ratings. While overall rating of surgical resident care was high, there may be room for improvement in certain S-CAHPS domains, indicating that patients are a valuable source of feedback regarding a resident’s progress in several core competencies. This feedback is essential to prepare trainees for a healthcare environment that ties patient satisfaction to hospital reimbursement.

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Chemoresistance in triple negative breast cancer is mediated via a therapeutically actionable YAP/RASAL2 pathway
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Purpose: Triple negative breast cancer (TNBC) is one of the most therapeutically intractable malignancies, owing to genetic heterogeneity and a lack of druggable targets. The primary systemic treatment option remains cytotoxic chemotherapies. While much work has focused on identifying TNBC patients who will respond to conventional chemotherapies, this effort does not address those who are unlikely to respond and therefore have the poorest clinical outcomes. Here, we sought to identify rational therapeutic options for treatment-refractory TNBC patients.

Materials & Methods: We performed bulk RNA-sequencing of 55 primary TNBC tumors collected from a Phase II clinical trial of single-agent platinum chemotherapy. Following the discovery of a gene signature that underlay tumors of treatment-refractory patients, as clinically defined by established response evaluation criteria (RECIST), we analyzed large-scale drug-response data to investigate if the signature correlated with sensitivity to any therapeutic agents. We then employed phospho-proteomic and biochemical approaches to uncover the molecular mechanisms of TNBC chemoresistance and collateral sensitivity.

Results: Gene expression profiling of primary TNBC tumors revealed that patients with de novo disease progression harbored a unique gene signature, which we termed the Highly Refractory Tumor (HRT) signature. Analysis of extensive dose-response data showed that the HRT signature predicted tumor cell sensitivity to multiple clinical MAP kinase kinase (MEK) inhibitors. Using an isogenic TNBC cell line model of chemoresistance, we confirmed greater sensitivity to MEKi in cisplatin-resistant cells compared to parental cells. Employing reverse-phase protein array phospho-proteomic analysis, we uncovered deregulation of the transcriptional cofactor Yes-Associate Protein (YAP) as a key mediator of these phenotypes. Convergent analyses involving chromatin immunoprecipitation and loss-of-function studies of YAP revealed RAS-GTPase activating protein RASAL2 as a direct transcriptional target promoted by YAP in chemoresistant TNBC. Correspondingly, a YAP signature and RASAL2 expression were significantly increased in tumors from patients experiencing rapid progression on platinum therapy. Strikingly, expression of RASAL2 in TNBC cells was sufficient to induce epithelial-mesenchymal transition (EMT) phenotypes, enabling chemoresistance. In tandem, expression of RASAL2 sensitized TNBC cells to MEKi through suppression of MAPK and PI3K signaling upon exposure to the inhibitors.

Conclusion: Chemoresistance in TNBC is mediated by hyperactive YAP, which transcriptionally promotes RASAL2 expression. RASAL2 expression confers EMT phenotypes associated with chemoresistance, but induces sensitivity to MEKi via attenuation of MAPK and PI3K signaling. These findings provide potential biomarkers and an actionable therapeutic approach for treatment-refractory TNBC patients.

Model of TNBC chemoresistance and collateral MEKi sensitivity

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Transplantation of diverse miPSC-derived RGCs into mouse models of Glaucoma
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Purpose:
Retinal ganglion cell (RGC) replacement has been proposed as an approach to recover lost vision in glaucoma and other optic neuropathies. While studies addressing axonal regeneration are progressing at fast pace, promising the possibility to rewire the eye-to-brain connection, RGC transplantation has only been published for primary cells - a strategy not suited for the clinic. Following our previous success in generating diverse RGC subtypes from pluripotent stem cells (iPSC and ES), we studied the ability of those RGCs to survive and integrate following transplantation within healthy and diseased hosts.

Materials & Methods:
RGCs were differentiated for 21 days within 3-dimensional retinal organoids, derived from Thy1-GFP iPSC (C57Bl/6 background). Thy1+ cells were isolated by magnetic micro-beads for syngeneic transplantation. As hosts, healthy adults, adults with RGC loss (NMDA and microbead-induced IOP elevation) and p2-p4 pups were used. RT-PCR, Flow Cytometry, Immunohistochemistry and Calcium Imaging confirmed molecular, functional identity of donor RGCs within re-plated cultures.

Results:
Transplantation success, measured as host retinas containing more than 10 donor RGCs, was highest in pups (83%, n=12). The survival of donor RGCs in healthy adult recipient retinas was higher (57%, n=14) than previously observed for primary RGCS (10%). Notably, transplantation success was even higher in models of RGC loss: microbead mediated elevation of IOP (67%, n=6) or NMDA toxicity injection (67%, n=6). On average, 2-3 weeks post-transplantation, host retinas contained a few hundred donor cells, extending both dendritic and axonal projections, which if cells integrated adjacent to the optic nerve entered the optic nerve head (Fig.1). Subtype diversity observed within transplanted RGCs was comparable to the initial donor population. In-vitro, RGCs displayed spontaneous and light-mediated activity, implying functional maturation pre-transplant.

Conclusion:
Thy1 positive, iPSC-derived RGCs are capable to survive post-transplantation within both, healthy and diseased host retinas and retain subtype specific identities. Following the observed electrical functionality of the generated RGCs in-vitro, our work opens the possibility for further transplantation studies eventually addressing the functionality of donor RGCs in-vivo.

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Exercise instructs hematopoietic progenitor cells
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Purpose: Physical activity beneficially affects metabolism and thereby positively influences cardiovascular health. However, how exercise affects the immune system is incompletely understood.

Materials & Methods: To address this question, we gave mice access to exercise wheels for six weeks.

Results: Here we show that voluntary exercise in mice alters the hematopoietic stem cell niche, resulting in increased bone marrow retention and quiescence factors. Mice show reduced hematopoietic stem and progenitor proliferation and differentiation, and overall reduced innate and adaptive immune cells. The exercise effect on the bone marrow niche initially persist during a subsequent 3-week sedentary phase, when exercise wheels are withdrawn. In settings that result in a skewed hematopoiesis, e.g. in ApoE deficient mice on high cholesterol diet, exercise can reduce the observed myeloid bias. Interestingly, while exercise reduces leukocytes in steady state, an acute challenge e.g. with endotoxin result in increased differentiation of quiescent progenitor cells and better clearance of inflammation.

Conclusion: These findings define a new mechanism by which regular physical activity positively transforms hematopoiesis and reduces cardiovascular risk.

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Tau PET imaging corresponds with neuropsychological performance to dissociate dorsal and ventral stream dysfunction in atypical Alzheimer’s disease

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Purpose: Posterior cortical atrophy (PCA), a “visual variant,” and logopenic Primary Progressive Aphasia (L-PPA), a “language variant” of Alzheimer’s disease (AD) present differently in terms of clinical phenotype and topographical distribution of paired helical filament tau compared to typical amnestic AD. While the spatial distribution of tau pathology has been associated with domain-specific cognitive deficits (e.g., language, memory) across the AD phenotypic spectrum, little is known about the relation of specific dorsal (parietal) and ventral (occipito-temporal) stream deficits to the topographic distribution of tau in the atypical variants of AD. We sought to relate task performance of visual/quantitative functions to tau deposition in regions of interest hypothesized to support these tasks.

Materials & Methods: Twelve individuals (6 diagnosed with PCA and 6 diagnosed with L-PPA based on neurological and neuroimaging exams) underwent neuropsychological evaluation and \(^{18}\text{F-FTP PET imaging. Bilateral FTP SUVR values were calculated in dorsal parietal regions, including the inferior parietal lobules (IPL), superior parietal lobules (SPL); a medial parietal region (precuneus); and a ventral stream region (Fusiform Gyrus). Bivariate correlations determined the association between tau signal in these regions and a measure of auditory calculations, a spatial attention task (VOSP Number Location Test) and an object naming test (MINT).}

Results: Poor performance on calculations was related to greater tau deposition in dominant (lh) dorsal stream parietal regions (IPL; \(r = -0.59, p=0.04\) and precuneus; \(r = -0.69, p=0.01\)) but not in non-dominant (rh) dorsal \((p>0.3)\) or ventral regions \((p<0.03)\). In contrast, poor performance on the spatial attention task was related to greater tau deposition in a non-dominant dorsal stream region (rh SPL; \(r = -0.76, p=0.004\)), and to a lesser extent in a dominant dorsal region \((p>0.05)\), but not in any ventral region \((p>0.1)\). Finally, poor performance on the object naming task was related to greater tau in a dominant ventral region (lh Fusiform Gyrus, \(r = -0.6, p=0.03\)), but not to any other ventral or dorsal region of interest \((all \ p’s >0.2)\)

Conclusion: These results support hypothesized syndromic variability within the atypical AD spectrum of PCA and L-PPA, associated with tau deposition in dorsal parietal and ventral temporal brain regions. With the knowledge of tau specificity in relation to cognition, these findings can be used to improve clinical tracking and model the spread of tau pathology in these atypical phenotypes as disease spreads.

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Targeting bacterial quorum sensing to improve intestinal barrier function following burn-site infection

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Purpose: Burn-site infections (BI), commonly due to Pseudomonas aeruginosa, have been associated with deranged intestinal integrity, allowing bacteria and their products to translocate from the gut to the circulation. The P. aeruginosa quorum sensing (QS) transcription factor MvfR (PqsR) controls the expression of many virulence factors, and the synthesis of several toxic products. However, the role of QS in intestinal integrity alterations has not been previously interrogated. In this setting, we utilized one of the anti-MvfR QS-inhibitors that we discovered, to assess the contribution of QS in the intestinal barrier dysfunction following BI.

Materials & Methods: Following induction of a 30% dorsal burn in C57BL/6 mice, a clinical P. aeruginosa isolate (PA14) was intradermally inoculated at the burn wound. MvfR inhibitor (M64) was administered at 2, 4, 8 and 16 hours following burn and infection. Mice were gavaged with Fluorescein Isothiocyanate-Dextran (FITC-Dextran). Permeability was assessed by serum FITC-dextran concentration, bacterial translocation to the mesenteric lymph nodes (MLNs), and tight junction alterations in the ileum. Intestinal inflammation was determined by ileum TNF-α, colon IL-6, and fecal lipocalin-2. Systemic inflammation, as indicated by serum endotoxin levels, and PA14 systemic dissemination were also assessed.

Results: Our results show that MvfR function exacerbates the post-burn intestinal hyperpermeability, increasing FITC-Dextran flow from the intestine to the circulation in thermally injured and infected mice. Inhibition of MvfR function through the use of our anti-virulence, anti-MvfR agents, significantly decreases the flux out of the gut, diminishes bacterial translocation from the intestine to mesenteric lymph nodes (MLNs), and improves tight junction integrity (Figure 1A). Moreover, antagonist administration alleviates the intestinal inflammation, as shown by reduced ileal TNF-α, colonic IL-6, and fecal lipocalin-2 concentrations (Figure 1B). In addition, it is associated with lower levels of circulating endotoxin and decreased P. aeruginosa dissemination from the burn wound to the ileum.

Conclusion: Collectively, our results show that inhibition of this QS system mitigates gut hyperpermeability by attenuating the derangement of morphological and immune aspects of the intestinal barrier, indicating that MvfR function is crucial in the intestinal integrity deterioration following P. aeruginosa burn-site infection. Thus, an anti-virulence approach targeting MvfR, could potentially offer a new therapeutic approach against multi-drug resistant P. aeruginosa infections following thermal injuries. Since this approach is targeting virulence pathways that are non-essential for growth or viability, it is expected to decrease the development of bacterial resistance, and preserve the beneficial enteric microbes, while improving intestinal integrity that is deranged as a result of burn and infection.

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Increased HCN Channel Activity in the Gasserian Ganglion Contributes to Trigeminal Neuropathic Pain

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**Purpose:** Trigeminal nerve damage-induced orofacial pain is severe and more resistant to standard pharmacological treatment than other types of neuropathic pain. However, the impact of hyperpolarization-activated cyclic nucleotide-gated (HCN) channel activity in the gasserian ganglion (GG) on trigeminal neuropathic pain has not been examined. We utilized an improved trigeminal nerve injury model, in which the distal infraorbital nerve was subjected to chronic constriction injury (dIoN-CCI), to investigate the role of HCN channels in trigeminal neuropathic pain.

**Materials & Methods:** Adult male Sprague–Dawley rats weighing 270-300 g were purchased from Charles River Laboratories. A Chronic constriction injury of infraorbital nerve (dIoN-CCI) was used to induce trigeminal neuropathic pain in rats. A guide cannula for intra-gasserian ganglion microinjection was implanted. HCN channel inhibitors or vehicle was microinjected into the GG of rats. Orofacial sensitivity to mechanical stimulation was tested using von Frey filaments. Face-grooming action was analyzed to assess the spontaneous nociceptive behavior. HCN mRNA expression in the GG was analyzed by real time qRT-PCR. HCN protein was analyzed by immunohistochemistry of the GG tissue sections.

**Results:** Unilateral ligation of infraorbital nerve induces nociceptive behavior such as mechanical allodynia and intense unilateral facial grooming, which lasted for weeks. Both 0.1 µg and 1 µg of ZD7288 (a HCN channel blocker) improved mechanical allodynia and the effect of 1 µg of ZD7288 lasted for 90 min. Measured at 30 min after 1 µg of ZD7288 injection, asymmetry facial grooming was also reduced in the dIoN-CCI rats. Ivabradine is a specific and selective HCN blocker currently being used to treat heart failure. At fourteen days after dIoN-CCI, the nociceptive threshold was significantly increased at 15 min after microinjection of 0.1 µg or 1 µg of ivabradine, which lasted for 90 min or 120 min respectively. We found that the mRNA levels of HCN1 and HCN2 were much higher than HCN3 and HCN4 in the GG. at fourteen days after dIoN-CCI, the counts of HCN1 and HCN2 immunopositive cells were increased in the ipsilateral GG of dIoN-CCI rats compared with sham rats.

**Conclusion:** All known HCN blockers cause bradycardia resulting from blocking the sinoatrial HCN4. Thus, broad HCN channel blockers may not be useful when applied systemically in neuropathic pain management. In contrast, administering a drug at the sites of peripheral pain transmission may avoid the side effects of systemic delivery. A recent study demonstrated that under the guidance of computed tomography (CT), a drug can be delivered precisely around the DRGs to decrease pain transmission in swine. Our study suggests that microinjection of an HCN blocker around the GG site might provide an alternative treatment of trigeminal neuropathic pain because the GG is enclosed in the Meckel cave which is readily accessible via needle access. This approach, coupled with the development of specific blockers of HCN1 and HCN2, would be clinically beneficial to patients with trigeminal neuropathic pain.

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In trauma-exposed individuals, self-reported hyperarousal predicts resting-state functional connectivity in frontocortical and paralimbic regions.

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\textbf{Purpose:} Traumatic-stress disorders are associated with CNS hyperarousal and impaired emotion regulation. In trauma-exposed individuals having symptoms that varied from absent to sufficient for PTSD diagnosis, we used resting-state functional connectivity (rsFC) to examine associations between self-reported hyperarousal and rsFC anterior salience and executive control networks.

\textbf{Materials & Methods:} Forty-two trauma-exposed participants (18-40y, mean=22.84±4.6y) completed psychological interviews that included the Clinician-Administered PTSD Scale (CAPS). A canonical self-report Hyperarousal Scale combined a published hyperarousal scale with the hyperarousal items of the PTSD Checklist and CAPS. Subjects underwent resting-state scans at 3T followed by seed-based rsFC analyses. Seeds were created for 5 fear-related regions – left and right amygdala, left and right anterior insular cortex (AIC), dorsal anterior cingulate cortex (dACC) – and 1 fear-regulatory region – ventromedial prefrontal cortex (vmPFC). Hyperarousal scores were used to predict connectivity between these seeds and regions within a frontocortical and limbic mask.

\textbf{Results:} Hyperarousal scores positively correlated with connectivity between: 1) left amygdala and right primary motor cortex; 2) right amygdala and pons; 3) right AIC and right superior medial frontal cortex; and 4) vmPFC and both supplementary motor cortex and right dorsolateral prefrontal cortex (dLPFC). Hyperarousal scores were negatively correlated with connectivity between: 1) left amygdala and right caudate body; 2) dACC and both the left dLPFC and right thalamus; and 3) right AIC and both the vmPFC, and right superior frontal cortex.

\textbf{Conclusion:} Among trauma-exposed individuals, hyperarousal predicted variation in connectivity of fear-and emotion-regulatory seeds with motor, frontal association and subcortical regions of the salience, executive control and motor networks.

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A High-Throughput Platform for Nerve Conduit Assessment
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**Purpose:** To present a novel platform for high-throughput histologic assessment of neural regeneration across conduits in a transgenic rodent model that expresses yellow fluorescent protein in peripheral axons.

**Materials & Methods:** A rapid, non-toxic, and stain-free frozen section protocol suitable for assessment of neural regeneration by brightfield and confocal laser scanning fluorescent microscopy was developed. Interposition repair of a sciatic nerve defect in Thy1.2 YFP-16 mice was performed using various types of bioengineered neural conduits, and regeneration assessed at six weeks.

**Results:** Processing time for axon counting was shortened from two weeks to three days, and stain costs eliminated. Confocal fluorescent microscopy images revealed excellent morphology of regenerating axons, with clear elucidation of permissive vs. repulsive conduit environments.

**Conclusion:** A rapid and cost-efficient platform for assessment of neural regeneration suitable for testing of novel neural conduit designs has been described.

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Integrated co-culture of hepatocytes and cholangiocytes as a 3D micro-organoid constructs: toward engineering liver bile channel system

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Purpose: Incorporating non-parenchymal cells with hepatocytes in liver bioengineering possess a more realistic model reflecting tissue architecture similar to the liver in vivo. Cholangiocytes forms bile ductules, which transport and modify the bile produced by hepatocytes. Current challenges facing establishment of a bile clearance system are the gap in investigating the interlink between formed bile canaliculi within the engineered liver model and engineered bile ductules, and tracking bile flow from hepatocytes till reaching ductular network, finally connecting the grafts to the ultimate intended host duodenum. Aiming to, develop a robust liver model that can bridge liver failure patients until transplantation.

Materials & Methods: Our approach starts by building up 3D co-culture spheroid model of hepatocytes/ cholangiocytes and studying the function and morphological arrangement of the cells within, then embedding these liver micro-organoids in a designed extracellular matrix for reconstruction of biliary networks. Spheroids' viability is assessed as well as Albumin synthesis and urea production for liver-specific function. We examine the spheroids under microscopy for follow up, histology, scanning and transmission electron microscopy and immunofluorescence studies.

Results: Collected data indicate that within spheroids, hepatocytes maintain viability and function with polarity restoration by forming bile canaliculi with presence of tight junctions, while biliary epithelial cells (BEC) form tubular structures around hepatocyte rich center. The BEC show characteristic monolayer lining of the outer and inner surfaces of the scaffold. Those within the embedded spheroids show expansion and similar growth pattern as the unattached spheroid culture. These tubular structures grow towards the surface of the scaffold forming interconnecting ductules like channels, which allow fluid exchange between organoids construct lumen and the outer environment.

Conclusion: These tubular structures grow towards the surface of the scaffold forming interconnecting ductules like channels, which allow fluid exchange between organoids construct lumen and the outer environment.

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Bivalent Oral Cholera Vaccine Induces Memory B Cell Responses

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Purpose: Cholera has become endemic in Haiti since its introduction in 2010. A killed whole cell bivalent oral cholera vaccine (BivWC) has now been used in multiple countries; however, a complete understanding of the immune response to this vaccine is still lacking. To determine whether this vaccine generates detectable circulating memory B cell (MBC) responses, we followed a cohort of 73 Haitian adults who received two doses of BivWC (Shanchol) for one year following vaccination.

Materials & Methods: The study was conducted in the Saint Nicolas Hospital, in St Marc, Haiti, an urban center in the Artilbonite Department. We assessed immune responses at day 0 (baseline), day 7 (7 days after the first vaccination), day 21 (7 days after the second vaccination), and again on days 44, 90, 180, and 360 following vaccination. We measured vibriocidal antibody titers at each time-point and used a standard enzyme-linked immunosorbent assay (ELISA) to measure plasma IgA, IgG and IgM antibody responses to V. cholerae –OSP. We measured MBC responses to V. cholerae-specific antigens using the Enzyme-Linked Immunosorbent Spot Assay (ELISPOT) from frozen peripheral blood mononuclear cells (PBMCs).

Results: We observed significantly elevated immunological responses to V. cholerae-specific antigens after vaccination. Vibriocidal titer peaked on day 21 after initial vaccination, and decreased in the subsequent time-points (day 44, 90, 180 and 360). However, unexpectedly, in aggregate these titers remained significantly elevated over baseline titers even at 1 year after vaccination. We observed a lower titer of antibody response targeting Inaba-OSP compared to Ogawa-OSP antibody titers, for both IgG and IgA isotypes. We also observed a robust and persistent increase in Ogawa OSP IgG responses up to a year after vaccination. In addition, there was a significant increase in circulating IgA MBC responses targeting the O-specific polysaccharide (OSP; Ogawa and Inaba) of Vibrio cholerae O1, starting 21 days following initiation of vaccination. We also observed an increase in the level of circulating IgG memory B cells targeting V. cholerae O1 Ogawa OSP starting at day 21 and remaining significantly elevated for 12 months following initiation of vaccination; the Ogawa serotype has been the predominant circulating strain of V. cholerae in Haiti.

Conclusion: We found that the BivWC vaccine induces a robust memory B cell (MBC) response to both Ogawa and Inaba V. cholerae serotypes. This study is the first demonstration of memory cell responses to any oral cholera vaccine. Prior evaluations of a previous generation cholera vaccine did not demonstrate significant MBC responses. In addition, the duration of these antibody responses are longer than any previous evaluated oral cholera vaccine in endemic or epidemic settings. This difference in immunogenicity between vaccines is consistent with clinical and field effectiveness trials that demonstrate significantly longer protection with the BivWC vaccine. MBC responses are associated with protective immunity during natural infection; our results provide an immunological marker and potential mechanistic basis for the longer term protection observed following BivWC vaccination.

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Chronic Hypoxia Restores Hypoxic Pulmonary Vasoconstriction in a Mouse Model of Leigh Syndrome

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**Purpose:** Hypoxic pulmonary vasoconstriction (HPV) is a physiological vasomotor response that maintains systemic oxygenation by matching perfusion to ventilation during alveolar hypoxia. Although mitochondria appear to play an essential role in HPV, the impact of mitochondrial dysfunction on HPV remains incompletely defined. Defects in the mitochondrial complex I NADH:ubiquinone oxidoreductase iron-sulfur protein 4 (Ndufs4) causes Leigh syndrome, which is the most common pediatric mitochondrial disease. Mice lacking Ndufs4 (Ndufs4⁻⁻) develop a fatal progressive encephalopathy that serves as a model for Leigh syndrome. We previously reported that breathing normobaric 11% O₂ prevents neurological disease and improves survival in Ndufs4⁻⁻ mice. In this study, we examined the effects of mitochondrial complex I dysfunction on acute HPV. To better characterize the beneficial effects of chronic hypoxia in Ndufs4 deficient mice, we also studied acute HPV in Ndufs4⁺/⁺ and Ndufs4⁻⁻ mice after breathing 11% oxygen for 3 weeks.

**Materials & Methods:** We used normoxia and hypoxia treated Ndufs4⁻⁻ mice and their wild-type littermates (Ndufs4⁺/⁺) to assess the contribution of complex I function to HPV. We measured the increase in left pulmonary vascular resistance index (LPVRI) induced by left mainstem bronchus occlusion (LMBO) and arterial oxygen partial pressure (PaO₂) in anesthetized and mechanically ventilated mice.

**Results:** Congenital deficiency of Ndufs4 impaired the ability of LMBO to increase LPVRI (% increase in LPVRI in response to LMBO in Ndufs4⁺/⁺ vs. Ndufs4⁻⁻ mice: 137 ± 5.6 vs. 79 ± 2, p < 0.01). In mice exposed to room air, PaO₂ during LMBO was higher in Ndufs4⁺/⁺ mice than in Ndufs4⁻⁻ mice (384 ± 32 vs. 190 ± 24 mmHg, p < 0.001). Exposure of Ndufs4⁻⁻ mice to 3 weeks of hypoxia did not change the ability of LMBO to increase LPVRI. In contrast, in Ndufs4⁻⁻ mice, chronic hypoxia restored the normal increase in LPVRI in response to LMBO (Ndufs4⁺/⁺ vs. Ndufs4⁻⁻ mice: 116 ± 13 vs. 107 ± 10, p = 0.90). In hypoxia treated animals, oxygenation during LMBO was similar between Ndufs4⁺/⁺ and Ndufs4⁻⁻ mice (300 ± 41 vs. 322 ± 25 mmHg, p = 0.65).

**Conclusion:** Normoxia treated mice with a congenital defect in mitochondrial complex I have impaired HPV. Breathing normobaric 11% O₂ for 3 weeks restored HPV and improved oxygenation in Ndufs4⁻⁻ mice. These results demonstrate that intact complex I is required for acute HPV. In the setting of mitochondrial dysfunction chronic hypoxia restores the ability of pulmonary vasculature to respond to acute hypoxia.

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Amyloid is associated with greater tau burden in clinically-normal females relative to males: Findings from two independent cohorts
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Purpose: We recently reported that clinically-normal (CN) females with high β-amyloid (Aβ) exhibit greater rates of cognitive decline than males at older ages, despite similar proportions of APOE ε4 and levels of Aβ-PET burden. The mechanisms driving sex differences in AD-related cognitive decline remain to be elucidated, with previous literature implicating female vulnerability to CSF total tau. The objective of this study was to understand if the presence of PET biomarkers differed by sex, and if interactions between sex and APOE ε4 were apparent in the Harvard Aging Brain Study (HABS) and the Alzheimer’s disease Neuroimaging Initiative (ADNI).

Materials & Methods: 209 CN HABS older adults (55–92 years, CDR=0, Female=61%) underwent Aβ imaging with PiB-PET, and tau imaging with Flortaucipir-PET, with median duration between=51 days (IQR=13-133 days). 103 CN ADNI older adults (63–94 years, CDR=0, Female = 52%, median=7 days; IQR= 2-34 days) underwent Aβ and tau-PET imaging with AV45 and Flortaucipir, respectively. Aβ was treated continuously according to summary measures taken from each study. Flortaucipir measures were computed as standardized uptake value ratios. We examined the following tau regions: the entorhinal cortex (EC), which exhibits early signs of tauopathy, and inferior temporal lobe (IT), a region likely to manifest AD-related tauopathy. We conducted separate linear regression models for each cohort, focusing on main and interactive effects of sex, Aβ and APOE on EC and IT tau, controlling for age.

Results: We found no main effect of sex on Aβ and EC-tau, however, IT-tau burden was lower in ADNI males than females (p=0.002). Females exhibited significantly higher EC and IT-tau burden than males at higher levels of Aβ burden (Figure1). The strength of this relationship was slightly stronger for EC-tau in both studies. A sex-APOE interaction with EC or IT-tau was not evident in either study (Figure2).

Conclusion: Sex did not exert a clear main effect on Aβ, EC or IT-tau. Female CN did, however, exhibit higher EC and IT-tau in the context of high Aβ burden. We did not find an interaction between sex-APOE on either tau region. These findings, replicated across cohorts, implicate a particular female vulnerability to tauopathy in preclinical AD, which may explain greater risk for cognitive decline at older ages.

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

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Purpose: Ovarian cancer is a life-threatening tumor in women as its diagnosis often occurs at a late stage. Although immunotherapy as an adjuvant to surgery and chemotherapy has been broadly investigated in ovarian cancer as a means of reducing tumor recurrence and improving survival, there remains a significant unmet need for combinatorial strategies to enhance the antitumor immune response. The purpose of this study was to develop a novel combination immunotherapy for ovarian cancer, utilizing our novel fusion protein to target and generate a cellular immune response to mesothelin (MSLN) in conjunction with blockade of the PD-1/PD-L1 checkpoint pathway to restore the function of cytotoxic T cells in order to enhance cancer control and prolong survival.

Materials & Methods: Luciferase-expressing ID8 cells were employed to establish an intraperitoneal ovarian tumor model in immunocompetent C57BL/6 mice. The efficacies of the MSLN-targeted immune-activating fusion protein (VIC-008), αPD-L1, and the combination were evaluated. Mice received 4 intraperitoneal (i.p.) treatments of VIC-008 from day 7 post tumor inoculation weekly, and 6 treatments of αPD-L1 i.p. every other day from 4 weeks post inoculation. Tumor growth was monitored by in vivo imaging of luciferase activity with an IVIS Spectrum. Survival time was calculated as life span from the day of tumor inoculation. In immunological studies, mice were sacrificed 7 weeks after tumor cell inoculation. Immune cells from lymph nodes, ascites and tumors were stained with antibodies against multiple immune cell markers and profiled by flow cytometry.

Results: VIC-008, αPD-L1 or combination treatment delayed tumor growth. The combination treatment resulted in the greatest prolongation in survival, followed by αPD-L1 treatment and then VIC-008 treatment. Improved survival was associated with increased levels of intratumoral CD3+CD8+ T cells (P<0.0001). The combination treatment also reduced the proportion of CD4+CD25+Foxp3+ Treg cells (P<0.0001) in the lymph nodes. An increased number of CD8+CD27+CD44+ memory T cells (P=0.0134) were observed in ascites in the combination treatment group. CD11b+CD11c+ dendritic cells were enriched in ascites in VIC-008 treatment (P=0.0019) and combination treatment groups (P=0.0010). More CD11c+CD38+ (M1) (P=0.0361) and fewer CD206+CD106+ (M2) (P=0.0285) macrophages were found in the tumors of the combination treatment group.

Conclusion: Our results suggest that, through activating dendritic cells and enhancing antigen presentation and cross-presentation, VIC-008 augments antitumor CD8+ T cell responses and facilitates generation of memory T cells when combined with PD-1/PD-L1 blockade, providing long-term antitumor effects. Our findings demonstrate for the first time a mechanistic rationale for combining MSLN-targeted immune-activating fusion protein VIC-008 and an immune checkpoint inhibitor αPD-L1 in treatment of ovarian cancer in mice, positioning this combination therapy as a potential promising new immunotherapeutic approach for ovarian cancer.

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

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**Purpose:** There is a significant unmet need for new treatment strategies for malignant mesothelioma (MM). Despite relevant advances in many cancer treatment areas, including improvements in diagnosis, staging, and the clinical course of treated patients, MM remains a highly lethal disease. The purpose of this study is to develop a combination immunotherapy for MM, which involves a fusion protein to target and evoke a cellular immune response to mesothelin (MSLN) and the blockade of CXCL12/CXCR4 pathway to mobilize cytotoxic effector cells into tumors.

**Materials & Methods:** The efficacy of the MSLN targeted immune activating fusion protein (scFv-MtbHsp70), FDA-approved small molecule CXCR4 antagonist AMD3100 (plerixafor), and the combination were evaluated in two syngeneic and orthotopic murine models of MM in immune competent C57BL/6 mice. Mice received 4 intraperitoneal (i.p.) treatments from 7 days post i.p. injection of luciferase-expressing 40L and AE17 cells. Tumor growth was monitored by *in vivo* imaging of luciferase activity with an IVIS Spectrum. Survival time was calculated as life span from the day of tumor inoculation. In immunological studies, mice were sacrificed 4 weeks after tumor cell inoculation. Immune cells from spleens and tumors were labeled with antibodies against CD3, CD4, CD8, CD25 and Foxp3 antibodies, and examined by flow cytometry. Splenocytes were stimulated with MSLN and assessed for intracellular IFN-γ production by flow cytometry.

**Results:** In both murine mesothelioma models, the fusion protein scFv-MtbHsp70 alone delayed tumor growth and prolonged mouse survival, which was associated with increased tumor infiltration by CD3+CD8+ T cells. Treatment enhanced the cytotoxic function of tumor-specific CD3+CD8+ T cells by evoking dendritic cell activation as well as antigen presentation and cross presentation. AMD3100 alone reduced the proportion of CD4+CD25+Foxp3+ Treg cells in tumors and decreased PD-1 expression on CD3+CD8+ T cells. The combination of the fusion protein and AMD3100 further significantly slowed tumor growth and enhanced mouse survival while augmenting tumor-specific CD8+ T-cell immune responses and abrogating intratumoral immunosuppression.

**Conclusion:** Our findings demonstrated for the first time the synergistic effect of combination of MSLN-targeted immune-activating fusion protein scFv-MtbHsp70 and AMD3100 in treatment of MM in mice. This is a new therapeutic strategy which may significantly prolong survival of patients with this disease.

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**TERT rearrangements identify a subset of aggressive meningiomas**

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**Purpose:** Although a significant proportion of aggressive meningiomas acquire TERT promoter (TERTp) mutations which drive TERT overexpression during progression, alternative mechanisms of telomere maintenance in meningioma are broadly unknown. TERT activating rearrangements are common in some aggressive cancers and associated with poor outcome. Therefore, we sought to assess TERT rearrangements in a large cohort of patients with progressive/high-grade meningiomas.

**Materials & Methods:** We determined the frequency of TERT mRNA overexpression in 126 temporally- and regionally-distinct specimens from 55 WHO grades II/III meningioma patients using reverse-transcriptase PCR. Subsequently, RNA sequencing was performed in samples with TERT overexpression to detect rearrangements. Additionally, the TERTp region was sequenced in all patients to assess hotspot mutations.

**Results:** We identified 9 samples from 3 patients (5%) with highly amplified TERT mRNA expression. RNA sequencing of these samples revealed a novel fusion RETREG1-TERT that was present in 2 patients, in addition to a previously-reported LPCAT1-TERT fusion in a third case (4 samples). One of the three patients had received a course of radiation treatment prior to the emergence of detectable mRNA fusion. In all cases the TERT rearrangements began in either exon 2 or 3, upstream of the reverse transcriptase domain that begins in exon 4, consistent with a proposed activating mechanism-of-action. In total, 10 patients (18.1%) harbored TERT alterations in our cohort: 3 TERT rearrangements and 7 TERTp mutations. Importantly, patients whose meningiomas harbored TERT alterations had a significantly worse overall survival (5.1 years, 95%CI 3.1–7.2) compared to TERT wild-type patients (18.5 years, 95%CI 14.6–22.4, p<0.001).

**Conclusion:** We discovered TERT rearrangements in a subset of aggressive meningiomas, including a novel RETREG1-TERT rearrangement. Two distinct mechanisms for TERT activation, TERT rearrangements and TERTp mutations were associated with a particularly poor outcome, suggesting a central role of telomere lengthening in the pathogenesis of aggressive meningioma. Detection of TERT alterations offers a basis for a more precise identification of patients at-risk for developing early progression of meningioma.

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Mitochondrial permeability uncouples elevated autophagy and lifespan extension
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Purpose: Recent work in the aging field has shown that activation of autophagy is mechanistically required in almost every genetic, dietary, and pharmacologic manipulation that extends lifespan. However, elevated levels of autophagy can also have negative effects, accelerating ischemia/reperfusion injury, inducing cell death and promoting cancer progression. How autophagy can have these seemingly disparate effects remains a central mystery in the autophagy field.

Materials & Methods: Using different C. elegans mutant or transgenic strains or RNAi of different genes, we measured development and lifespan in these worm strains. mTORC2 mutant worms and cells from Sgk1 knockout mice were used for analyzing autophagy by western blotting or microscopy. Liver injury was analyzed by measuring AST/ALT levels in the serum, detection of cell death under ischemia/reperfusion conditions.

Results: Elevated levels of autophagy unexpectedly shorten lifespan in C. elegans lacking functional serum and glucocorticoid-induced kinase-1 (sgk-1) because of increased mitochondrial permeability. Remarkably, in sgk-1 mutants, inhibition of autophagy or mitochondrial permeability transition pore opening (mPTP) restores normal lifespan. Low levels of mitochondrial permeability are required in all situations examined in which elevated autophagy promotes longevity. Genetic methods to induce mPTP opening completely block autophagy-dependent lifespan extension resulting from caloric restriction or loss of germline stem cells. Finally, mitochondrial permeability also transforms autophagy into a destructive force in mammals, as mice lacking hepatic Sgk1 show a marked enhancement of hepatocyte autophagy, mPTP opening and death with ischemia-reperfusion injury.

Conclusion: Autophagy fails to extend lifespan and promote health when mitochondrial permeability is compromised, and that mTORC2 and SGK1 are critical to limit this permeability in vivo. Leveraging mitochondrial permeability and SGK1 function may represent a viable therapeutic strategy to maximize the benefits of autophagy on health and lifespan.

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The stable glucagon analog dasiglucagon is well-tolerated and as effective as recombinant human glucagon when delivered by the bionic pancreas in response to insulin excess

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Purpose:
We have developed an automated artificial pancreas (AP) system, the bionic pancreas (BP), which was designed with the goal of improving glycemic control and reducing the burden of care in people with type 1 diabetes. The instability of currently approved recombinant glucagons after reconstitution has been a barrier to the commercialization of the bihormonal artificial pancreas systems. Dasiglucagon is a glucagon analog that is stable in an aqueous formulation, making it suitable for pump use. We compared dasiglucagon to freshly reconstituted recombinant human glucagon (Eli Lilly) in the bihormonal bionic pancreas (BHBP) in 12 adults with T1DM in a randomized, two-period crossover trial.

Materials & Methods:
Ten subjects completed both arms of the study and contribute to efficacy analyses. Each 8-hour study period stress-tested the anti-hypoglycemic action of the BHBP under conditions that increased the need for glucagon by starting with participants in the fasted state and giving extra insulin (up to 2X normal basal rate, full bolus for lunch) via a separate pump without informing the BHBP. Structured exercise started 3 hours after lunch. Plasma glucose levels were measured via the YSI and samples for glucagon concentrations were obtained at pre-determined intervals. Participants were evaluated hourly for nausea and pain at both the insulin and glucagon infusion set sites

Results:
The primary endpoint was safety and tolerability and key secondary endpoints addressed glycemic regulation. Adverse events were mild or moderate in intensity and were similar between groups; the most frequent besides hypoglycemia was nausea. No subjects developed antibodies against either drug. No infusion set occlusions occurred. Under these highly stressed conditions designed to provoke hypoglycemia, there were no significant differences in percent of time below 60 mg/dL (13±17% vs 20±15%; p=0.25) and percentage of time in the 70-180 mg/dL range (71±24% vs 62±16%; p=0.34) between dasiglucagon and recombinant glucagon. The delivered dose over 8 hours and number of carbohydrate interventions were also comparable.

Conclusion:
In conclusion, dasiglucagon delivered by the bihormonal bionic pancreas, was well tolerated, and provided anti-hypoglycemic action similar to recombinant human glucagon under challenging conditions. This, along with its stable formulation in aqueous solution, makes it a suitable option for use in the BHBP.

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Treatment adherence, competence, and modifications to Cognitive Processing Therapy in a diverse community health clinic: Associations with clinical change
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**Purpose:** Clinicians in community settings have reported a perceived need to modify evidence-based protocol (EBP) treatments to address contextual challenges. Understanding which components can be modified and which should be maintained is paramount to treatment implementation. This study examines associations between therapist adherence, competence, and modifications to Cognitive Processing Therapy (CPT), an EBP for Posttraumatic Stress Disorder (PTSD), and patient outcomes, delivered in routine clinical care at a diverse community setting.

**Materials & Methods:** Data were derived from an NIMH-funded implementation-effectiveness hybrid study of CPT for PTSD in a diverse community health center. Providers (n=19) treated patients (n=72) as part of routine clinical care. Patients completed the Posttraumatic Stress Disorder symptoms (PCL-S) and Patient Health Questionnaire-9 (PHQ-9) at baseline, after each CPT session, and post-treatment. CPT sessions (n=463) were rated for treatment adherence, competence, and for therapist treatment-consistent or inconsistent modifications per session. Latent growth curve modeling was used to assess the effects of three treatment factors on changes in PCL-S and PHQ-9 scores over the course of treatment: (1) average adherence rating per session, (2) average competence rating per session, and (3) average number of treatment-consistent modifications made by the therapist per session.

**Results:** Providers were on average 45.7 years old (SD = 13.8), primarily female (78.9%), non-Hispanic (84.2%), white (78.9%), social workers (73.7%) who had worked an average of 15.3 years (SD = 12.4) as a mental health provider at the study site. Patients were on average 39.1 years old (SD = 13.6), primarily female (69.4%), Latina (48.6%), and with a high school education (34.7%). Patients attended an average of 8.3 CPT sessions (SD = 4.04), with patients experiencing significant reductions in both PCL-S ($\beta = -1.03, p < .001$) and PHQ-9 ($\beta = -0.51, p = .008$) scores during treatment. Overall, therapist adherence and competence were high, though ratings decreased over time suggesting potential drift. Therapists made on average 1.5 treatment-consistent, and 0.4 treatment-inconsistent modifications per session.

Results show that high adherence ratings were associated with greater reductions in depressive symptoms [$B = -2.20$ points for every 10% increase in adherence (-3.88, -0.50), $\beta = -0.25$], whereas higher competence ratings were associated with greater reduction in PTSD symptoms [$B = -0.44$ points for every 10% increase in competence (-0.83, -0.05), $\beta = -0.20$]. Higher numbers of treatment-consistent modifications were associated with higher reductions in PTSD [$B = -0.90$ points per modification per session (-1.51, -0.29), $\beta = -0.31$] and depressive [$B = -0.37$ points per modification per session (-0.64, -0.10), $\beta = -0.31$] symptoms.

**Conclusion:** The treatment effects in patients’ PTSD and depressive symptomatology improvement were comparable to those of controlled clinical trials of CPT and other evidence-based trauma treatments. The results highlight the importance of differentially assessing therapist adherence, competence, and modifications to EBP in community settings, and how these relate to different clinical outcomes. The findings also suggest that effective EBP delivery in routine care may require minor adaptations to meet patient needs or to ensure that patients are able to understand and benefit from interventions, consistent with previous studies. Greater attention to adherence, competence, and modifications can enhance training and consultation to prepare providers to tailor interventions while retaining core components of the intervention.

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Prenatal Exposure to Acid Suppressant Medications and Risk of Recurrent Wheeze at 3 years of Age in Children with a History of Severe Bronchiolitis

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Purpose: The use of acid suppressant medications, such as proton pump inhibitors (PPI) or histamine-2 receptor antagonist (H2RA), is common throughout the lifespan, including during pregnancy. Prenatal exposure to these medications has been associated with offspring development of asthma. Infants with severe bronchiolitis are at 3-to 4-fold higher risk for developing recurrent wheeze and subsequent asthma. In this high-risk population, it is not known if prenatal exposure to acid suppressant medications further increases risk of these respiratory outcomes. We tested the hypothesis that prenatal exposure to acid suppressant medications increases risk of recurrent wheeze among children with a history of severe bronchiolitis.

Materials & Methods: We enrolled 1016 infants hospitalized for severe bronchiolitis in a multi-center, US-based cohort from 2011-2014. Children are being followed for the development of recurrent wheeze (age 3 years) and subsequent asthma (age 6 years). Recurrent wheeze was defined per US asthma guidelines as parent report of at least 2 corticosteroid-requiring exacerbation in 6 months, or having at least 4 wheezing episodes in one year that last at least 1 day and affect sleep. (The cohort participants are too young to study risk of asthma.) After excluding children without complete exposure and outcome data, 900 children (89%) remained in the analytic cohort. Time to event analysis was performed using Cox-proportional hazards with stratification by age at enrollment and adjustment for maternal history of atopic disease (asthma, rhinitis, food allergy, eczema), maternal smoking during pregnancy, low birthweight, gestational age at birth, mode of delivery, multiple gestation, race/ethnicity, income and insurance status as potential confounders.

Results: Of the 900 infants studied 144 (16%) were exposed to PPI or H2RA in utero. 119 (83%) of these mothers reported use of these medications for at least 2 months during pregnancy. Recurrent wheeze developed in 56 (39%) of the exposed children and 233 (31%) of the unexposed children by age 3 years (unadjusted hazard ratio 1.37; 95%CI, 1.02-1.83). Even after adjustment for potential confounders, prenatal exposure to acid suppressant medications increased the risk of developing recurrent wheeze (adjusted hazard ratio 1.40; 95%CI, 1.02 -1.92).

Conclusion: In this high-risk cohort of children with a history of severe bronchiolitis, prenatal exposure to acid suppressant medications further increased the risk of developing recurrent wheeze by 3 years of age.

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Therapeutic insight into Mucolipidosis IV via \textit{in vitro} glia models

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**Purpose:** Mucolipidosis IV (MLIV) is a devastating neurologic childhood disease with dramatic unmet medical need. MLIV is an early-onset lysosomal storage disorder that causes motor and cognitive deficiencies, as well as visual impairment and eventually blindness. At present, there is neither a specific treatment for MLIV, nor a complete mechanistic model explaining its pathology. Using the \textit{Mcoln1}\textsuperscript{-/-} mouse model, we have determined that neuroinflammation is an early and profound manifestation of MLIV. Therefore, we are developing \textit{in vitro} models for \textit{Mcoln1}\textsuperscript{-/-} primary glia. We are currently using these \textit{in vitro} models as a pre-screening tool for potential therapeutic drugs for pre-clinical trials. Our recent data shows promise of a successful pre-clinical trial of fingolimod in our \textit{Mcoln1}\textsuperscript{-/-} mice. Using these models, we intend to screen additional drugs for potential pre-clinical trial candidates.

**Materials & Methods:** Primary astrocytic cultures are obtained from P1 \textit{Mcoln1}\textsuperscript{-/-} and \textit{Mcoln1}\textsuperscript{-/+} pup cortices. Microglial cultures are obtained by magnetically labeling and isolating CD11B\textsuperscript{+} cells from young adult \textit{Mcoln1}\textsuperscript{-/-} and control mouse brains using the Miltenyi MACS system. Cells are cultured in media containing factors which are known to promote homeostatic gene-expression profile of either astrocytes or microglia. Once cells are confluent, they are treated with fingolimod through the media. Media and cell lysates were collected for multiplex analysis of cytokine/chemokine expression and phosphoprotein signaling. Some cells were fixed, stained, and imaged on a Leica confocal microscope for morphological and molecular analysis.

**Results:** Both astrocytic and microglial models have been separately developed and show strong phenotypical physiology of MLIV, including high proinflammatory signaling as well as lysosomal storage dysfunction. These models are not only phenotypically representative of the disease, but are also providing mechanistic insight into the glial function in MLIV. We have shown that fingolimod, an FDA approved drug for multiple sclerosis, can ameliorate MLIV pathology. Our preliminary data shows that fingolimod reduces pro-inflammatory signaling and cytokine expression in cultured primary astrocytes from MLIV mice. Furthermore, fingolimod was also able to correct lysosomal impairment, which is a primary consequence of \textit{Mcoln1} loss.

**Conclusion:** Two phenotypically representative \textit{in vitro} models for MLIV brain glia have been developed and are currently being used to gain insight into the mechanistic pathology of the disease. Through these models, we have demonstrated that fingolimod ameliorates lysosomal dysfunction and reduced pro-inflammatory signaling. Our data shows promise of a successful pre-clinical trial of fingolimod in our \textit{Mcoln1}\textsuperscript{-/-} mice. Using these established models, we intend to screen additional drugs for potential pre-clinical trial candidates.

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Characterization of the Chromatin Landscape of Human Parathyroids and Identification of Direct Targets of Glial Cells Missing Homolog 2 (GCM2)

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Purpose: On a DNA level, the binding of transcription regulators to specific sites provides the fundamental mechanism for achieving tissue-specific expression. Consequently, these regulatory regions have emerged as key players in disease pathology. However, transcription regulators specific to the parathyroid glands, which produce the key hormone for regulation of calcium and phosphate, have not yet been systematically characterized. The transcription factor Glial Cells Missing Homolog 2 (GCM2) has emerged as the master regulator of parathyroid development, but its role in the mature organ is unknown. Therefore, the investigation into the chromatin landscape of the parathyroids and the target genes of GCM2 is fundamental for understanding parathyroid function.

Materials & Methods: We used tissue from human parathyroid adenoma to map global chromatin accessibility by DNase-seq profiling, genome-wide active and repressive histone modifications, and comprehensive analysis of GCM2 binding sites by Chromatin immunoprecipitation followed by sequencing (ChIP-Seq).

Results: We identified the locations, targets and possible binding partners of GCM2. We found that GCM2 primarily bound to active promoters or enhancers in parathyroids, and often co-targeted genes along with GATA3, MAFB, and VDR. We systematically identified parathyroid-specific enhancers and the transcription factors that bound to them. We used luciferase assays to validate the function of some of these enhancers; transfection of reporter vectors into HEK293 cells yielded an increase in expression compared to empty vector controls for all enhancers tested. For example, a short region about 5 kilobases upstream of the PTH gene, and a 160 basepair element within the 8th intron of the Vitamin D receptor (VDR), were able to drive luciferase expression 2-fold and 40-fold higher than the empty vector control, respectively.

Interestingly, parathyroid-specific enhancer elements were identified in the intronic region of the calcium-sensing receptor (CASR), which showed a 10-fold increase in expression in luciferase assays. These enhancer regions are a direct target of GCM2 and overlap with an SNP variant recently identified in a genome-wide association study (GWAS) of blood parathyroid hormone (PTH) levels. Upon incorporating the SNP into the enhancer sequence, we found that luciferase activity doubled. The mechanism by which the variant operates remains elusive.

Conclusion: These datasets and analyses provide a rich resource for understanding the mechanism of parathyroid gland regulation through distinctive transcription networks and functional DNA elements. Furthermore, knowledge of the chromatin landscape of the parathyroid glands has significant implications for understanding the genomic mechanisms that predispose an individual to diseases of the parathyroid.

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Tau Induces Increased Formation and Dysfunction in Small Diameter Capillaries in Aged Tau P301L Mice

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Purpose: Alzheimer’s disease pathology is characterized by amyloid beta plaques, tau-containing neurofibrillary tangles and neuronal cell death. Mounting evidence indicates that these changes are exacerbated by vascular abnormalities with cerebral amyloid angiopathy, hypertension, cholesterolemia, and diabetes being among the best known contributors to weakened and dysfunctional cerebral vasculature (BUÉE et al., 1997; Kalaria, 1999; Zipser et al., 2007). The contribution of tau neurofibrillary tangle pathology to vascular change is less clear. Previously, we have observed an increase in the density and tortuosity of blood vessels in aged Tg4510 mice, which overexpress tau. We hypothesized that this increased density was due to aberrant angiogenesis and was contributing to a state of chronic hypoxia.

Materials & Methods: We performed qPCR arrays on 84 hypoxia and angiogenesis-related genes from Tg4510 (n=3) and wild-type (n=3) mice at 15 months of age. In separate cohorts, we then dissociated brains from aged mice and used magnetic bead separation to isolate endothelial cells (CD31-positive), microglia (Cd11b-positive), astrocytes (asca-2 positive) and neurons. Purity of cell-type specific preparations was performed by further qPCR analysis. Changes in gene expression identified by arrays were verified by western blot.

Results: Of the 84 genes that were assessed, seven were significantly upregulated and one was significantly downregulated (p<0.05) in total brain preparations. Among upregulated genes were VegfA, Serpine1, Plau. Endothelial cells isolated from Tg4510 animals exhibited the greatest overall changes in gene expression. Similar changes were confirmed in human gene expression data sets comparing patients with high tau pathology versus low tangle pathology.

Conclusion: Alterations in the expression of genes classically associated with hypoxia and angiogenesis support our hypothesis that increased blood vessel density is due, at least in part, to the formation of new vessels. Future studies are aimed at understanding how these blood vessel changes may accelerate cognitive decline in tauopathies and suggest a novel avenue for therapeutic development.

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Investigation of ECG-Gated Non-invasive Cardiac Arrhythmia Ablation Using Double Scattering Proton Beam
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Purpose: Conformal delivery of radiation to a cardiac target is challenging due to respiratory and cardiac motion and the proximity to radiation sensitive organs-at-risk (OARs) including right coronary and left anterior descending arteries. Although recent studies showed non-invasive cardiac arrhythmia ablation for atrial fibrillation and ventricular tachycardia with radiotherapy modalities, mitigation techniques for cardiac motion have not been studied thoroughly. The purpose of this study is to evaluate the dosimetric advantage of using cardiac gating on ECG signals as a motion mitigation method for proton radiotherapy of targets in the heart.

Materials & Methods:
Cardiac gated 4D CTs were obtained at end-expiration, resulting in 10 cardiac phase CTs. OARs and seven different targets were contoured on each phase for 4D planning, and 3D target volumes were generated on the average intensity-projection for a non-cardiac gated comparison. For each target, a reference cardiac phase was selected to determine optimal beam angles for double-scatter proton radiotherapy in terms of target coverage and OAR sparing. Dosimetric parameters for cardiac gated and non-gated plans were compared. Target size and location were also evaluated to investigate the feasibility of a respiratory and cardiac double gating delivery.

Results:
Due to the anatomical location of the heart, the angle of incoming beams played an important role in dose homogeneity and OARs dose. Figure 1 presents a good example of the tradeoff among target coverage, mean heart dose, mean left ventricular dose and left lung dose with various beam angles. Since proton beams deposit their energy on the target without exit doses, the incoming beam angles play an important role in sparing OARs, compared to x-ray beams. The beam angle or the combination should be chosen where all the requirements are well-balanced. With the optimal beam angle, targets in left ventricle and interventricular septum can benefit from cardiac gating by reducing the mean heart dose and mean left ventricular dose by 35% shown in Figure 2.

Conclusion:
This work evaluated cardiac gating on ECG signals as a motion mitigation technique for proton radiotherapy in cardiac arrhythmia ablation. It demonstrated that the choice of beam angle is important to spare OARs, and that cardiac gating may be beneficial for some targets out of the seven studied target locations in the heart to reduce cardiac toxicity.

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Measurement of retinal capillary oxygenation in mice

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Purpose: (1) Characterization of in vivo oxygenation at the micro-scales under normal vs. challenged conditions; (2) Studying retina an extension of the brain. Visual processing starts in retina, which is made out of thin layers of cells with high-metabolic demand and complex microvasculature. Moreover, the advantage of noninvasive optical access to retina makes it an expedient model to study metabolism, neurovascular coupling, and mechanisms driving pathologies in brain as well as eye.

Materials & Methods: We measured the absolute oxygen partial pressure (pO₂) in retinal microvasculature of anesthetized mice under normoxia and hyperoxia. Intravascular pO₂ in individual capillaries, arterioles, and venules at various depths was measured noninvasively through the dilated pupil by using two-photon phosphorescence lifetime microscopy. To our knowledge, this is the first demonstration of absolute pO₂ imaging in retina capillaries in vivo. The oxygen-sensitive phosphorescent nanoprobe (Oxyphor 2P) was excited at 950 nm, and emission at 757 nm was collected with a long-working-distance objective lens. We used a clear ultrasound gel as the immersion medium. An eye lubricant gel was applied and a flat glass microscope cover slip was gently placed on mouse cornea for refractive index matching, without using additional optical correction mechanisms. We also recorded microvascular angiograms by using FITC-labeled blood plasma. The transverse and axial distances within the measured tissue volume were calibrated with the help of a model of the eye optical structure.

Results: We demonstrated absolute pO₂ measurements of individual capillaries, arterioles, and venules at various depths, noninvasively in retina of anesthetized C57BL/6 mice under normoxic and hyperoxic conditions. We confirmed our results with systemic blood-gas measurements. We used a single off-the-shelf objective without utilizing any additional optical correction mechanism other than refractive index matching with immersion

Conclusion: We, for first time, demonstrated noninvasive in vivo measurements of absolute pO₂ in retina capillaries. Our two-photon phosphorescence lifetime microscope can easily be combined with other imaging methods that are used for blood flow measurements, such as Optical Coherence Tomography. This would allow us to quantify metabolic rate of oxygen. Our noninvasive method for measuring the absolute pO₂ in retinal microvasculature in the rodent eye will lead to improved understanding of oxygen delivery at the microvascular scales, and mechanisms that are driving development of various eye disorders.

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Methicillin-resistant *Staphylococcus aureus* causes sustained collecting lymphatic vessel dysfunction

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**Purpose:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of morbidity and mortality worldwide and is a frequent cause of skin and soft tissue infections (SSTIs). Lymphedema—fluid accumulation in tissue caused by impaired lymphatic vessel function—is a strong risk factor for SSTIs. SSTIs also frequently recur in patients and sometimes lead to acquired lymphedema. However, the mechanism of how SSTIs can be both the consequence and the cause of lymphatic vessel dysfunction is not known.

**Materials & Methods:** Here, we used the endemic USA300 strain of community-associated MRSA (CA-MRSA), a common cause of serious bacterial infections in the United States, to establish a model of localized MRSA infection. We used intravital imaging to determine whether MRSA infections inhibit lymphatic vessel contractility and lymph flow. In vivo studies used C57BL/6, iNOS⁻/⁻ C57BL/6, MyD88⁻/⁻ C57BL/6, LysM<sup>gfp/gfp</sup> C57BL/6, and alpha SMAP-DsRed/C57BL/6 mice to assess the effects of host-derived molecules to lymphatic contractility after infection. Alpha SMA-DsRed transgenic mice were visualized and immunofluorescence staining of alpha SMA⁺ lymphatic muscle cells (LMCs) of WT mice was performed to analyze LMC coverage of lymphatic vessels on multiple days after infection. To define the molecular mechanisms related to this response, we performed cell viability and cell lysis assays and measured killing of LMCs by MRSA-conditioned supernatant from wild type and mutant MRSA strain(s). Similar experiments were performed using recombinant MRSA toxins.

**Results:** Intravital imaging in mice revealed an acute reduction in both lymphatic vessel contractility and lymph flow after localized MRSA infection. Moreover, chronic lymphatic impairment is observed long after MRSA is cleared and inflammation is resolved. Associated with decreased collecting lymphatic vessel function was the loss and disorganization of lymphatic muscle cells (LMCs), which are critical for lymphatic contraction. In vitro, incubation with MRSA-conditioned supernatant led to LMC death. Proteomic analysis identified several accessory gene regulator (agr)–controlled MRSA exotoxins that contribute to LMC death. Infection with agr mutant MRSA resulted in sustained lymphatic function compared to animals infected with wild-type MRSA.

**Conclusion:** Our findings suggest that agr is a promising target to preserve lymphatic vessel function and promote immunity during SSTIs.

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Novel near-infrared fluorescence agent for the in vivo detection of activated platelets

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Purpose: Advancing our understanding of human coronary artery disease requires new imaging reagents that can be used in patients for studying thrombosis microstructure in relation to the molecular mechanisms that underlie its initiation, progression, and clinical complications, including deep vein thrombosis (DVT) and sudden cardiac death. The most advanced intracoronary imaging approaches such as intravascular ultrasound and optical coherence tomographic imaging do not specifically detect activated platelets, a key cell that drives thrombosis.

The goal of this study was to develop and validate a new platelet-targeted imaging agent that enables high-resolution near-infrared fluorescence (NIRF) imaging of activated platelets (Fig. 1). The novel agent could be used in conjunction with a catheter-based NIR fluorescence imaging system providing a powerful tool to detect thrombus prone stents.

Materials & Methods: A NIRF conjugate of tirofiban (Tf), a clinical IIb/IIIa antagonist, was synthesized via the chemical modification of the sulfonamide fragment of the molecule with a benzoic acid moiety and subsequent addition of the PEG-modified fluorophore, CyAl5.5 (ex/em 675/695nm). The efficacy of the conjugate was validated in vivo in the FeCl3-induced model of femoral thrombosis, and compared to the free dye or blocking with the parent drug Tf. After injection, serial intravital fluorescence microscopy (IVFM) was carried out for one hour, followed by histological examination of binding.

Results: A NIR fluorescent conjugate of Tf was successfully synthesized via an optimized multi-step synthesis, with the identity and purity of the agent fully characterized after the final HPLC-based purification. IVFM characterization of conjugate efficacy showed an increase in NIRF signal in the thrombus over the initial 30 minutes, followed by a slow decrease until the endpoint of the experiment (1 h). The NIRF signal was 92% ablated by preinjection of excess Tf. Free dye did not display any localization to the thrombus. Histological analysis showed significant accumulation within the thrombus that co-localized with GPIIb/IIIa expression.

Conclusion: A novel NIR fluorescent Tf conjugate has been developed that specifically binds to GPIIb/IIIa, and can enable the detection of activated platelets in experimental thrombi in vivo. This novel agent provides the tools for assessment of GPIIb/IIIa activation in a broad range of biological processes and may also aid in the detection of thrombosis-prone stents. While OFDI can identify large protruding thrombi on a structural basis, the greater biological specificity and sensitivity afforded by NIRF molecular imaging of platelets using this agent should enable specific discrimination of key thrombus-associated molecules that overlie stent struts following implantation, a capability that is currently not possible with standalone OCT or OFDI. It is likely that clinical applications of this imaging reagent can be realized in the near future; with the cardiac catheterization NIR fluorescence imaging systems.

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

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Purpose: Cortical capillary blood flow and oxygenation are highly heterogeneous. Mapping the absolute capillary blood flow and oxygenation along the capillary path is a key step towards understanding how oxygen is transported and delivered in a complex microvascular network to enable adequate tissue oxygenation. In this work, we applied two-photon microscopic imaging of intravascular oxygen partial pressure (PO2) to measure both oxygen concentration and red blood cell (RBC) flux in cortical arterioles, capillaries, and venules.

Materials & Methods: The PO2 measurements with high signal-to-noise ratio were enabled by a novel oxygen-sensitive phosphorescence probe, PtG-2P. Imaging was performed in awake, head-restrained C57BL/6 mice (n=15), through a chronic sealed cranial window centered over the E1 whisker barrel.

Results: We obtained a detailed mapping of the resting state cortical microvascular PO2 in all arterioles and venules, and both PO2 and RBC flux in most capillaries down to 600 μm depth from the cortical surface (n=6,544 capillaries across all mice). Capillary RBC speed and density were also extracted and all measurements were co-registered with the microvascular angiograms. We characterized the distributions of capillary PO2 and flow as a function of branching order and cortical depth.

Conclusion: The results show strong positive correlation between oxygenation and flow in the capillary segments, with an increased correlation in downstream capillaries. We have also observed homogenization of both oxygenation and flow in deeper cortical layers, which may imply a mechanism to improve oxygen delivery without increasing global blood flow in the area with increased metabolism.

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Sequential ALK Inhibitors Can Select for Lorlatinib-Resistant Compound ALK Mutations in ALK-Positive Lung Cancer

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**Purpose:** The cornerstone of treatment for advanced ALK-positive lung cancer is sequential therapy with increasingly potent and selective ALK inhibitors. The third-generation ALK inhibitor lorlatinib has demonstrated clinical activity in patients who failed previous ALK inhibitors. However, acquired resistance to lorlatinib is expected to occur. The purpose of this study is to clarify clinically relevant mechanisms of resistance to lorlatinib.

**Materials & Methods:** To define the spectrum of ALK mutations that confer lorlatinib resistance, we performed N-ethyl-N-nitrosourea (ENU) mutagenesis screening of Ba/F3 cells expressing EML4–ALK. Then, we analyzed paired pre-treatment and progression biopsies for 18 patients relapsing on lorlatinib to confirm the clinical relevance of our findings. Furthermore, to determine the evolutionary origin of ALK resistance mutations in patients treated with sequential ALK inhibitors, we performed whole exome sequencing (WES) on serial biopsy samples from three patients.

**Results:** ENU mutagenesis generated numerous crizotinib-resistant clones and those harbored a variety of single ALK kinase domain point mutations, including the majority of crizotinib resistance mutations identified in clinical specimens. However, no lorlatinib-resistant clones harboring single ALK mutations emerged under comparable conditions. In similar screens with EML4-ALK containing single ALK resistance mutations, numerous lorlatinib-resistant clones emerged harboring compound ALK mutations. Together these results suggest that ALK mutations conferring lorlatinib-resistance readily emerge from single-mutated ALK, whereas unlikely emerge from non-mutant ALK. Two of the identified compound mutations in the screen, C1156Y/L1198F and G1202R/L1196M, were found in the following analysis of clinical samples. Independently generated Ba/F3 cell lines expressing those compound mutations showed resistance to lorlatinib. Especially, G1202R/L1196M was resistant to all available ALK inhibitors. In the analysis of 18 clinical samples, five (28%) harbored compound ALK resistance mutations, including three with a double ALK mutation and two with a triple ALK mutation. Four of the five cases had the paired pre-lorlatinib specimen, and all of them harbored a single or double ALK resistance mutation present in the compound mutant post-lorlatinib specimen. The WES on serial biopsy demonstrated that a founder single ALK mutant clone in the pre-lorlatinib tumor gave rise to a lorlatinib-resistant compound ALK mutant subclone. These data elucidate that these compound ALK mutations develop in a stepwise fashion in patients treated with sequential ALK inhibitors.

**Conclusion:** We have shown through in vitro cell based mutagenesis screening and molecular analysis of patient samples that treatment with sequential first-, second-, and third-generation ALK inhibitors fosters the development of diverse compound ALK mutations, some of which are highly refractory to all available ALK inhibitors. It is tempting to speculate that upfront treatment with the third-generation inhibitor lorlatinib may be able to prevent the emergence of single and subsequently compound ALK mutations, potentially improving clinical outcomes.

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Objectives: To utilize electronic health records (EHR) to conduct studies in patients with rare, complex diseases such as systemic lupus erythematosus (SLE), electronic phenotypic algorithms are needed to accurately identify these patients. Our objectives were to assess the portability of published rule-based algorithms to our EHR and to use machine learning to develop algorithms for SLE using both structured (codified) data and natural language processing (NLP) of narrative data.

Materials & Methods: 400 charts with >1 ICD-9 code for SLE (710.0) were randomly selected from the Partners Biobank. Two rheumatologists identified gold standard cases of definite and probable SLE (based on American College of Rheumatology Classification Criteria/rheumatologist diagnosis) and applied published rule-based algorithms to a training set of 200 charts (e.g., 1st algorithm: >3 ICD-9 codes for SLE, no ICD-9 codes for dermatomyositis or systemic sclerosis, ANA ≥1:40, ever use of disease modifying anti-rheumatic drugs, and ever use of steroids).

Results: 1,322 patients (2.29% of the Partners Biobank) were identified with >1 ICD-9 SLE code. Within the combined training and validation cohorts (n=200 each), 28.5% had definite SLE, and 41% had definite or probable SLE. The positive predictive values (PPVs) of published rule-based algorithms were low in our EHR (e.g., <50% for identifying definite SLE and ≤65% for definite/probable SLE among the top algorithms). The codified-only algorithms developed via penalized logistic regression had high PPVs (90% for definite SLE at 97% specificity, 87% negative predictive value (NPV) and 92% for definite/probable SLE at 97% specificity, 73% NPV). AUCs were 0.947 and 0.922 for the definite SLE and definite/probable SLE coded-only algorithms, respectively (Final models, Tables 1, 2). Models with NLP data did not improve performance.

Conclusion: Previously published rule-based SLE phenotype algorithms performed poorly in our EHR. Potential explanations include differences in SLE case definitions, variation in the use of billing codes, differences in the way medications were used and recorded, and variability in ANA assays across institutions. Our algorithms developed using penalized logistic regression models had excellent predictive characteristics in our internal validation cohort. Next steps include external validation of these algorithms. Careful consideration of EHR characteristics, case definitions, and goals of the research using the EHR cohorts (e.g., whether a more sensitive or specific algorithm is preferred) must be taken into account when applying algorithms to identify SLE patients in the EHR.


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What Machines Can Read: Sex Identification from Hand and Wrist Radiographs
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Purpose: Machine learning extracts meaningful patterns from medical images without use of explicit hand-crafted features. This approach opens up new possibilities to find patterns that have not been noticed by humans. Skeletal sexual dimorphism is widely used in various fields including forensics and anthropology as well as endocrinology, sexual health, and behavioral science. In hand and wrist, tendencies toward higher second-to-fourth digit ratio (2D:4D) and smaller carpal bones in females compared to males are reported in previous studies. However, no discrete feature has been found in hand and wrist that clearly distinguishes male from female. To explore the ability of deep learning algorithms to detect subtle differences in medical images that has not been recognized by human radiologists, we trained and tested a deep convolutional neural network (DCNN) with hand and wrist radiographs to predict sex.

Materials & Methods: We compiled a dataset of 10,607 radiographs of hand and wrist from a cohort of pediatric and adult patients, ranging from 5 to 80 years of age. A total of 7,461 (3,841 females and 3,620 males) radiographs were used for training, and 1,573 images (809 females and 764 males) separate from the training data were randomly selected for validation. Images from the remaining 1,573 cases (809 females and 764 males) were reserved for testing. The images were labeled solely with the sex of the subject, and any other information such as age was not provided. Following a series of preprocessing steps to segment a region of hand and wrist, images were analyzed using a deep CNN. We fine-tuned an ImageNet VGG-16 on our training dataset by a stochastic gradient descent (SGD) with a minibatch size of 64, a weight decay of 0.005, and a base learning rate of 0.001. The learning rate was decayed by a value of 0.1 by three steps to attain a stable convergence of train loss function. The best CNN, selected based on the validation loss, then provided automated prediction of sex, which was compared to the sex in the patient’s medical record.

Results: Of the 1,259 radiographs tested, the algorithm predicted sex with 95.4% accuracy (95.2% in females and 95.7% in males). The focus is mostly on the carpal bones in female, whereas the focus is dispersed on second or third metacarpal bones or second metacarpophalangeal joint in male (fig. 1).

Conclusion: A deep learning algorithm identifies sex with high accuracy from hand and wrist radiographs in children and adults.

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Fig. 1. Attention maps for prediction of sex from hand and wrist radiographs. The attention maps observed did not appear to be associated with age.
Defining Correlates of Humoral Immune Protection Against EBV

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Purpose: Epstein-Barr virus (EBV)-associated cancers including Burkitt, Hodgkin and non-Hodgkin lymphoma pose a deadly health threat, especially among HIV-1 infected populations. However, a protective vaccine to prevent EBV infection and or limit disease has yet to be developed. While previous vaccine efforts have focused on the induction of either neutralizing antibodies or CD8+ T-cell responses to prevent or kill infected cells, mounting evidence points to a potentially critical role for cytotoxic antibodies, able to deploy the antiviral activity of the innate immune system, in the control and clearance of EBV. Thus, here we aimed to define whether non-neutralizing antibody functions, able to recruit a broad array of innate immune effector functions, might selectively evolve following EBV infection.

Materials & Methods: Systems serology profiling was performed in a cohort of 12 EBV-infected patients that were enrolled during the acute stage of infection and sampled for a year. Both functional and biophysical assays were performed to define cross-sectional and longitudinal differences in the humoral immune response against acute- and latency- associated EBV antigens.

Results: As anticipated, IgG1 and IgG3 antibody subclasses, known to drive enhanced antiviral function, predominated the IgG immune response to the EBV viral envelope protein (gp350/220), capsid antigen (p18), early antigen (p47/54) and latent protein (EBNA 1). However, variability was observed among infected subjects suggesting the existence of different responder groups or patterns. While all EBV protein-specific antibodies were unable to recruit monocyte-dependent phagocytosis, p18-specific antibodies induced low levels of phagocytosis by neutrophils (ADNP) early in infection (Figure 1, 0-2 weeks post enrollment; average median fold over background response (SD)= 2.33±0.16). Moreover, ADNP was associated with p18-specific IgM antibodies, indicating that an IgM, rather than an IgG response to this target, may be largely responsible for this antiviral function (r=0.77, p=0.044). Further, EBNA 1-specific IgMs were found to correlate more strongly (r=0.99, p=0.000013) with the severity of EBV symptoms than the previously described p18-specific IgA suggesting a potential role of IgMs in the pathogenesis of EBV infection.

Conclusion: Our systems serology approach identified ADNP as a first antibody mechanism of action against EBV with the potential to be exploited for future treatment and vaccine strategies.

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Psychological Well-Being and Type 2 Diabetes.
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Purpose: Positive psychological characteristics such as optimism, positive affect, gratitude, and related constructs may play an important role in health. The aim was to review the association of positive psychological constructs with the outcome of patients with type 2 diabetes.

Materials & Methods: We performed a literature review of recent medical literature of positive psychological constructs among patients with type 2 diabetes.

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

Conclusion: Continued work is needed to ascertain whether positive psychology interventions can truly impact functioning, blood sugar, and overall health in this key population.

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Systemic and local immune response against commensal bacteria.
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**Purpose:** The host must be able to mount protective immune responses against pathogenic microbes while maintaining tolerance to commensal microbes under homeostatic conditions. The failure to maintain this tolerance is a major underlying cause of inflammatory bowel diseases (IBD). Very little is known about how these immune cells normally tolerate these microbial antigens. Our aim is to understand how T cell tolerance is established to different commensal bacteria at unprecedented level of physiological significance and to investigate the role of microbial antigen specific CD4 T cells at homeostasis and inflammatory context.

**Materials & Methods:** Commensal bacteria specific T cells were tracked and characterized from harvested immune tissues of mice using peptide:MHC II tetramer reagents corresponding to I-Ab restricted epitopes. Because endogenous specific T cells exist at very low frequency, these cells were enriched using the magnetic beads that bind the fluorescent tetramers. They were then enumerated and phenotyped by flow cytometry in Gut Associated Lymphoid tissue (GALT) versus systemic lymphoid environments in steady state. The local tolerance was also challenged in mice immunized by oral gavage with antigen peptides. Regulatory populations were carefully checked and assessed using mice with 2 different reporters for Foxp3 and IL-10 expression.

**Results:** Using the appropriate tetramer for different epitopes, we enumerated and characterized specific T cells from different tissues in naïve mice. Our preliminary data shows that T cells targeting the peptide SFB570-580 of Segmented filamentous bacteria extracellular protein are mainly detected in the Lamina propria while FlgE specific T cells, an immunodominant flagellin epitope of *Helicobacter hepaticus* (FlgE) are present in different GALT tissues (mesenteric lymph node and lamina propria). However, T cells specific for CBir1, commensal-derived flagellin expressed by a subset of the Clostridium XIVa bacteria, reside primarily in spleen and peripheral lymph nodes (Fig 1) while they are deleted in the lamina propria. Moreover, these data pointed out Tr1 population as an important source of IL-10 production in the lamina propria.

**Conclusion:** Our data showed that commensal bacteria specific T cells are differently localized and compartmentalized in different sites regarding the anatomic location of bacterium. They also suggested that different commensal bacterial species have distinct effects on intestinal T cells. They also pointed out Tr1 population as key player in intestinal homeostasis against different commensal bacteria.

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Neuronal cell therapy to restore colorectal motility in a novel animal model of enteric neuropathy
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Purpose: Neuronal cell therapy offers an innovative approach for the treatment of Hirschsprung disease (HSCR), a congenital disorder characterized by the lack of enteric nervous system (ENS) in the distal colon, called colonic aganglionosis, resulting in functional colonic obstruction. Testing the effect of cell therapy on gut motility in HSCR is hampered by the poor survival of existing mouse models, which die within the first few weeks of life, limiting the time window available for analysis after cell transplantation. The broad objective of this project is to develop a novel, clinically relevant in vivo model of colonic aganglionosis and use it to demonstrate restoration of gut function following enteric neural stem/progenitor cell (ENSC) transplantation.

Materials & Methods: We generated a novel model of ENS ablation based on transgenic expression of diphtheria toxin receptor (DTR) in neural crest-derived cells and administration of diphtheria toxin (DT). We crossed Wnt1-Cre mice with R26R-iDTR reporter mice, thus generating a Wnt1-iDTR transgenic line in which active Cre recombination should render Wnt1-expressing neural crest cells sensitive to human. 4 µl of 1 ng/µl DT was injected into the wall of the mid-colon of Wnt1-iDTR mice via laparotomy to create focal colonic aganglionosis.

Results: Treatment of neural crest cells isolated from Cre Wnt1-iDTR mice with DT (10 ng/ml) for 24 hours resulted in a marked increase in expression of the apoptotic marker, cleaved caspase-3. Similarly, intraperitoneal administration of DT (40 µg/kg) to Cre Wnt1-iDTR mice resulted in pronounced intestinal dilatation, absence of coordinated patterns of colonic migrating motor complexes, and enteric neuronal loss, as confirmed by immunostaining with neuronal marker Hu. To limit neural crest cell injury to a focal region of the ENS, we injected DT into the wall of the mid-colon of these mice via laparotomy. 1 ng/µl DT resulted in focal loss of ENS that was maintained at 8 weeks, as confirmed by absence of neuronal marker, Tuj1, in the myenteric plexus. Focal loss of ENS was also found to alter GI motility.

Conclusion: We have established this in vivo mouse model of focal colonic aganglionosis. In future studies, we will transplant ENSCs isolated from Wnt1-tdT mouse intestine into the colon of our novel model of ENS ablation and perform functional assays for quantitative assessment of GI motility following cell transplantation.

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Genetically encoded calcium biosensors reveal cell signaling dynamics during kidney glomerular morphogenesis.
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**Purpose:** Podocytes are highly specialized epithelial cells in the kidney glomerulus that, along with capillary endothelial cells, play a critical role in maintaining the glomerular filtration barrier. Nephrotic syndrome genes, including TrpC6 and PLCe1, lead to defects in the glomerular barrier function and affect podocyte calcium signaling. However, the role of calcium signaling during podocyte development *in vivo* remains unknown. Here we aim at understanding the role of calcium signaling during glomerular development using live, *in vivo* cell imaging of zebrafish.

**Materials & Methods:**

**Results:** By confocal imaging, we found that immature podocytes (48 hours post fertilization) are motile and interact with the dorsal aorta to form glomerular capillaries. By 4 days post fertilization (dpf) podocytes stabilize and the filtration barrier is functionally mature. Using the genetically encoded fluorescent biosensor GCaMP to monitor *in vivo* podocyte calcium signaling during development, we found that calcium release is a prominent feature of glomerular morphogenesis. Notably, we observed podocyte calcium transients starting at 2.5 dpf, when blood filtration starts in the glomerulus, and continuing up to 4 dpf, suggesting a critical role for calcium signaling activity in podocyte remodeling. Using inhibitors to dissect the calcium transients we found they were due to release of calcium from IP3-dependent intracellular calcium stores and not influx via voltage gated membrane channels. To determine whether calcium signaling was dependent on cell interactions in the glomerulus, we performed a knockdown of *cloche*, a bHLH-PAS factor required for vascular development, leading to larvae lacking endothelial cells. We observed a significant decrease of calcium transients in *cloche* podocytes, suggesting that endothelial cells are critical to trigger podocyte signaling. Using an unbiased, whole glomeruli RNAseq transcriptome approach, we identified candidate signaling ligands and receptors potentially responsible for developmental podocyte calcium signaling. Using this approach, we identified the serotonin receptor 5-HTR2C 5-fold enriched in kidney glomeruli. Notably, we observed that amperozide, a selective 5-HT2 receptor antagonist, lead to a significant reduction in podocyte calcium transients, suggesting an unexpected contribution of vascular serotonin signaling to glomerular morphogenesis.

**Conclusion:** This work establishes a novel link between endothelial cells and kidney podocytes in the formation of the glomerular blood filter. Using biosensor fish as a discovery platform for novel receptor ligand interactions, our studies present new leads for treating glomerular disease and promoting organ repair.

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Lymph node metastasis contributes to distant metastasis in cancer mouse models

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Purpose: The presence of lymph node metastasis in patients with solid tumors is associated with tumor aggressiveness, poorer prognosis and the recommendation for systemic therapy. However, whether tumor cells exit the lymph node and contribute to distant metastases remains controversial.

Materials & Methods: In this study, we used syngeneic murine cell lines representing breast and melanoma cancer that spontaneously metastasize to the lymph node. We engineered these cells to express Dendra2, a photoconvertable protein. Dendra2 is a green-emitting fluorescent protein that can be converted to emit red light by exposure to 405nm light. Once tumor cells spontaneously metastasized to the lymph node from the primary site, a 405nm laser diode was used on 5 consecutive days to convert Dendra2-cancer cells from green to red fluorescence, restricting the light exposure only to the metastatic lymph node. This technology allowed us to specifically trace the fate of cancer cells in the lymph node and beyond to other organs.

Results: We show that spontaneous lymph node metastasis from breast cancer and melanoma mouse models can leave the lymph node and enter the blood circulation. We identified micrometastatic disease in the lung that originated from the lymph node in both models. We hypothesized that cancer cells escape the lymph node by directly invading lymph node blood vessels, as opposed to draining through the efferent lymphatic vessel. Immunohistochemical analysis of metastatic lymph nodes revealed isolated cancer cells in close association with CD31+ blood vessels, within high endothelial venules (HEVs) and breaching the vascular basement membrane. Quantitative analysis showed that 23±2% of isolated cancer cells were within 5 μm of a blood vessel, compared to only 11±1% using a predictive model of randomly distributed cells in the lymph node (p<0.05). Further, 6±2% of the cancer cells were inside blood vessels defined as cells within the lumen of blood vessels and having cell centroids more than 3 μm from the blood vessel endothelium. Finally, we analyzed lymph nodes with large metastatic lesions from patients with head and neck cancer and identified cancer cells that were closely associated with and inside blood vessels. To further confirm that metastatic cancer cells in a lymph node have affinity for lymph node blood vessels, we used multiphoton intravital microscopy to measure cancer cell migration in an optical lymph node window in mice. Dendra2 expressing metastatic cancer cells are first seen in the subcapsular sinus of the lymph node and later invade the cortex of the lymph node where they accumulate around rhodamine-dextran labeled blood vessels. Cancer cells can be observed in directed migration toward blood vessels as well as moving inside blood vessels.

Conclusion: Together, our data show for the first time that in spontaneous breast and melanoma mouse models, tumor cells in the lymph node can invade blood vessels, exit the node and colonize the lung.

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Associations between cerebral blood flow and structural and functional brain imaging measures in individuals with neuropsychologically defined mild cognitive impairment

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Purpose: Cerebral blood flow (CBF) is an indicator of regional neurovascular processes throughout the brain with signal weightings based on the health of the vascular system as well as the regional metabolic demands, however, little is known about the mechanisms that contribute to blood flow reductions in individuals with mild cognitive impairment (MCI). Here we examine regional patterns of CBF differences in individuals with neuropsychologically defined MCI compared to age-matched cognitively healthy older adults.

Materials & Methods: CBF data from pseudo-continuous arterial spin labeling (ASL) perfusion magnetic resonance imaging (MRI) and amplitude of low frequency fluctuations (ALFF) obtained from blood oxygenation level dependent (BOLD) imaging was obtained in 21 cognitively older adults with MCI and 19 age-matched cognitively healthy older adults. CBF was calculated using Bayesian Inference for Arterial Spin Labeling MRI (BASIL) with a cerebrospinal fluid (CSF) region used as a reference to calculate the equilibrium magnetization for arterial blood in each individual perfusion image; WMSA were quantified using T1/T2 image data with a procedure in the Freesurfer image analysis suite; cortical thickness was obtained using the Freesurfer recon-all procedure. Voxel-wise ALFF was calculated as the average square root of the low-frequency (0.009-0.08 Hz) power spectrum in each individual. Functional images were registered into a native T1-weighted MRI and then mapped into the cortical surface by using spherical registration. Surface-based group differences in CBF, ALFF, and cortical thickness between individuals with MCI and cognitively healthy older adults as well as associations among measures were performed by surface-based general linear models.

Results: Individuals with MCI had significantly reduced CBF that was associated with white matter lesion volume. In contrast, there was no relationship between WMSA and CBF in cognitively healthy adults. ALFF was regionally altered in MCI compared to cognitively healthy adults and was related to regional cortical thickness, but no relationship between CBF and ALFF was found in either group.

Conclusion: Although speculative, given the presumed vascular etiology of white matter lesions, these results may suggest that deterioration in vascular health is a causal mechanism of reduced CBF in MCI which may also be an important component of the pathological etiology of this condition. Taken together, a combined CBF/WMSA metric may be a useful marker reflecting an increased risk of cognitive deterioration in individuals with elevated risk for the development of Alzheimer’s disease.

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Immune Profiling of *Coxiella burnetii* Vaccination and Infection by Mass Cytometry

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**Purpose:** *Coxiella burnetii* (*Cb*), a highly infectious and resilient intracellular bacterial pathogen, is the cause of Q fever, which can require months of antibiotic treatment. The current *Cb* vaccine is approved in Australia alone and reactogenicity can occur in persons previously exposed to the bacterium. The Q-VaxCelerate consortium was assembled to develop an effective and less reactogenic Q-fever vaccine.

**Project objectives** include multi-parameter immune cell profiling to define a signature of immune protection in animal models of infection and in humans exposed to *Cb*.

**Materials & Methods:** To characterize the immune response to *Cb* infection or vaccination in both human and mouse samples we developed an approach utilizing Cytometry by Time Of Flight mass spectrometry (CyTOF) to measure >35 immune-parameters simultaneously. Donor samples from Oss, The Netherlands, which experienced an Q-fever outbreak from 2007-2011, were grouped by clinical history, anti-*Cb* antibody titers, and IFN\(\gamma\) response to killed *Cb* in vitro. PBMCs were incubated with killed *Cb*, and analyzed by flow cytometry and mass cytometry (CyTOF). To assess the immune response to *Cb* in mice, blood samples were collected prior to and after vaccination with whole killed *Cb* and following challenge with live *Cb*. CyTOF analysis was conducted on blood samples collected, and bacterial load and spleen pathology assessed at sacrifice.

**Results:** *In vitro* stimulation with killed *Cb* showed greater T-cell responses in seropositive donors. An ongoing study will characterize the immune responses from additional donors to profile across exposure status, clinical history, and HLA type. Initial analysis of murine samples indicates that vaccination results in an innate inflammatory response, a Th1-biased CD4 response and anti-*Cb* antibody. Following challenge, unvaccinated mice exhibit enhanced inflammatory responses in both innate and T-cell populations as compared to vaccinated mice. Robust adaptive responses to challenge in vaccinated mice are evident in the increased expression of T-bet in B-cells and decreased bacterial burden and splenomegaly.

**Conclusion:** Analysis of murine data has provided insights into the immune response to vaccination and challenge. Continued analysis using semi-supervised algorithms will provide a more detailed description of the immune response to *Cb*. Analysis from ongoing human studies will shed light on the various clinical outcomes of Q-fever. Insights gained from these studies will inform the design and assessment of candidate vaccines for *Cb*. Together these data reveal novel hallmarks of immune responses to *Cb* during and following infection and vaccination, with the potential to identify host-based biomarkers of protection against Q.

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Targeting FUS protein to mitigate RNA processing alterations linked to ALS and frontotemporal dementia.

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Purpose: Mutations in the FUS gene are responsible for familial cases of Amyotrophic Lateral Sclerosis (ALS) with young onset and very rapid disease progression. The protein encoded by the FUS gene, is a mostly nuclear RNA binding protein, which is abnormally accumulated in the cytoplasm in ALS patients with FUS mutations and in approximately 10% of sporadic patients with frontotemporal dementia. Cytoplasmic mislocalization of FUS is associated with alterations of RNA processing including expression and splicing abnormalities. Our goal is to identify therapeutic factors that restore FUS nuclear localization and reverse RNA alterations linked to ALS FUS mutations.

Materials & Methods: RNA-sequencing was performed using neurons directly converted from fibroblasts of FUS-ALS patients and control individuals to identify RNA processing alterations associated with disease. Identified RNA changes are used as functional readouts to determine the therapeutic potential of compounds that restore nuclear localization of mutant FUS. A high content screen with more than 3,000 kinase inhibitors and FDA-approved molecules was performed using fibroblasts from ALS patients with P525L-FUS mutation and neurons differentiated from CRISPR-edited isogenic neural progenitor cells (NPC) with or without P525L mutation. FUS cellular localization was visualized by immunofluorescence staining and imaged using Opera Phenix High Content Screening System. The therapeutic potential of Kapb2, the nuclear import factor of FUS that was recently shown to have disaggregase properties (Guo et al. Cell in press), was tested for its ability to reverse RNA processing alterations and prevent the abnormal formation of FUS-containing stress granules.

Results: We have defined a set of alterations in mRNA processing that delineate a disease-dependent signature in neurons directly converted from ALS patients with various FUS mutations (P525L, R521H and H517Q). Lentiviral-mediated expression of Kapb2 in R521H-FUS-ALS patients fibroblasts showed a partial reversion of the RNA profile linked to ALS mutation. Kapb2 overexpression in R521H-FUS neuronal progenitors also reduced the number of FUS-positive stress granules after arsenite treatment. In addition, small molecules triggering the restoration of nuclear FUS were identified by a high-content screen in patient cells and isogenic neurons expressing endogenous levels of mutant FUS. Several compounds were confirmed for their dose-dependent ability to increase the nuclear cytoplasmic ratio of FUS protein in patient cells. Notably, we identified several PKC inhibitors that increase the nuclear localization of FUS in P525L fibroblasts.

Conclusion: ALS-related mutations in FUS lead to cytoplasmic mislocalization and specific RNA processing alterations in neurons derived from ALS patients. We have identified molecules, including FDA approved compounds that restore FUS nuclear localization and represent potential candidates for developing a novel therapeutic approach in ALS.

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Role of GP73 in liver fibrosis

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Purpose: Liver fibrosis is the end result of nearly every chronic liver disease. If left unchecked, it can progress to cirrhosis, and ultimately hepatocellular carcinoma (HCC). HCC is the fast-growing cause of cancer-related mortality in the United States. Golgi Protein – 73kDa (GP73/GOLM1) is a resident cis-Golgi Type II membrane protein that is nearly undetectable in normal hepatocytes. However, its expression increases in patients with chronic hepatitis C and HCC in the context of fibrosis. In this project, we aim to investigate the role of GP73/GOLM1 protein as a potential therapeutic target for liver fibrosis.

Materials & Methods: GP73 expression was analyzed in two rodent models of hepatic fibrosis. In the first model, carbon tetrachloride (CCl4) was administered via oral gavage to male C57BL/6 mice for 8, 12, or 18 weeks to recapitulate fibrosis, cirrhosis, and subsequent HCC development. In a second model, a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD) consisting of 60% kcal fat and 0.1% methionine was used to reproduce non-alcoholic steatohepatitis (NASH) and fibrosis. Male C57BL/6 mice were fed this diet for either 10 or 14 weeks, and a subset of animals received CDAHFD for 10 weeks followed by normal chow for 4 weeks to model disease resolution. A hepatocyte-specific Golm1 knockout (KO) mouse model was created using the Albumin-driven Cre-loxP system and confirmed via genotyping. Golm1 floxed and KO mice were placed on CDAHFD for 12 weeks to induce NASH and fibrosis. Fibrosis was analyzed using Sirius red staining for collagen deposition and qPCR to assess fibrosis markers such as collagen Type 1, Alpha 1 (Col1a1) and alpha-smooth muscle actin (Acta2).

Results: In the CCl4 model, GP73 expression levels corresponded to fibrosis stage and tumor development. Similarly, in the CDAHFD model, GP73 expression increased significantly at 10 and 14 weeks compared to controls. A subsequent reduction in GP73 was also observed during disease resolution after CDAHFD withdrawal. These results correlated with other markers of fibrosis including Acta2 and Col1a1. In the Golm1 KO and floxed control mice that were fed CDAHFD, we observed that KO mice presented with reduced and localized collagen deposition in the livers compared to the controls as analyzed by collagen proportional area.

Conclusion: GP73 has been well documented as a serum biomarker for HCC, but recent studies have shown that its potential as a marker is limited to patients with a background of fibrosis. This study addresses the role of GP73 in fibrosis using hepatocyte-specific Golm1 KO mice and chemical and diet-induced models of fibrosis. In this study, we demonstrated that GP73 expression is correlated to the stage of fibrosis and subsequent HCC. Our data suggests that GP73 could serve as a potential therapeutic target for fibrosis. We are now using these Golm1 KO mice to further understand its role in disease pathogenesis.

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Learning Not to Fear: Mindfulness Improves Retention of Fear Extinction

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Purpose: Mindfulness based stress reduction (MBSR) programs have been widely utilized to ameliorate psychiatric and stress-related symptoms, however the neural mechanisms that underlie these improvements are still largely unknown. Mindfulness meditation involves refraining from cognitive avoidance and thus provides a basis for internal exposure to aversive stimuli. Thus, we hypothesized that mindfulness-based interventions create a context akin to behavioral exposure and thereby alter participants’ neurobiological responses to the aversive stimuli.

Materials & Methods: We tested this hypothesis using a 2-day fear conditioning and extinction protocol with skin conductance response as the index of extinction retention (ERI), in a randomized longitudinal study design. Meditation-naïve participants completed either 8-week MBSR (n=42), or stress management education (SME, n=25) programs. Behavioral changes and alterations in neural activation patterns associated with extinction memory from pre to post interventions were assessed using fMRI.

Results: Both interventions decreased stress levels, while MBSR resulted in further improvements in anxiety, emotion regulation, rumination, mindfulness, and self-compassion. ERI analyses demonstrated that both interventions improved memory for extinguished stimuli. Both interventions were associated with changes in brain activity from pre to post in ventromedial prefrontal cortex and in the hippocampus during the recall of extinguished stimuli. Compared to the control intervention, the MBSR intervention was associated with the differential engagement of a cluster in posterior parietal cortex. Critically, for the MBSR group, neural activity within this cluster correlated with the total amount of meditation practice engaged during the intervention. Further investigation of functional coupling patterns revealed an enhanced connectivity between dorsal anterior cingulate and supramarginal gyrus, the two major components of the ventral attention network, especially in the earlier phases of recall following the MBSR intervention.

Conclusion: MBSR improves extinction memory predominantly though enhancing neural activity in regions associated with attentional input to memory during extinction recall. Considering that the ability to recall that a stimulus is no longer associated with threat is critical for healthy emotional functioning, these results suggest the improvement in this ability may be a key mechanism through which mindfulness meditation ameliorates psychiatric and stress-related symptoms.

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Characterizing anhedonia in adolescents using diffusion MRI and a cluster-based approach

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Purpose: The Boston Adolescent Neuroimaging of Depression and Anxiety (BANDA) project is a disease human connectome project (dHCP) study on adolescents with depression and/or anxiety and healthy controls. We focus on an age that is critical for brain development and for the onset of mental illness. Our transdiagnostic approach is consistent with NIMH research domain criteria making these data a valuable resource for investigating imaging biomarkers of depression and anxiety. White matter (WM) tracts can be estimated from diffusion MRI (dMRI) by following the preferential direction of water molecules that are restricted by myelin. Here we present results from the dMRI data of the first set of BANDA subjects using AnatomiCuts, our novel method for unsupervised whole-brain WM streamline clustering based on anatomical similarity. We find associations of anhedonia symptoms with diffusion measures in left-hemispheric bundles connecting the anterior cingulate, thalamus, and prefrontal cortex.

Materials & Methods: Subjects aged 14-15 were scanned on a 3T Siemens Prisma at MGH to obtain structural MRI (sMRI) data (.8mm iso) and dMRI data (1.5mm iso, 197 directions, b=1500/3000). We estimated 500K WM streamlines from dMRI. We used AnatomiCuts, our novel hierarchical clustering method that groups streamlines into anatomically meaningful bundles without relying on an atlas, but only on each subjects' cortical and sub-cortical segmentations (see Fig.1). Our anatomical similarity is based on the structures streamlines go through or next to all along the streamlines' trajectories for different directions (anterior, posterior, left, right, etc.). This allows us to find corresponding clusters across subjects without inter-subject registration. We use the Hungarian algorithm to find cluster's correspondences across subjects. Anhedonia symptoms were assessed with the Snaith-Hamilton pleasure scale.

Results: We identified 17 subjects with anhedonia and 32 age- and sex-matched non-anhedonic subjects. The groups did not differ in IQ (p=0.32). The dMRI data did not differ in translational motion (p=0.26), rotational motion (p=0.31), or SNR (p=0.44). We obtained a hierarchical tree of 200 tract clusters using AnatomiCuts. To reduce multiple comparisons, we pruned the tree to obtain the top 50 clusters. We compared fractional anisotropy (FA) and mean/axial/radial diffusivity (MD/AD/RD) for each of the 50 clusters between groups, with age and sex as regressors. Statistical significance in two clusters survived Bonferroni correction for the number of clusters and measures (cluster 1: p[RD]=0.0007, p[FA]=0.0001; cluster 2: p[RD]=0.0003, p[MD]=0.0007). Post-hoc analyses on the children nodes of these two clusters (at the 200-node level, see Fig.2) showed that significant differences were localized in bundles connecting the anterior cingulate, supramarginal, precentral, postcentral, transverse temporal, thalamus, peri-callosal, superior frontal, and insular cortex.

Conclusion: Anhedonia, the inability to feel pleasure, is one of the main symptoms of depression. Functional and morphometric MRI studies of anhedonia have shown effects in prefrontal, anterior cingulate, precentral, and insular cortex, and the striatum. Studies using dMRI have shown FA decreases in the cingulum and thalamic radiations, with conflicting findings in the uncinate fasciculus. Our analytic approach, which explores the whole brain beyond just the main highways, while also allowing a finer-grained subdivision of bundles, both confirms and augments prior findings. The BANDA data set containing 225 subjects will be released to the research community for further analysis of anxiety and depression disorders. AnatomiCuts allows the full brain analysis of WM structure without the need of normalization to a template space or across populations, is not restricted to a subset of well-known WM bundles, and is therefore a powerful tool to characterize diseased populations' onsets for early diagnosis.

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Development of a time-domain finite-element model of acoustic wave propagation within the cornea
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**Purpose:** Upon mechanical stimuli, such as an air-puff, mechanical waves are generated and propagate in the cornea. These acoustic waves can be described as planar Lamb waves or Rayleigh surface waves, depending on their acoustic wavelengths compared to the corneal thickness. Rayleigh waves propagate along the epithelial surface with speeds mainly related to the shear modulus of the corneal tissue, independent of the corneal thickness. Therefore, measuring Rayleigh wave speeds is a promising way of estimating the corneal elasticity. Here we introduce a finite-element model to assess the effect of individual parameters on the corneal wave propagations to better generate and detect Rayleigh waves.

**Materials & Methods:** We used COMSOL to simulate acoustic wave propagation. We first verified the method for a simple plate model of the cornea. We then developed a more complete model of eyeball consisting of cornea, limbus, sclera, and aqueous and vitreous humors. The model considers the solid-fluid interaction between solid tissues and liquids, the viscoelasticity of solid components, the viscosity of aqueous and vitreous humors, and the intraocular pressure. We varied different parameters and observed the wave propagation on the corneal surface.

**Results:** Simulation results from the flat cornea model agreed with the theory of elastic plates. This simple model was also used to verify different boundary conditions and the effect of fluid domain under the cornea on the wave propagation. The model produced time sequence of the excitation of mechanical vibrations and propagation of acoustic waves. The typical temporal resolution was 1/20th of the acoustic period. The time domain method allows us to investigate transient and time-varying evolution of acoustic waves. Among 40 cycles simulated for each condition, the first few cycles showed how the wave propagates through cornea and other components, while the last few cycles were picked for data analysis to obtain different wave properties. The transient responses depend on the duration, width, and types of excitation sources.

**Conclusion:** We have developed a model to simulate wave propagations in the cornea. Using this model, we have investigated the effect of various mechanical and anatomical parameters on the wave propagations in the cornea. This tool will be useful to design devices to generate optimal mechanical stimulus and to interpret experimental data of corneal elastography.

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Association of High Human Leukocyte Antigen -B and -C Expression Level with Prolonged Overall Survival in Colorectal Cancer

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Purpose: Human Leukocyte Antigen (HLA) Class I expression is frequently defective in cancer cells. HLA Class I antigens play a crucial role in the interactions of tumor cells with the hosts’ immune cells. Papers describing HLA Class I expression level in primary resected colorectal cancers (CRC) were reviewed to understand the mechanisms regulating it and also to evaluate its prognostic significance.

Materials & Methods: Five of the 1265 papers reviewed met our eligibility criteria. They included use of immunohistochemistry with monoclonal antibodies to evaluate HLA-A and HLA-B/C expression in CRC tumors and availability of survival data. Primary data from these publications were obtained for re-analysis.

Results: The 2966 CRC patients were representative of the general CRC patient population in terms of gender, age and disease stage. BRAF gene mutation was found in 15.8% of patients and DNA mismatch repair gene, which led to microsatellite instability (MSI), was found in 14.1% of patients. BRAF gene mutation was found in 27.5% of patients with downregulated HLA-B/C expression and 24.6% of patients with downregulated HLA-A expression but only 14.0% of patients with high HLA-B/C expression and 11.7% of patients with high HLA-A expression. Similarly, MSI was present in 26.3% of patients with downregulated HLA-B/C expression and 13.6% of patients with downregulated HLA-A but only 11.4% of patients with high HLA-B/C expression and 8.7% of patients with high HLA-A expression. Chi-square analyses showed that both BRAF gene mutation and MSI were found to have a significant association with downregulated HLA-B/C expression (p=0.008 and p<0.001 respectively) and with downregulated HLA-A expression (p<0.001 and p=0.034 respectively). Median overall survival (OS) of the 2322 patients with high HLA-B/C expression was significantly longer than that of the 401 patients with low HLA-B/C expression and of the 195 patients with non-detectable HLA-B/C expression (93.0 months, 72.8 months and 43.0 months respectively, p<0.001). Multivariable Cox’s regression analysis confirmed this association and demonstrated that the patient cohort with non-detectable HLA Class I expression had a significantly shorter OS than the one with low HLA Class I expression (HR=1.67, 95% CI 1.33-2.10, p<0.001).

Conclusion: Activation of the MAP kinase pathway triggered by the BRAF mutation may play a role in the HLA Class I downregulation. Since high HLA-B/C expression level is a favorable prognostic biomarker, CRC might benefit from strategies which inhibit MAP kinase pathway activation.

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Liver X Receptors are essential to thymic function and T cell development

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Purpose: Atherosclerosis is a lipid-driven inflammatory disease and a root cause of myocardial infarction and stroke. T cells both limit and aggravate atherosclerosis, owing to their ability to recognize oxidized lipoproteins. Implicit in all these approaches is the idea that T cells contribute to disease only after they mature to effector cells in the periphery. However, T cells are born in the thymus, and we hypothesize that cardiovascular risk factors (e.g. high cholesterol) impairs thymic function, producing a pool of aberrant T cells that drive inflammation. We will focus our inquiry on Liver X Receptors (LXRs), transcription factors that orchestrate reverse cholesterol transport via induction of cholesterol efflux genes Abca1 and Abcg1.

Materials & Methods: Thymuses, blood and spleens from C57BL6/J, LXRαβ−/−, Foxn1Cre/+LXRαfl/flβfl/fl, CD4Cre/+LXRαfl/flβfl/fl and LckCre/+Abca1βfl/flAbcg1βfl/fl mice from 2-5mo age were used for flow cytometry and cholesterol biochemical assays. For atherosclerosis, low-density lipoprotein receptor (Ldlr)−/− mice were reconstituted with wild-type or LXRαβ−/− bone marrow and fed a high cholesterol diet (1.25%) for 16 wk, and aortas/thymuses harvested for histochemistry and flow cytometry.

Results: In mice lacking LXRs, thymuses completely involuted by 5 months of age. Prior to involution, thymuses were hyperplastic and engorged with cholesterol, and thymocyte development was markedly impaired. We wondered whether thymic epithelial cells (TECs) were responsible for thymic involution - as their quantity determines lymphopoietic capacity and hence declining numbers cause involution. We found LXR−/− TECs proliferated more and were also highly apoptotic, suggesting that LXRs maintained TEC homeostasis and that defective TEC cholesterol metabolism compromised thymic integrity. Conditional and bone marrow LXR knockout mice confirmed that LXR deficiency in TECs caused rapid thymic involution but did not alter T cell development, whereas T cell LXR-deficiency only impaired T cell development. T cell-specific LXR-deficiency also reduced peripheral T cell numbers, despite previous reports describing that the loss of LXRβ or Abcg1 enhanced T cell proliferation. However, conditional Abca1/Abcg1 T cell-knockouts also confirmed reduced T cell numbers. Furthermore, LXR-dependent Abca1/Abcg1 expression was crucial for the maintenance of T cell quiescence. Finally, LXR-deficient, LDLR−/− mice developed enhanced atherosclerosis compared to control animals, characterized by thymic involution and a striking attenuation of aortic T cell accumulation with no differences in monocyte/macrophage levels, suggesting that T cells and the thymus contribute to this phenotype.

Conclusion: In summary, we have uncovered novel roles of LXRs and cholesterol metabolism in T cell biology and thymic function. Future studies will elucidate mechanisms underlying how these T cell/TEC cell-specific LXR deficiencies 1) causes thymic involution, 2) impairs T cell development, function, and survival and 3) accelerates atherosclerosis.

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Purpose: The prevalence of food allergies is estimated to be 2–10% of the population with rates increasing and self-reported rates climbing much higher than reported prevalence. In the United States, 30% of allergic children suffer from multiple food allergies. Each year there are an estimated 203,000 food allergy-related emergency department visits in the United States, including 90,000 cases of anaphylaxis. Novel therapeutic approaches, including oral immunotherapy with or without anti-IgE antibody (Omalizumab), are being pursued. However, the accepted standard of care is to strictly avoid allergenic foods and administer emergency medication upon accidental exposure. In response, we developed a portable, point-of-use detection technology, termed integrated exogenous antigen testing (iEAT). The system consists of a disposable antigen extraction device coupled with an electronic keychain reader for rapid sensing and communication.

Materials & Methods: The portable iEAT system comprising a keychain reader, an extraction kit, and a smartphone app (Fig.A). The analytical capabilities is comparable to a benchtop system. Allergens are extracted by optimized TECP-based extraction buffer with 20s, enriched on immunomagnetic beads and labeled with a second antibody conjugated with an oxidizing enzyme (HRP). After mixing with chromogenic mediators (TMB), the enzyme catalyzes the TMB oxidation and H2O2 reduction. The oxidized TMB is then reduced by receiving electrons from the electrode, and the generated current was measured (Fig.B). The mini-reader is extensible to multichannel for parallel measurements. Measured currents were converted to allergen concentrations according to preloaded lookup tables of the device (Fig.C). All the calibration curves were comparable to standard ELISA (Fig.D). It not only detects and displays results, but also wirelessly communicates via bluetooth to transmit results and information to a cloud server for web-based data collection and sharing among users.

Results: The analytical performance for rapid detection of varying target allergen dose are better than the significant chance of adverse reaction, requiring a precautionary statement, regulated by U.S. Food and Drug Administration and Voluntary Incidental Trace Allergen Labeling (Table 1). We applied the iEAT device for testing panels of packaged staple foods, desserts (Fig.E), and randomly field testing foods from restaurants and cafeteria. The profiling results showed the expected allergens, such as gluten in hamburgers and pizza, but we also detected unexpected antigens contributed by food processing. Salad contained gluten, likely from the salad dressing. We also identified ovalbumin and casein in beer, which is presumably added as food additives; egg-white is used to improve the foam characteristics, and casein is used to stabilize beer during the brewing process (Fig.F). In testing gluten-free menu items (color-coded), we observed widely varied gluten levels, and some items had gluten levels far exceeding the regulatory limit. The test results were stored in the cloud server to create a customized restaurant map (Fig.G). Compared with other common methods, the device is inexpensive (<$40) with assay costs of <$4 per antigen (Table 2).

Conclusion: We developed the point-of-care iEAT system to detect five major food antigens in peanuts, hazelnuts, wheat, milk, and eggs. Antigen extraction and detection with iEAT requires <10 min and achieves high-detection sensitivities (e.g., 0.1 mg/kg for gluten, lower than regulatory limits of 20 mg/kg). While we focused on specific protein antigens, the current assay format could also be modified to detect small molecules, toxins, or nucleic acids by changing affinity ligands (e.g., aptamers, oligonucleotides); creating detection panels for food safety (e.g., pesticides) and for food-source identification (e.g., DNA-based testing). The device could have many interesting applications, such as verifying food origins, confirming the absence of contaminants, or supporting dietary restrictions for religious purposes. We envision that the portable iEAT technology will allow for more rigorous and evidence-based analysis of food products, enhance consumer protection, reduce accidental allergy exposure, and identify problems in our food supply chain.


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Distinguishing immunomodulatory commensal viruses in healthy and disease intestine
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**Purpose:** Although the microbiome has been established as an important regulator of health and disease, the role of commensal viruses that inhabit human intestine (the “virome”) is largely unknown. The fecal virome is altered in inflammatory bowel disease (IBD) and depletion of viruses or host viral receptors enhances intestinal inflammation in mice. How the virome contributes to host homeostasis or impacts gut inflammation is unknown.

**Materials & Methods:** We isolated viral like particle (VLP) from ileostomy or colon resection samples from healthy, ulcerative colitis (UC) or Crohn’s disease (CD) donors and compared how healthy or IBD intestinal viromes differentially impact host immunity. To identify the components of the virome that provide homeostasis, or trigger inflammation, we unbiasedly identified the virus-derived RNAs bound directly to the host virus receptor Retinoic acid inducible gene–I (RIG-I) in healthy and IBD human and mouse intestine using RIG-I crosslinking immunoprecipitation (CLIP) protocol.

**Results:** Viruses isolated from fresh ileostomies or colon resections from CD or UC patients triggered enhanced interferon (IFN) and pro-inflammatory tumor necrosis factor (TNF) production from macrophages than viruses isolated from healthy controls that triggered more anti-inflammatory IL-10. RNA isolated from IBD VLPs that was transfected into macrophages triggered more pro-inflammatory response compared to healthy controls, while VLPs from healthy, non-IBD individuals triggered significantly more anti-inflammatory response than IBD VLPs. Using CLIP protocol, we identified a number of eukaryotic virus families stringently attached to RIG-I in the intestine that changed during inflammation. Interestingly, some prokaryotic viruses were RIG-I-bound demonstrating that bacteriophages have important immunomodulatory roles independent of their host bacteria. Thus, our data reveal that commensal viruses trigger host immunity that is altered to a more pro-inflammatory response in IBD.

**Conclusion:** Viruses commensal to the intestine are not ignored by the host and are capable of triggering an innate immune response. Determination of the precise viral components that interact with the host helps in understanding the role of the virome in regulating immune homeostasis and paves the way for the development of novel precision-based therapies that harness the healthy virome or the specific innate immune pathways activated by it.

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The brainstem response to respiratory-gated auricular vagal afferent nerve stimulation (RAVANS) at ultrahigh-field (7T) fMRI and its effect on vagal autonomic outflow


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**Purpose:** Transcutaneous electrical stimulation of the auricular branch of the vagus nerve (ABVN) has been used as a neuromodulatory therapy for multiple disorders, and primary brainstem relay occurs in nucleus tractus solitarii (NTS). We have previously proposed that ABVN stimulation effects are optimized by gating stimulation to the respiratory cycle, with increased NTS response shown for exhalation-gated RAVANS (eRAVANS) compared to inhalation-RAVANS (iRAVANS). Here, we exploit the enhanced spatiotemporal resolution afforded by ultrahigh-field functional MRI (7T fMRI) to evaluate brainstem response during RAVANS, concurrently estimating stimulus-evoked instantaneous vagal outflow with a point-process algorithm.

**Materials & Methods:** 16 healthy subjects experienced two 8-minute fMRI runs, with moderately strong eRAVANS or iRAVANS delivered to the left cymba concha of the ear (450µs pulse width at 25 Hz, 1s duration). A third resting state run was used to deliver sham stimulation (no current delivered), to control for respiratory cycle influence on medullary response. Brainstem BOLD fMRI data were collected on a 7T scanner (1.2mm isotropic voxels, 38 coronal slices, TR=0.99s, 500 volumes), concurrently with ECG and respiration monitoring at 500Hz. Images were preprocessed and analyzed modeling the hemodynamic response with an optimal basis set (FLOBS), and group statistics were obtained with non-parametric methods (randomization). Instantaneous estimate of the high-frequency component of heart rate variability (HF-HRV) was used as a metric for parasympathetic activity.

**Results:** Group maps demonstrated an increased response during eRAVANS compared to iRAVANS and Sham conditions in ipsilateral medulla, consistent with purported NTS and nucleus ambiguus (NAmb). Vagal outflow increased in response to eRAVANS compared to iRAVANS and Sham, providing promising basis for therapeutic applications of eRAVANS in disorders involving autonomic disruption.

**Conclusion:** High spatial resolution fMRI and high temporal resolution HF-HRV allowed for non-invasive characterization of brainstem and cardiovagal response to tVNS, which was significantly modulated by respiratory rhythm. Our approach developed promising tools for parameter optimization of this neuromodulatory therapy.

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Fluidic shear stress induces chemoresistance in Ovarian Cancer in a 3D microfluidic model

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**Purpose:** Ovarian cancer (OvCa) is the leading cause of death from gynecologic cancer in the United States, with a 5-year survival rate of only 40%, which has remained unchanged for decades. Recurrence, metastasis, and chemoresistance are often observed despite efforts to improve outcomes. Poor prognosis of OvCa is commonly associated with the development of ascites and micrometastases of tumor nodules within the abdominal cavity. Previously, it was shown that ascitic fluid movement confers molecular and phenotypic changes on tumor nodules due to shear stress. This includes induction of epithelial-mesenchymal transition (EMT) in OvCa cells (Rizvi et al., 2013). In this project, we investigated whether the flow-induced modulation of the biological features of the OvCa contributes to chemoresistance in a 3D microfluidic platform.

**Materials & Methods:** We established a 3D OvCa culture (NIH:OVCAR5) on stromal beds of Matrigel under both static and flow conditions. Tumor nodules were grown for seven days followed by treatment with a therapeutically relevant range of doses of carboplatin, an FDA-approved drug for OvCa, for an additional four days. The residual live tumor after chemotherapy was quantified by *in situ* live cell imaging using Calcein-AM to stain viable 3D nodules. Under flow, tumor nodules grew in multiple layers in the microfluidic channels, therefore, confocal fluorescence mosaic images were taken in multiple z-planes and stitched together. In static cultures, images from the brightest focal plane were used to determine the viable tumor area of the 3D nodules overlaid on the Matrigel beds. Image analysis was performed using custom MATLAB-based algorithms. In parallel experiments, 3D tumors grown under static or flow conditions were harvested from the Matrigel beds to characterize changes in molecular expression by Western Blots. Cellular uptake of carboplatin (platinum) was quantified by inductively coupled plasma mass spectrometry (ICP-MS). Finally, using *in silico* simulation, we estimated the shear force experienced by the nodules and the fluid dynamics in the microfluidic channels.

**Results:** We compared the response to carboplatin of OvCa nodules grown in static and flow 3D cultures. The concentration of carboplatin that decreased tumor viability by 82% (500 µM) in static culture, showed resistance in tumor grown under flow. Tumor viability in under static and flow conditions were 18 % and 47%, respectively, after 500 µM carboplatin treatment. Chemoresistance in 3D nodules grown under flow was also observed at a dose of 250 µM carboplatin. We investigated whether the EGFR/ERK signaling pathway, which has been implicated in treatment resistance, was involved in the flow-induced chemoresistance observed here. It was found that the expression of phosphorylated-ERK1/2 (at Thr202/Tyr204), a downstream protein in the EGFR pathway, was drastically increased in Ovcar5 cells grown under flow compared to the static cultures in all the experimental replicates while the total EKR expression remained the same. Interestingly, phospho-ERK1/2 levels were further increased in cells treated with carboplatin in both flow and static culture groups. Previous studies suggest that ERK1/2 activity could also be regulated by focal adhesion complex proteins, such as vinculin, paxillin, and focal adhesion kinase (FAK). We found that FAK was slightly decreased in cells under flow and significantly decreased when cells were treated with carboplatin in both flow and static cultures groups. In addition, paxillin and vinculin were also downregulated in flow culture and further decreased when cells were treated with carboplatin. Drug uptake studies indicate that there was a trend towards elevated carboplatin uptake by tumors under flow compared to nodules grown in static culture. However, the difference in carboplatin uptake was not statistically significant suggesting that the chemoresistance was not due to limited drug uptake. Finally, *in silico* modeling was used to evaluate the fluid dynamics in the microfluidic channel and estimate the forces experienced by the nodules.

**Conclusion:** It was found that OvCa cells grown under sustained flow-induced shear stress were more resistant to chemotherapy than the same tumors grown under static conditions. Chemoresistance was associated with enhanced expression of phospho-ERK1/2, a pro-survival protein. Drug-uptake studies suggest that physical stress-induced chemoresistance in cells under flow was not due to limited platinum uptake. These findings suggest that treatment strategies should be designed to include the effects of physical stress in the tumor microenvironment with a goal of developing rationally designed combinations using mechanistically distinct modalities.

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Burn-induced Microglia Activation is Associated with Motor Neuron Degeneration and Muscle Wasting in Mice
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**Purpose:** Severe burn injury (BI) involves multiple organ system dysfunction due to systemic inflammation even in the absence of sepsis, creating a complex network of metabolic interactions. These changes are associated with increased morbidity and mortality, and seriously affecting prognosis and having long-term health issues, which lead to significantly decreased quality of life of burned patients. The pathophysiological responses to severe BI include muscle mass loss and muscle weakness leading to decreased functional neuromuscular abnormalities. Most studies have focused on muscle itself with very little attention to the role of central nervous system or specifically the spinal cord in the muscle changes and neuromuscular dysfunction. This study tested the hypothesis that severe BI induces activation of microglia and release of cytokines and chemokines, which leads to degeneration of motor neurons in spinal cord ventral horn and consequently cause skeletal muscle wasting.

**Materials & Methods:** Mice (n=80) were randomly divided into four groups: Sham BI group alone, scald BI group (third-degree burn injury with 35% total body surface area) alone, BI with immobilization (by external plastic casting) group and Sham BI group with immobilization group. The spinal cord and skeletal muscle tissues were harvested on day 7 and 14 after each treatment. The microglia marker IBA1 and motor neurons in the ventral horns of the lumbar spinal cords (L2-4) were assayed by immunofluorescence staining for neurons or Nissl stain. Tunnel staining and NeuN were used to quantitate apoptosis (degeneration) and motor neuron number, respectively. The lumbar spinal cords were harvested to evaluate cytokines and chemokines expression using QPCR; and the muscle weights of gastrocnemius, tibialis anterior and soleus muscles were tested at day 7 and 14 after euthanization to test for muscle atrophy.

**Results:** The ventral horn motor neurons apoptosis occurred in the spinal cord. Compared to sham BI, the number of motor neurons decreased about 10-20% at day 7 and 14 after treatment, especially during the concomitant presence of immobilization. The microglia density around motor neuron in ventral horn increased significantly at both day 7, 14. This increase was more prominent in BI with immobilization. Moreover, the expression of inflammatory cytokines IL-1, TNF-α and chemokines CXCL2 were sharply induced by sever burn injury, compared with sham control. Corresponding the gastrocnemius, tibialis anterior and soleus muscles masses were decreased ~14- 38% at 7 and 14 days, in severe burn or burn plus immobilization group, respectively.

**Conclusion:** Severe burn injury induces activation of microglia and degeneration of motor neurons in spinal cord ventral horn, which causes corresponding skeletal muscle wasting. Our result also shows that severe burn injury or plus immobilization is sufficient for activation and proliferation of spinal cord microglia which release inflammatory cytokines and chemokines. The connection between microglia activation and motor neuron loss requires further study.

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Excess of rare protein truncating variants in patients with amyotrophic lateral sclerosis
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**Purpose:** Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease characterized by degeneration of motor neurons leading to progressive weakening of limb, bulbar, and respiratory muscles. Genetics is an important risk factor for ALS with 5-10% of patients reporting a positive family history. Until recently, ALS gene discoveries were made through large multigenerational pedigrees. We have assembled the largest ALS exome case-control study consisting of 15,722 individuals (5,073 ALS cases and 10,649 controls) to identify independent statistically significant genetic risk factors.

**Materials & Methods:** We processed our initial dataset of 15,722 samples through a rigorous quality control pipeline using Hail, an open-source, scalable framework for exploring and analyzing genomic data, to perform rigorous quality control analysis, making our dataset 11,703 individuals (3,864 cases and 7,839 controls). We complemented our analysis with a much larger set of exome sequencing data consisting of >50,000 samples from DiscovEHR, a source of sequencing data from healthy individuals, and >45,000 individuals ascertained from non-psychiatric or non-brain related studies, as part of the Exome Aggregation Consortium (ExAC). We performed an exome-wide analysis to determine whether there was an enrichment of a specific class of variation in ALS cases. We also assessed multiple different biologically relevant gene sets. We evaluated (1) constrained genes, which are under strong purifying selection; (2) genes known to confer risk to ALS; (3) genes associated with clinically overlapping diseases; and (4) genes in which their expression is specific to the brain.

**Results:** We observed an excess of rare protein truncating variants (PTV) in ALS cases, which typically lead to loss of protein function. The PTV enrichment observed in ALS cases was primarily concentrated in highly constrained genes; however, when removing the effects of constrained genes, we still observed a significant abundance of PTVs, suggesting that there are residual effects harbored elsewhere. Accordingly, we examined (1) known ALS genes, (2) genes associated with other motor neuron diseases such as primary lateral sclerosis, progressive muscular atrophy, progressive bulbar palsy, and spinal muscular atrophy, as well as diseases with overlapping phenotypes such as Parkinson’s disease, frontotemporal dementia, Pick’s disease, and Alzheimer’s disease, and finally, (3) genes with specific brain expression, none of which showed an elevation in PTVs. Finally, we conducted single gene burden analyses within our dataset (3,864 cases and 7,839 controls) and an additional larger cohort of ancestry matched individuals ascertained from non-psychiatric or non-brain studies, for a total of 28,910 controls, in which, multiple variants harbored within one gene enables us to potentially identify novel ALS genes. In this analysis, we observed the most significant genes as SOD1, NEK1, and FUS, which are known ALS genes. Interestingly, with the additional controls, multiple novel genes approached statistical significance. DNAJC7, which is a highly constrained gene had 4 PTVs in cases and 0 in total controls, 28,910.

**Conclusion:** DNAJC7 encodes a molecular chaperone, DnaJ heat shock protein family (Hsp40) member C7, and like all 50 DNAJ proteins, which are also classified as HSP40 proteins, facilitates protein maintenance and quality control, such as folding of newly synthesized polypeptides, and clearance of degraded proteins. Specifically, DNAJs act as co-chaperones for HSP70 proteins by regulating ATPase activity, aid in polypeptide binding, and prevention of premature polypeptide folding. When these processes are not regulated, misfolding and accumulation of degraded proteins occur leading to aberrant protein aggregation, one of the pathological hallmarks of neurodegenerative diseases like Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, prion disease, and ALS.

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Developing flow cytometry-based assays to identify determinants of FUS nucleocytoplasmic localization and cellular morphological changes in amyotrophic lateral sclerosis

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**Purpose:** Mutations and/or cytoplasmic mislocalization of FUS are associated with frontotemporal dementia (FTD) and the most aggressive juvenile forms of amyotrophic lateral sclerosis (ALS). Accumulating evidence support that cytoplasmic mislocalization of FUS confers gain of toxic functions that are crucial for the motor neurons death, but the factors influencing aberrant nucleocytoplasmic transport of FUS, and the morphological changes induced by FUS mislocalization are still elusive.

**Materials & Methods:** Here, we have developed a flow cytometry based protein localization/aggregation tracking assay for sorting out cells with restored nuclear localization of FUS after therapeutic intervention with small molecules or pooled genome wide CRISPR/Cas9 genetic screen. Using this approach, we intend to identify the determinants of FUS cytoplasmic mislocalization. For the development of assay, we have used the pulse shape analysis to monitor localization/aggregation of the FUS mutant protein in patient cells. The fluorescence forward scatter pulse for each cell was examined for their width and height/area. The width (time of flight) of the cells represents the localization of FUS protein, cytoplasmic FUS gives wider width compared to nuclear FUS. However, the height/area of pulse represents intensity of signal or aggregation of protein, in this case the intensity is higher with cytoplasmic FUS than nuclear FUS. In parallel to the assay development, we have examined the morphological changes induced by FUS mutations (R495Xfs36, P525L, and R521H mutated patient cells) using imaging flow cytometry. Here we have quantified the bright field cell size; intensity/pixel of cytoplasmic and nuclear FUS; DNA area, intensity, and granularity of mutant cells and compared them with other three control cell lines.

**Results:** We have observed that our flow cytometry-based protein localization/aggregation tracking assay was able to separate mixed population of control and mutant cells. With this assay we can discriminate separate gates for control and mutant populations. Subsequently we have validated the assay by testing a small molecule that we previously identified for its ability to increase the nuclear localization of mutant FUS. We observed clear separation between the non-treated and treated mutant cells. Similarly, we have used our developed assay for sorting the P525L mutant fibroblast cells edited with CRISPR/Cas9 pool library to identify genetic determinants of FUS cellular localization. Cells with increased nuclear localization were collected and will be analyzed by high-throughput sequencing to identify genes modifying nucleocytoplasmic transport of FUS. On the other hand, using flow cytometry imaging, we found that FUS mutations increase the size of the cells, with a decrease of DNA granularity and intensity consistent with chromatin decompaction.

**Conclusion:** In summary, we have developed an assay that can separate cells with cytoplasmic FUS from cells with nuclear FUS. This assay can be used for therapeutic development or CRISPR/Cas9 genetic screens. Thus, our new approach has a potential to reveal crucial pathways associated with FUS mislocalization and other ALS associated RNA binding proteins that may be therapeutically targeted.

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Normothermic Machine Perfusion of Human Kidneys with no Oxygen Carrier

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Purpose: The increasing demand for kidneys has led to a greater use of marginal donors which are at greater risk of subsequent discard. Normothermic Machine Perfusion NMP has presented a promising solution for per-transplant assessment and reconditioning of kidney grafts that would otherwise be discarded. The aim of this feasibility study is to test whether an oxygenated nutrient rich cell culture medium with no added oxygen carriers can be sufficient to sustain the ex-vivo viability and function of a human kidney graft, compared to a hemoglobin based perfusate.

Materials & Methods: 10 Human Kidneys (8 DBD, 3 DCD) were perfused up to 6 hours at 37°C using a pressure-controlled system. 6 kidneys were perfused with pRBC based solution, while 4 kidneys were perfused with no oxygen carrier. Our perfusion evaluation parameters included renal artery flow/resistance, urine production, and macroscopic appearance. Pre and post perfusion histology readings were taken. Hourly tissue biopsies were collected for molecular assessment. Inflow and outflow perfusate samples were examined for chemistries and oxygen content difference.

Results: Both groups displayed comparable behavior regarding oxygen consumption (p = n.s). The two groups also showed a congruent decline in creatinine over time. Urine production was similar between both groups (p = n.s). The renal artery resistance in the pRBC group was initially higher compared to the no-oxygen carrier group, but decreased to below 0.3 mmHg/min/mL after 3 hours of perfusion (p = n.s).

Conclusion: The initial data of this ongoing study demonstrates that human kidneys can be safely preserved with NMP using no oxygen carriers for up to 6 hours. Nutrient rich cell culture media supplied with sufficient dissolved oxygen appears to be satisfactory for maintaining graft viability and function.

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Interactive effects of vascular risk and β-amyloid burden on neocortical tau burden in clinically normal elderly


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Purpose: Cerebrovascular disease promotes cognitive decline in older individuals, both alone and in combination with Alzheimer’s disease (AD) pathology. However, whether cerebrovascular disease accelerates cognitive decline by directly promoting AD pathology remains unclear. Leveraging newly available positron emission tomography (PET) imaging, we examined whether a well-validated measure of systemic vascular risk predicts regional tau burden in combination with elevated β-amyloid (Aβ) burden in a sample of clinically normal elderly.

Materials & Methods: We acquired Aβ (11C-Pittsburgh Compound B) and tau (18F-Flortaucipir) PET imaging in 152 clinically normal older adults (mean age = 73.5±6.1 years). Vascular risk was quantified using the office-based Framingham Heart Study cardiovascular disease risk algorithm (FHS-CVD). Aβ-PET and FHS-CVD were performed at baseline; tau PET was acquired 2.98 years (SD±1.1) after study entry. We examined tau PET signal in the entorhinal cortex (EC), an early site of age-related tau accumulation, and the inferior temporal cortex (ITC), an early site of AD-associated neocortical tau deposition. Linear regression models examined Aβ burden and FHS-CVD as interactive predictors of tau PET signal, controlling for age, sex, APOE ε4 status, and the time interval between baseline and tau PET.

Results: We observed a significant interaction between FHS-CVD and Aβ burden in relation to the ITC (p < 0.001), whereby the combination of higher FHS-CVD and elevated Aβ burden predicted increased tau PET signal in this region. The interaction between FHS-CVD and Aβ burden was not significant in the EC (p=0.16).

Conclusion: Higher Aβ burden and FHS-CVD may synergistically promote neocortical tau deposition, representing a possible mechanism to account for the accelerated cognitive decline observed in individuals with both elevated amyloid and high vascular risk.

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Figure. Plots illustrate the predicted trajectories from the full regression model. For visualization purposes, low and high levels of Aβ burden are represented based on values at the 25th percentile and 75th percentile, respectively. The interaction was significant in the inferior temporal cortex (left panel), such that combined higher FHS-CVD and Aβ burden was associated with higher tau PET signal in the ITC. The effect was not significant in the entorhinal cortex (right panel).
Inner mitochondrial membrane protein Bcl-2 as a potential major player in autism and cancer
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Purpose: Previous published studies revealed marked overlaps between autism and cancer in genes, signaling pathways, and mitochondria. We used systems analysis approaches to examine these levels for possible common mechanisms that contribute to pathophysiology.

Materials & Methods: Genes in four categories—autism, cancer, mitochondrial localized, and signaling pathways—were examined. 990 Autism genes were downloaded from Gene Human Module of SFARI (Simons Foundation Autism Research Initiative), and 699 cancer genes from Cancer Gene Census. 1158 genes encoding proteins with strong support for localization in mitochondria were downloaded from MitoCarta2.0. Seven signaling pathways, Calcium, Wnt, MAPK, PI3K-Akt, mTOR, Ras and insulin signaling pathways were included in the study, chosen based on studies linking them all to both autism and cancer; the 981 genes that participate in these seven pathways were downloaded from KEGG Pathways. We then looked for overlaps among the genes from the four categories.

Results: Numbers of genes shared: Autism/cancer 101; mitochondria/ signaling pathways: 24; autism/mitochondria: 40; cancer/mitochondria: 25; autism/signaling pathways: 113; cancer/signaling pathways: 130. We noted that the group of genes shared between autism and cancer has 28 genes in common with signaling pathway genes, and 3 genes (BCL2, FHIT, and SND1) in common with mitochondrial genes. BCL2(B-cell lymphoma 2) is the only gene that is present in both the “genes shared between autism and cancer” and the “genes shared between mitochondria and signaling pathways” groups.

Conclusion: The number of mitochondrial localized genes (3 genes) shared between autism and cancer is much smaller than number of signaling pathway genes (28 genes) shared between autism and cancer. This smaller overlap may suggest a more specific role for mitochondria to autism or cancer. With BCL2 being the only gene shared by the “genes shared between autism and cancer” and the “genes shared between mitochondria and signaling pathways” groups, a potentially important role in autism/cancer mechanisms is suggested. Through identifying how, when and where BCL2 and other shared genes from different the different categories and grouping contribute to autism and cancer, we may increase our understanding of the causes and mechanisms of these conditions.

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Cell-specific drug delivery for the prevention of pulmonary fibrosis in vivo
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**Purpose:** The development of nanoparticle-mediated vectors for the cell-specific delivery of inhibitors of allow for the probing of the role of RhoA and its associated kinases in fibrosis. This study validates the utility of peptide-targeted nanomaterials for the targeted inhibition of pro-fibrotic pathways in cell types of interest.

**Materials & Methods:** The nanoparticles were prepared by W/O emulsion method where the polymers and hydrophobic drugs were dissolved in DCM/DMF and 2% PVA solution was added slowly with vigorous stirring. The stirring was continued for overnight at room temperature. PVA was removed by washing with water and centrifuge at 2000 rpm. This step was repeated for several times to remove PVA and as well as excess amount of CyAl5.5 conjugated polymer. The formulation was lyophilized for 2 days and characterized to measure particle size and drug loading content.

Fibroblast-specific peptide-targeted particles were synthesized using an emulsification technique, resulting in nanoagents ~80 nm in diameter and incorporating an inhibitor of myocardin related transcription factor (CCG-1423), a factor directly downstream of RhoA implicated in fibrosis, at 10% loading (w/w).

**Results:** In vitro, the resulting particles showed avid uptake by primary cardiac myofibroblasts, as compared to controls. Subsequently, in vitro inhibitory efficacy was examined using western blot analysis, demonstrating significantly decreased α-smooth muscle actin and connective tissue growth factor in the cells incubated with the targeted particles, as compared to non-targeted controls. The targeting ability of the particles have been examined in vivo in both the heart (myocardial infarction) and lung (pulmonary fibrosis) fibrosis models, and showed high specificity for the cells of interest. Investigations of in vivo therapeutic efficacy shows significant reduction of SRF, hydroxyproline and collagen in the lung tissue. We have also observed the survival rate for the targeting therapeutic particles treated groups is 90% in opposed of 50% for the control groups.

**Conclusion:** Nanoparticle-mediated delivery of inhibitors of pro-fibrotic pathways allows for the probing of these pathways in a cell-specific manner for the determination of their role in the initiation and progression of fibrosis. Additionally, the modularity of the nanoparticulate platforms permits the facile interchange of inhibitors or targeting ligands for the extension of this project to other areas of interest.

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EEG-Based Brain Age and Its Relation with Mortality
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Purpose: Aging constitutes one of the most pressing global issues. Markers of aging are urgently needed to understand aging mechanisms, to identify individuals at increased risk of age-related cognitive impairment and death, and to develop effective tools to improve brain health. Here, we test whether an electroencephalogram (EEG)-based biomarker of brain age independently predicts mortality.

Materials & Methods: Brain age was determined in adults ≥ 39 years old using machine learning analysis, developed on a large dataset of overnight sleep EEGs (N=1736) from the Sleep Heart Health Study. The brain age index (BAI) was obtained by subtracting chronological age from EEG-based brain age. Kaplan-Meier survival curves were used for graphical presentation of time to death, and log-rank statistics were used to assess difference by brain age index status. Cox proportional hazards survival analysis regression models were used to calculate adjusted hazard ratios for mortality.

Results: The average follow-up period was 11.9 years during which 8% (n=137) of subjects died. Mean BAI was significantly higher in the deceased group compared to the alive group [1.273 (s.d.= 7.024) years vs -0.109 (s.d.= 7.037) years, P<0.05]. Having a higher BAI score (i.e. brain age older than one’s chronological age) was significantly associated with increased mortality risk, even after adjusting for covariates (including age, sex, race, smoking status, body mass index, hypertension, diabetes, and education level) (adjusted hazard ratio [aHR], 1.032; 95% confidence interval [CI], 1.005-1.060). Each extra year of BAI (i.e. having an EEG-based brain age – chronological age of +1) yielded a 3.2% relative increase in the risk of death. Other variables significantly associated with mortality included current smoking (aHR, 2.804; 95% CI, 1.693-4.645), female gender (aHR, 0.586; 95% CI, 0.401-0.858), and education level (aHR, 1.305; 95% CI, 1.007-1.690).

Conclusion: Brain age index (BAI), a new EEG-based brain age biomarker, is an independent predictor of mortality, with higher BAI (i.e. an older brain) being associated with increased mortality. This helps to validate BAI as a novel biomarker of a subject’s brain health, and may hold important insights into the individual aging process and risk of death.

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Sialyl-Tn contributes to the pathology of ovarian cancer and is a potential therapeutic target.

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**Purpose:** Ovarian cancer (OVCA) is the leading cause of death from gynecological malignancies in the U.S. Unfortunately, patients often relapse with chemoresistant disease. Tumor associated carbohydrate antigens (TACA) are a class of cancer-specific targets found on the cell surface of many solid tumors. The TACA Sialyl-Thomsen-nouveau antigen (STn) is rarely expressed in adult tissues but it is expressed in various epithelial cancers including ovarian. Highly specific humanized STn antibodies were developed by Siamab Therapeutics, Inc. (Newton, MA) and conjugated to the antimitotic agent monomethyl auristatin E (MMAE) to generate a humanized anti-STn ADC. The efficacy and specificity of the anti-STn ADC antibody was assessed *in vitro* and *in vivo* to discern its potential as a therapeutic option for women with OVCA.

**Materials & Methods:** SKOV3 and ID8 +STn clonal cell lines were created by transfection of the pBABE_Puro-ST6GalNAc1, and pBABE_Puro, plasmid into wild type cells. Post transfection, cells were selected using 5 μg/ml Puromycin and clones were harvested to determine STn expression by flow cytometry. Anti-STn ADC efficacy was tested on the wild type OVCA cell lines Ovcar3, Ovcar4, OV90, SKOV3STn+, ID8STn+, as well as ST6GalNAc1 transfected clones, SKOV3STn+ and ID8STn+. Cells were either treated on day 2, after plating, and harvested on day 3 or treated on days 2 and 5 and harvested on day 6 for MTT analysis. Additionally, carboplatin resistance was assessed in ID8STn+ and ID8STn+ cells by treating them with 25 μM of carboplatin on days 2 and 5 then harvested on day 7 to measure cell proliferation. Indirect measure of OVCA cell proliferation was done using MTT assays in which cells were treated with anti-STn-ADC, Isotype-control ADC, or carboplatin; stained with 10% MTT solution; incubated at 37 °C for 3 to 4 hours; and the colorimetric change was read by a luminometer at 540 nM. Invasion potential of cells was determined using Matrigel transwells in which 75,000 cells were seeded in media without Fetal Bovine Serum (FBS) in the transwell and inserted into wells in media with FBS as an attractant, except for the negative control which lacked FBS in the well. At 72 and 96 hours, the cells in the wells were fixed and stained using crystal violet. Five fields at 4x were taken at random and the cells were counted using Image J software to determine the number of cells that passed through the transwell and attached to the well. For *in vivo* assessment of immunocompromised mice were subcutaneously injected with 2 different human PDX models (MTR41 and MTR152). Once tumors formed in these mice, they were treated with anti-STn-ADC, isotype-control ADC (each at 5 mg/kg, weekly), or vehicle. Tumors were harvested at the end of the study, processed, and analyzed for STn expression. Similarly, ID8 cells were subcutaneously injected into C57B16 mice to form tumors. Tumors, ascites, bone marrow, and spleen cells were harvested, processed, and assessed for STn by flow cytometry. Bone marrow and spleen from age matched mice were also harvested and assessed for STn as a control group.

**Results:** While there was no effect of anti-STn ADC treatment on STn low SKOV3WT cells *in vitro*, there was a dose-dependent decrease in MTT activity in Ovcar3, Ovcar4, OV90 and SKOV3STn+ cells. Mice hosting MTR41 tumors had a quick and robust response (50% decrease in tumor volume in ~4 weeks) to treatment compared to mice hosting MTR152 tumors which responded minimally in the same amount of time. Furthermore, flow cytometric analysis of the residual tumor cells revealed that mice treated with anti-STn ADC had fewer STn positive cells compared to isotype-control ADC treated mice in both PDX models. To better understand the functional aspects of STn in a syngeneic model the murine ovarian cancer cell line ID8 was used. ID8WT cells have <4% STn and STn levels were increased through stable expression of ST6GalNAc1 to produce the ID8STn+ cells. Utilizing the murine version of the anti-STn ADC, 3 and 6 day MTT assays showed a dose-dependent decrease in ID8STn+ cell viability compared to ID8STn- cells. Moreover, ID8STn+ cells had ~30% decrease in MTT activity after carboplatin treatment compared to ID8STn+ cells suggesting that increased STn levels promoted carboplatin resistance. Furthermore, ID8STn+ cells had increased migratory capability compared to ID8STn- cells in invasion assays. Remarkably, when ID8WT cells were injected into mice and allowed to form tumors, there was an inherent increase in STn levels (53%) compared to <4% *in vitro*. Lastly, select mice with the ID8WT tumors developed ascites which had 38% STn+ cells.

**Conclusion:** In summation, we have demonstrated specificity and efficacy of the humanized anti-STn ADC. This is important since elevated STn is associated with chemoresistance and metastatic potential in OVCA.

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Patient-clinician concordance in social mirroring circuitry underpins non-verbal communication and placebo analgesia in the context of pain treatment – a fMRI hyperscanning study

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Purpose: The patient-clinician relationship can powerfully shape patients’ clinical outcomes, but the brain basis for this is unknown, and psychosocial aspects of pain treatment have typically been studied in patients in isolation. We hypothesized that patient-clinician concordance in social mirroring circuitry, such as ventrolateral prefrontal cortex (vlPFC) and temporoparietal junction (TPJ), during pain treatment, supports non-verbal communication and placebo analgesia.

Materials & Methods: We simultaneously recorded functional Magnetic Resonance Imaging (fMRI hyperscanning) in 21 patient-clinician dyads (fibromyalgia patients and acupuncturists), who interacted via video transfer, during clinician-initiated treatment (real/sham electro-acupuncture) of patients’ evoked pain. Using MRI-compatible cameras, participants were enabled to communicate non-verbally throughout the scan.

Results: Patients’ pain, as well as clinicians’ vicarious pain, was decreased during both real and sham treatment compared to overt no-treatment (pain+treatment, relative to no-treatment). Furthermore, patients’ placebo analgesia (no-treatment–sham) correlated with clinicians’ perceived efficacy (no-treatment–sham). A conjunction analysis of brain responses of patients (receiving pain+treatment) and clinicians (providing treatment) demonstrated activation of vlPFC, aINS, and TPJ for both patients and clinicians. Using ROI extraction from the group vlPFC conjunction cluster, we found that the number of co-activated voxels (treatment–no-treatment) between patients and clinicians correlated with patients’ placebo analgesia, within dyads. Furthermore, patient-clinician concordance in right TPJ correlated with self-reported emotional expressiveness, frequency of eye-contact, and patients’ placebo analgesia.

Conclusion: In conclusion, our findings suggest increased patient-clinician concordance in vlPFC and right TPJ during pain treatment can support patient-clinician communication and ultimately placebo analgesia.

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**Immunoisolation and long-term function of human stem cell-derived β cells co-encapsulated with CXCL12 in alginate in a murine model of type 1 diabetes**

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**Purpose:** Functional insulin-producing β cells (SC-β cells) derived in vitro from human pluripotent stem cells represent a potential treatment for type 1 diabetes (T1D). To avoid systemic immunosuppression, alginate microencapsulation of islet-β cells is widely explored as a safe delivery vehicle for β-cell replacement. However, microencapsulated islet-grafts retrieved from patients have been characterized by pericapsular fibrotic overgrowth that causes islet cell death and graft failure. We investigated whether the immunomodulatory chemokine, CXCL12, could prevent the foreign body immune response that causes pericapsular fibrotic overgrowth and enhance long-term function of alginate-encapsulated SC-β cells without immunosuppression in a murine model of T1D.

**Materials & Methods:** We assessed the impact of CXCL12 on glucose-stimulated insulin C-peptide secretion (GSIS), β-cell gene expression and cytokine-induced apoptosis. We evaluated glycemic correction by SC-β cells co-encapsulated with and without CXCL12 in clinical grade alginate in pre-sensitized, immunocompetent streptozotocin-induced diabetic C57BL/6 mice. Microcapsules were recovered 154 days post-transplantation and analyzed for local immune responses, viability and differentiation of the SC-β cells.

**Results:** CXCL12 potentiated GSIS, induced β-cell function gene expression and reduced cytokine-induced apoptosis of SC-β cells. Diabetic mice implanted with SC-β cells co-encapsulated CXCL12 maintained euglycemia over 150 days, whereas those bearing encapsulated SC-β cells without CXCL12 reversed to hyperglycemia in less than 100 days (P<0.05). Phase contrast microscopy revealed dense pericapsular overgrowth on retrieved microcapsules without CXCL12. In contrast, microcapsules that incorporated CXCL12 showed little to no pericapsular overgrowth. Fluorescent microscopy revealed the presence of macrophages and myofibroblasts in the pericapsular overgrowths, reminiscent of the fibrotic response. Furthermore, only microcapsules that incorporated CXCL12 contained viable and differentiated SC-β cells, characterized by dual staining for the classical β-cell markers C-peptide and NKX6.1.

**Conclusion:** CXCL12 potentiates the function and survival of SC-β cells, and co-encapsulation of SC-β cells with CXCL12 in clinical grade alginate evades the fibrotic immune response and pericapsular overgrowth, resulting in long-term function and glycemic correction. This lays the groundwork for further preclinical translational evaluation of this novel approach in non-human primates.

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Obesity and Maternal Mortality in the United States
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Purpose: The United States has the highest maternal mortality ratio of all high-income countries in the world. Obesity has been associated with adverse pregnancy outcomes and increased maternal morbidity, and it has been proposed as a contributing factor to the increasing maternal mortality ratio (MMR). The objective of this study was to investigate the relationship between obesity on maternal mortality in United States.

Materials & Methods: We performed a quasi-experimental study using a panel dataset constructed from publicly available data from the Centers for Disease Control and Prevention (CDC). All 50 states and the District of the Columbia contributed observations to the dataset for each year between 2003 and 2015. The number of live births per year and the proportion of pregnancies by maternal age, race, and common comorbidities (hypertension and diabetes) were extracted from the CDC’s natality database for each state. Maternal deaths were identified in the CDC’s mortality database (ICD-10 codes O00-O99, A34). The proportion of women aged 18-54 who were obese (BMI ≥ 30) in each state was estimated from annual Behavioral Risk Factor Surveillance System survey-weighted data. We estimated the causal effect of obesity on risk of maternal mortality using a two-way fixed-effects Poisson regression model, adjusting for state and year fixed effects. Standard errors were adjusted for state-level clustering. Sensitivity analyses were performed, adjusting for maternal characteristics, socioeconomic characteristics of the state, and for the use of different death certificate versions.

Results: The average annual number of births per state varied widely, ranging from 6,252 to 526,752 (median 56,591). The median state obesity rate over the period was 24.3 (interquartile range (IQR) 21.9-27.1). The national average obesity rate increased by 0.75% each year between 2003 and 2015 (p<0.001). The state median MMR 19.7 per 100,000 births averaged over the period (IQR 14.4-27.1). On average, the national MMR increased by 1.15 per 100,000 births each year between 2003 and 2015 (p<0.001). For every percent increase in a state’s obesity rate, the risk of death increased by 6.4% (incidence rate ratio 1.064, 95% confidence interval 1.023-1.106). In the stepwise sensitivity analyses, the association between obesity rate and risk of maternal mortality remained significant after adjusting for maternal factors (IRR 1.057, 95%CI 1.026-1.088), extrinsic socioeconomic factors (IRR 1.047, 95%CI 1.021-1.074), and the adoption of the 2003 revised death certificate (IRR 1.039, 95%CI 1.019-1.061).

Conclusion: Using a model adjusting for year and state-level fixed effects, maternal mortality increased by approximately 6.4% for every percentage increase in a state’s obesity rate. The obesity epidemic is contributing to the increasing MMR in the United States. Policies directed at lowering state obesity rates among reproductive-aged women may result in reduced maternal mortality.

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

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**Purpose:** Schizophrenia, a debilitating psychiatric disorder affecting nearly 1% of the general population, has been demonstrated to have a substantial genetic component. While over a hundred common risk loci of small effect have been implicated, analyses of rare protein-coding variants have had limited success in identifying individual genes, presumably owing to power limitations.

**Materials & Methods:** Here, we present the Schizophrenia Exome Sequencing Meta-Analysis (SCHEMA) Consortium, a large global collaboration to aggregate, generate, and analyze high-throughput sequencing data of schizophrenia to advance gene discovery. Our study actively recruited from diverse global populations, and includes individuals of European, Latin American, East Asian, Ashkenazi Jewish, and African American ancestry. To date, we have sequenced and processed the whole exomes of 25,000 cases and 50,000 matched controls using a standardized protocol, yielding one of the largest sequencing data sets of a complex trait to date.

**Results:** We first show that schizophrenia cases carry a substantial excess of rare damaging variants in genes demonstrated to be under strong selection, with a notable enrichment in genes for broader neurodevelopmental disorders. We present the first gene-based burden results from our exome meta-analysis, and implicate at least two individual genes in which ultra-rare damaging coding variants confer substantial risk for schizophrenia. Finally, we present a new results browser that allows for easy viewing of identified variants and gene-based results.

**Conclusion:** In summary, we introduce the largest multi-center effort to aggregate sequencing data of a psychiatric trait, and the initial results from the harmonization and analysis of over 75,000 exomes.

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The role of peroxidasin in liver fibrosis development during NASH

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**Purpose:** NAFLD is a growing epidemic worldwide, which afflicts at least 17–33% of the US population. A subset of NAFLD patients will progress to non-alcoholic steatohepatitis (NASH), and this group is at risk for further progression to liver fibrosis. NASH results from chronic damage to the liver in conjunction with inflammation that leads to the accumulation of ECM proteins. These ECM proteins are stabilized by enzymes into a fibrotic scar. Peroxidasin (PXDN) is an ECM-associated enzyme that is known to stabilize ECM proteins through cross-linking of collagen molecules. In this project, we aim to investigate the expression of PXDN during the development of liver fibrosis in animal models.

**Materials & Methods:** In the first model of liver fibrosis, male C57BL/6 were fed either normal chow as control (n=3) or a choline-deficient, L-amino acid-defined, high-fat diet consisting of 60 kcal% fat and 0.1% methionine by weight (CDAHFD) for 12 weeks to induce NASH and fibrosis (n=3). In the second model of liver fibrosis, male C57BL/6 were injured with CCl4 by oral gavage to cause liver fibrosis after 12 weeks (n=3). All animals were sacrificed at 12 weeks. At the time of sacrifice, livers were harvested for pathologic and morphometric assessment of fibrosis and q-PCR analysis of Pxdn enzyme expression.

**Results:** In the CDAHFD model, we have demonstrated that steatosis in mice develops after 2 weeks followed by NASH with early stage fibrosis at 6 weeks, and NASH with advanced fibrosis by 12 weeks. In addition, we observed that Pxdn expression increased significantly as a function of fibrosis stage in the CDAHFD model similar to other markers of fibrosis including collagen Type 1, Alpha 1 (Col1a1) and alpha-smooth muscle actin (Acta2). In the CCL4 model, we observed that Pxdn expression increases in the fibrotic liver. In both models, PDXN staining was dramatically up regulated in and around the myofibroblasts in the fibrotic bands. Immunostaining of PXDN showed that it is present in the endoplasmic reticulum of human and mouse hepatic stellate cells and is increased during their activation to myofibroblasts.

**Conclusion:** This is the first report describing the expression of the PXDN enzyme during development and stabilization of liver fibrosis. We showed that activated stellate cells express and deposit PXDN in the fibrotic area in injured mouse liver. Our data indicates a potential role of PXDN in collagen crosslinking during liver fibrosis and we are now using Pxdn knockout mice to further explore its role in disease pathogenesis.

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Dietary Fat Quality and Genetic Risk of Type 2 Diabetes
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Background Type 2 diabetes (T2D) is a complex disease driven by lifestyle and genetic factors. The extent to which dietary fat quality may modify T2D genetic burden on the incidence of T2D is unknown.

Methods We used Cox proportional-hazards models to calculate adjusted hazard ratios (HRs) for T2D among 103,206 participants of European descent from 15 prospective cohort studies. T2D genetic risk profile was characterized by a 68-variant genetic risk score (GRS) weighted by published effect sizes. Diet was recorded using validated cohort-specific dietary assessment tools.

Findings During a median follow-up of 12 years, 20,451 participants developed T2D. The relative risk of T2D per increment of 10 risk alleles in the GRS was 1.68 (95% confidence interval [CI] 1.62-1.74). Increasing monounsaturated fat intake in place of carbohydrates was associated with a higher risk of T2D (HR per 5% of energy 1.08, 95% CI 1.02-1.15), while increasing polyunsaturated or total ω-3 fat intake in place of carbohydrates was associated with a lower risk of T2D (HR per 5% of energy 0.92, 95% CI 0.85-1.00; and HR per increment of 1g/d 0.95, 95% CI, 0.92-0.99, respectively). We did not observe evidence of significant interactions between dietary fat subtypes and GRS on the risk of T2D.

Interpretation In the present long-term prospective study including 103,206 participants, our results support that genetic risk profile and monounsaturated fat intake were each associated with a higher risk of T2D, whereas polyunsaturated fat intake was associated with a lower risk of T2D. Findings from this study suggest that dietary fat recommendations do not need to be tailored to individual T2D genetic risk profile for the primary prevention of T2D, and that dietary fat subtypes associate with the risk of T2D across the spectrum of T2D genetic risk.

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Comparing mean continuous blood glucose monitoring data within individuals wearing a bihormonal bionic pancreas or using conventional care
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Purpose: In our multi-center trial published in 2017, we showed that the bionic pancreas (BP) reduced the group mean for CGM glucose and the percent of time spent <60 mg/dl. We applied a new statistical approach to these data to parse out which individuals would benefit most from using the BP.

Materials & Methods: In the trial, 39 participants completed two 11 day study arms in random order during which they used the BP or their own insulin pump according to their usual care (UC). Subjects wore a CGM in both study arms. For the new analysis, data was binned into 60 minute blocks allowing for an hourly hypoglycemia rate to be calculated. For each patient we compared the average CGM glucose and average percent of time <60 mg/dl between the BP and UC arms using autoregressive time series models.

Results: Analysis of the data from population means showed that relative to UC, the BP was associated with a reduction in the mean glucose level (162±29 versus 141±10 mg/dl, p<0.0001) and reduced time <60 mg/dl (1.9±1.7% versus 0.6±0.6%, p<0.0001). Comparing each individual subject to themselves (FIGURE 1) showed 72% of subjects had a statistically significant reduction in mean CGMG, 51% had significant reduction in % <60 mg/dl, 44% had a significant reduction in both, and 97% had a significant reduction in at least one of those outcomes. In the six cases in which the mean CGM glucose was higher in BP than UC, the percentage of hypoglycemia was less in BP than UC and significantly less in 2/6 cases. In every case in which the CGM glucose mean was higher with BP than UC the BP mean was still less than 154 mg/dl. If subjects did not benefit more with the BP for average CGM it was only because they were below the target of 154 mg/dl in UC.

Conclusion: This comparison of BP and UC on a per subject basis provides new prospective on the efficacy of the BP system that can tell us which individuals could benefit most from using the BP.

Figure 4: Individual Analysis for the Multi-Center Trial Data Comparing UC to BP. Subjects are arranged on the X axis according to the increasing rank order of the difference between UC and BP. The X axis in the percent time spent less than 60 mg/dl is percentage and the x axis in the mean CGM data is displayed in mg/dL. 72% of subjects had a statistically significant reduction in mean CGMG, 51% had significant reduction in % <60 mg/dl, 44% had a significant reduction in both, and 97% had a significant reduction in at least one of those outcomes.

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Noninvasive optical monitoring of cerebral perfusion to predict the outcome of mechanical thrombectomy –Towards personalized acute stroke treatment

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Purpose: Stroke is a leading cause of mortality and long-term disability in the world. Ischemic stroke, responsible for 87% of all strokes, is caused by a clot obstructing the blood flow to the brain. A recent breakthrough in the management of ischemic stroke is advancement of mechanical clot removal from large arteries (mechanical thrombectomy) under contrast agent enhanced cerebral angiography. Mechanical thrombectomy is shown to have a significantly better outcome compared to conventional drug treatment at resolving large clots. However, a significant portion of patients with complete clot removal (recanalization) suffer from poor outcome and the mechanism behind it is not well understood. It is hypothesized that hyperperfusion is the main cause of poor outcome in patients with complete recanalization. This raises the importance of a noninvasive bedside blood flow monitor to quantitatively measure cerebral blood flow (CBF) during and after mechanical thrombectomy to detect possible hyperperfusion phases and treat the individual patient accordingly.

Materials & Methods: In this project, we aim to utilize diffuse correlation spectroscopy (DCS), a relatively novel optical technique that noninvasively estimates the blood flow, to continuously monitor CBF of 20 patients during mechanical thrombectomy under angiogram. DCS has been validated against gold standard imaging techniques (perfusion MRI, Doppler ultrasound, Xenon-enhanced computed tomography, etc.). We have developed an angiogram-compatible 3-D printed optical probe that attaches to the forehead skin using a piece of adhesive tape and delivers the light (785 nm) through the source fiber. The compatibility of the optical probe in the angiogram has been tested and verified by a team of interventional radiologist and neurosurgeons. The laser power is in the safe range, specified by American National Standards Institute (ANSI). The delivered light experiences multiple scatterings and penetrates to the scalp and skull and reaches the cortex. The diffused photons will be collected at 3 cm distance from the source by optical fibers that are coupled to single photon counting units. The blood flow can be estimated using the physical model of the speckle fluctuations of the diffused light due to the movement of scatterers (red blood cells).

Results: Data from one representative patient with right MCA (middle cerebral artery) stroke. Top- left panel, illustrates the ischemia in the MCA territory before mechanical thrombectomy procedure. The top-right confirms the complete re-canallization in MCA territory. The bottom panel shows the changes of blood flow has a large increase (reaching to more than 300% of baseline) after removing of the clot. The blood flow stabilizes after few minutes to values 140% of the baseline. The blood flow changes match the timing of the interventions and the images. These results indicate that DCS signal is sensitive to the cerebral perfusion and can provide a quantitative tool for cerebral perfusion monitoring during thrombectomy.

Conclusion: Our results demonstrate the feasibility of using DCS during thrombectomy procedure. The DCS monitor enables the surgeon to observe the blood flow changes in real-time and alarm the surgeon of the open vessel immediately after the clot removal. In current practice, after each attempt of clot removal the surgeon should inject a contrast agent and take angiogram images to examine the opening of the vessels. Moreover, we aim to correlate the values of optically measured blood flow with the outcome of therapy to better identify the settings that lead to poor outcome. The goal of these analysis is to develop certain indicators of the optimum hemodynamic changes that lead to the best therapeutic outcome towards a personalized treatment plan.

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Abstract Title: Sudden Cardiac Death Among Persons Living with HIV with Heart Failure without an Implantable Cardioverter defibrillator.

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Purpose: Heart failure (HF) is associated with an increased risk for sudden cardiac death (SCD). Persons living with HIV (PHIV) are at an increased risk of HF and, among PHIV without HF, the risk of SCD is increased 4-fold; whether the risk for SCD among PHIV with HF is increased is unknown. Therefore, the aim of this study was to characterize the risk of SCD among PHIV with HF.

Materials & Methods: We leveraged a single center academic registry of patients with HF to identify patients with and without HIV. Patients with an implantable cardioverter defibrillator (ICD) were excluded. The outcome of interest was SCD. Within PHIV, we tested the association of the traditional and HIV-specific factors with SCD. Sub-group analyses were performed by CD4 count and viral load (VL) and LVEF (<35%, 35-49%, ≥50%).

Results: Of 2,308 patients with HF, 1,982 (86%) did not have an ICD; of these, there were 315 PHIV and 1,667 uninfected controls. Of PHIV, 91% were prescribed an ART (mean CD4 count: 338±243 cells/mm³; 65% virally suppressed). Compared to controls, PHIV had higher baseline rates of coronary disease (CAD) and cocaine use, and a 4-fold increased rate of SCD (20 vs. 5%, p<0.001; median follow-up 19 months), an absolute rate of 13% per year of SCD. Among PHIV, CAD, cocaine use, low LVEF, absence of beta-blocker use, and HIV-specific parameters (CD4 count, VL) were predictors of SCD. Among PHIV, those with a detectable VL (and lower CD4) had higher SCD rates (43 vs. 8%; 33 vs. 8.3%, respectively, p<0.001 for both). Similar outcomes were observed by strata of LVEF.

Conclusion: PHIV with HF are at a markedly increased risk for SCD. This finding was consistent across strata of LVEF. Cocaine use and absence of beta-blocker prescription were predictors of SCD among PHIV.

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Figure:

Survival curves comparing sudden cardiac death among (A) PHIV and uninfected controls and PHIV with a CD4 count ≥200 cells /mm³, (B) PHIV and uninfected controls and PHIV with an undetectable viral load (<200 copies/ml), (C) PHIV with a CD4 ≥200 cells /mm³ vs. patients with CD4 count <200 cells /mm³, (D) PHIV with an undetectable viral load with those with a detectable viral load.
Radiomics of Coronary Artery Calcium in the Framingham Heart Study

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Purpose: Clinicians and Radiologists use relatively few metrics to quantify images. Presence of coronary artery calcium (CAC) is indicative of coronary artery disease and has been proven to add prognostic value in asymptomatic patients. The novel methodology in quantification of coronary calcium plaques features enables radiomics-based computed tomography (CT) imaging phenotyping and provides the opportunity to assess its ability to identify individuals at greater risk for adverse outcomes. Here, the objective of this study is to apply radiomics coupled with supervised machine learning in defining prognostic importance of CAC (measured by Agatston Score (AS) in CT-based phenotypes associated with adverse health outcomes.

Materials & Methods: An Asymptomatic community-based Framingham Heart Study (FHS) cohort whom underwent chest and abdomen CT imaging between 2002-2005 with median follow-up: 9.1(7.8–10.1) years. Participants are from the offspring and third-generation cohorts including total of 624 who had no known prior events and presented with CAC in CT. Subsequently, this cohort was randomly split into discovery and validation cohorts. CAC plaques were segmented and 2120 coronary computed tomography-based features were extracted via radiomics algorithms characterizing shape, first order statistics and textural differences of calcium plaques.

Results: The discovery and validation cohorts consisted of participants with average age of 58.1±11.1(210 (66.1%) men) and 59.2±11.2(188 (61.4%) men) years, respectively. Minimum redundancy maximum relevancy feature selection resulted in top 20 ranked features emphasizing on coarseness of plaque texture. Random Forest models calculated Radiomics scores (RS) per patient to have values of 0 to 1 with three tertiles: T1=0.0065-0.066, T2=0.066-0.108 and T3=0.108-0.71. As compared to the T1 reference group, radiomics scores in T2 and T3 remained robust in association with greater all-cause mortality risks after adjustment for traditional Framingham risk factors in validation cohort (T2:hazard ratio (HR)=8.9, p=0.04, T3:HR=17.2, p=0.005). Radiomics score combined with Agatston score (AS) had a significant incremental value to AS in prediction of events in participants with AS<300 (p=0.03).

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Figure 1. Comparison of performance between Agatston score (AS), Radiomics Score (RS) and the Combined (AS+RS) scores for participants <300.
Traumatic Auditory and Vestibular Injury Following Head Injury: A New Take on an Old Diagnosis

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Purpose: Inner Ear ‘concussion’ has long been recognized as one of the possible consequences of head injury. While hearing loss and vestibular dysfunction in the setting of head trauma with concurrent temporal bone (TB) fracture is generally well described, less is known about their pathophysiology due to head injury without TB fracture, such as following mild traumatic brain injury (TBI). Dating back to the descriptions of boxers afflicted with Punch Drunk Syndrome in the 1920s, later known as dementia pugilistica, athletes involved in contact sports that sustain mild head injury, such as concussions, have routinely indicated symptoms of audiovestibular dysfunction. Moreover, military personnel and civilians also commonly report tinnitus and dizziness following head injury. Taken together, while hearing loss and vestibular dysfunction secondary to head injury without temporal bone fracture is a recognized clinical phenomenon, the precise mechanism remains unknown and under-investigated. Herein, we hypothesize that 1) auditory and vestibular symptoms following head injury are associated with decreased quality of life (Aim 1); and, 2) pathological changes occur in the cochlea and the peripheral vestibular organs following head injury (Aim 2).

Materials & Methods: For Aim 1, individuals with a history of mild traumatic brain injury and audiovestibular symptoms were prospectively evaluated at a tertiary care rehabilitation hospital using validated patient reported outcome measures: Hearing Handicap Inventory for Adults (HHIA), Tinnitus Handicap Inventory (THI), Dizziness Handicap Inventory (DHI), and 36-Item Short Form (SF-36) survey. SF-36 scores were converted into health utility values (HUV) using a validated algorithm, where 0.3 is defined as poor health and 1.0 is defined as perfect health. For Aim 2, TBs of separate cohort of patients with a history of head injury without TB fracture were evaluated by light microscopy. In auditory system, the evaluation included counts of spiral ganglion cells (SGC) with age-matched controls, as well as quantification of hair cells (HC) and pillar cells. In vestibular system, otopathologic evaluation included counts of Scarpa ganglion cells (ScGC) in the vestibular nerves with age-matched controls, vestibular HC and/or dendrites degeneration in vestibular end organs. The presence of cochlear and/or vestibular endolymphatic hydrops and obstruction of the endolymphatic duct were also assessed.

Results: In the prospective clinical study, twenty-five consecutive met inclusion and exclusion criteria. The average age was 52.6 ±13.6 and 36% were female. The most common symptoms reported were hearing loss (84%), dizziness (80%), tinnitus (56%) and hyperacusis (52%). Mean HUV was 0.61 ±0.10. HUV was negatively associated with HHIA scores (Pearson’s correlation r = -0.42, p=0.036) and DHI scores (r = -0.65, p<0.001). THI was not associated with decreased HUV (r =-0.19, p=0.37). For pathologic study, individuals with a history of head injury had an average of 65% of SGC loss (range: 43%-84%) compared to controls. Two of the six (33%) cases had severe loss of HC and pillar cells along the length of the cochlea, and four of the six (67%) cases demonstrated moderate-severe loss at the basal turn of the cochlea. In the vestibular system there was an average of 46% of ScGC loss (range 44 to 60%) compared to historical age-matched controls, and 50% of TBs (n=3/6) had degeneration of HC and/or dendrites of vestibular-end organs. Additional findings include cochlear and/or vestibular hydrops in three of the six (50%) TBs, and blockage of the endolymphatic duct (17%, n=1 TB).

Conclusion: Patients with a history of head injury demonstrate significant inner ear symptoms that is associated with decrease in healthy utility and quality of life. Otopathologic analysis in patients with history of head injury without TB fracture demonstrated peripheral cochlear and vestibular otopathology. Findings have implications for mechanism of peripheral cochlear and vestibular pathology in patients following head injury.

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Supercooling of Human Livers to Extend the Preservation Time for Transplantation

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Purpose: Optimizing preservation of donor organs has the potential to dramatically improve the outcome of organ transplantation by diminishing the donor organ shortage, enabling near perfect global HLA matching, permitting recipient immune tolerance induction, and allowing transplantation in an elective surgical setting. For livers, the current preservation standard, static cold storage at +5°C (SCS) allows for a maximum preservation time of 12 hours. Recently, it has been shown that rat livers can be preserved in a supercooled state at -6°C for 3 days with 100% survival after transplantation. The main goal of this study is to translate this supercooling protocol to human livers. We hypothesized that preservation duration of human livers can be extended from 12 to 24 hours, by storing the organ at -4°C in an ice-free, supercooled state, followed by recovery with sub-normothermic machine perfusion (SNMP).

Materials & Methods: Human livers, rejected for transplantation, were recovered from conventional SCS using SNMP before they were loaded with the cryoprotectant agent (CPA) 3-O-methylglucose (3OMG) using SNMP. Subsequently, the liver was gradually cooled to 4°C and flushed with oxygenated UW supplemented with the CPAs polyethylene glycol (PEG), glycerol and trehalose. The livers were supercooled and stored for 20 hours at -4°C. After supercooling the CPAs were washed out and the livers were recovered during 3 hours of SNMP. Pre- and post-supercooling SNMP conditions were identical. Vascular resistance, blood gas parameters, electrolytes, urea, liver enzymes and bile production were measured every 30 minutes during pre- and post- supercooling SNMP. Bilateral wedge biopsies for the measurement of mitochondrial energy charge and conventional histology were also sampled throughout the protocol.

Results: Mitochondrial energy charge, an important marker for transplant success, was similar pre- and post-supercooling. Also, we observed no difference between arterial resistance and oxygen consumption comparing pre- and post-supercooling SNMP. Viable livers produced bile pre-supercooling and continued bile production post supercooling. Similarly, histology shows minimal necrosis, no edema and limited endothelial injury. We also measured common markers of liver damage, such as ALT and AST, which were the same pre and post supercooling.

Conclusion: Preliminary results demonstrate the feasibility of storing whole human livers in the supercooled state to double the preservation duration as compared to clinical standards. Moreover, we identified important bio-stabilizing agents and perfusion conditions which are critical for success.

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**Proton therapy for brain tumors: how to measure where the protons stop**

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**Purpose:** At the Francis H. Burr Proton Therapy Center of MGH, cancer patients are irradiated with protons accelerated up to 230 MeV by an isochronous cyclotron. These stop at a certain depth, the proton range, and deposit no dose behind. This is very helpful to spare normal tissue and organs at risks in the vicinity. However, there are inherent uncertainties in the calculated stopping point, which prevent from taking full advantage of proton therapy. To counteract this limitation, a prompt gamma-ray spectrometer is under development, which aims at measuring the proton range during the patient treatment based on the detection of prompt gamma-rays, a by-product of the proton irradiation. The ultimate goal is to improve quality assurance, increase the precision of the beam delivery, so that patients can benefit from lower dose and side effects in healthy tissue.

**Materials & Methods:** Eight scintillation detectors detect the energy and time of prompt gamma-rays, emitted in nuclear interactions between the accelerated protons and nuclei of the tissue. As seen in the right image, this gamma-ray camera is placed in front of the patient, perpendicular to the beam axis. It is mounted on a motorized frame that rotates according to the beam incidence angle. A tungsten collimator in an open slit configuration is used to obtain the prompt gamma-ray emission density map.

The system is characterized with phantom experiments at the gantry treatment room of the proton center. A field of 2 Gy is delivered to a water and a polyethylene phantom, with a proton beam current of 2 nA at nozzle exit. Heterogeneities are inserted in half of the irradiated field to simulate range shifts. The trigger rate of each detector ranges up to 1 Mcps and the pile-up fraction is about 15%. Thus, the application of dead time and pile-up correction factors is mandatory.

The detected gamma-rays include background events due to neutron interactions and activation. The time structure of the prompt gamma-rays is exploited to subtract the background from the actual signal. Then, the energy spectrum is fitted to obtain the magnitude of the different gamma-ray lines (with discrete energies) and compared with a Monte Carlo simulated model. The intensity ratios between the different lines is used to calculate the proton range and elemental tissue composition. This information can be used for verifying that the proton range is as expected according to the treatment plan, or to detect potential range deviations due to changes in the patient anatomy or patient misalignment with respect to the beam.

**Results:** The measured proton range is measured with a statistical precision (90% confidence level) of 2 millimetres for a beam spot with 4x10^8 protons. The absolute accuracy yields 1 mm. Range shifts of 5.2 and 2.3 mm in half of the field are successfully detected. Differences in material composition (oxygen and carbon concentration) are identified, too.

**Conclusion:** At the Francis H. Burr Proton Therapy Center of MGH, a camera prototype based on prompt gamma-ray spectroscopy is under development to measure the proton range during tumor treatment. Phantom experiments have been conducted to test the camera performance prior to the application in actual patient treatments. The results show a precision and accuracy of the measured range of few millimetres, which encourages a first clinical study and opens up the possibility of enhanced precision in beam delivery, and potentially, reduced dose and toxicity in healthy surrounding tissue.

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Deep Learning Pseudo-CT Synthesis for Pelvis PET/MR Attenuation Correction

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**Purpose:** Accurate attenuation correction (AC) for whole-body (WB) applications, particularly in the pelvic area, continues to be the most important methodological challenge in the PET/MR field. In the commercially available PET/MR scanners, MR data acquired using a Dixon-VIBE sequence (18 sec) are used to generate segmented attenuation maps (μ-maps) including a limited number of tissue classes (background, fat, lung and soft tissue) with uniform attenuation properties. Bone tissue is currently misclassified as soft tissue or air. We propose a Deep Learning (DL) approach to synthesize a pseudo-CT to be used for PET/MR AC based only on the standard Dixon-VIBE images.

**Materials & Methods:** MRI-CT-PET matching data sets from 19 patients were used in this study. Preprocessing of the images included: i) MRI bias correction, ii) intra-subject registration, and iii) Reslicing of the images to a fixed FOV. Figure 1 provides a schematic representation of our convolutional-deconvolutional network, using the 4 standard Dixon-VIBE images (in phase, out of phase, fat and water) as inputs to generate the corresponding pseudo-CT slice. As accurate bone synthesis is critical, we included a mask to increase the importance of bone voxels in the loss function. The Hounsfield units of the pseudo-CT and resliced CT images were converted to linear attenuation coefficients (LACs) to generate the corresponding μ-maps: \( \mu_{DL} \) and \( \mu_{CT} \), respectively. Additionally, the μ-maps generated using the method currently available on the Biograph mMR scanner (\( \mu_{Dixon} \)) were also available for comparison. AC factors in sinogram space were generated from all these μ-maps and used to correct the PET data for the validation subjects.

**Results:** We trained the model using fifteen subject datasets and computed the pseudo-CT for the remaining four subjects. Figure 2 shows the MR-based μ-maps fused with the PET images and the relative change (RC) maps for one representative subject. The DL method showed a statistically significant improvement over the Dixon method (paired t-test, \( p<0.05 \)).

**Conclusion:** We present a DL method for AC that avoids the over-simplification of most of the previously proposed approaches. The DL implementation allows for real-time pseudo-CT synthesis, making this approach suitable for clinical use. Our preliminary results suggest that the DL-based pelvis μ-map is more similar to the CT-based one than that obtained using the Dixon-based method. The bias was reduced when compared to approach currently used for clinical PET/MR studies. Additionally, it could be used to retrospectively perform AC using the existing Dixon-VIBE data. Further improvement of the current model can be achieved by increasing the number of training sets and fine-tuning the bone and weight parameters.

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