omics analysis furthers explore local regulatory networks in pursuit of novel drug targets of ES. METHODS/STUDY POPULATION: In our pilot, eight induced pluripotent stem cell lines were obtained, differentiated into neural crest cells, and then transduced with a lentivirus expressing GFP-2A-EWS/FLI1. We compared wild type (WT) to EWS-FLI1-induced cells and then compared cell survival, gene expression, and EWS-FLI1 binding differences at varying levels of EUR / AFR ancestry admixture. We will build on this pilot data by expanding the number of cell lines and measuring chromatin state. Subsequently we will refine our understanding of the relationships between local ancestry, epigenetic and gene expression changes, and phenotype in tumor progression via integration of multi-omics datasets. Our systems genomics approach will utilize directed local regulatory networks in a Bayesian structure learning framework. RESULTS/ ANTICIPATED RESULTS: Induction by EWS-FLI1 resulted in gene expression changes enriched in known ES gene sets. Higher %EUR ancestry correlated with prolonged maintenance of EWS-FLI1. We identified thousands of ancestry-linked changes to gene expression and EWS-FLI1 binding. Eighty of these genes are both differentially expressed and differentially bound based on AFR ancestry admixture level and may be some of the early critical targets that initiate the cascade of molecular changes in ES. We will identify novel drug targets, with potential cross functional use of known drugs. Once we have developed directed local regulatory networks, we will use them to test in silico potential perturbations due to small molecules or novel drugs and predict expression changes. DISCUSSION/SIGNIFICANCE: With a limited number of cell lines, we identify 80 ancestry-linked candidate loci for functional validation through genome engineering. As EWS-FLI1 itself has proven elusive to direct targeting, studying its immediate downstream effects has the potential for establishing new druggable biologic pathways for treatment of ES.

256

Addressing the Underdiagnosis of Familial Hypercholesterolemia

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OBJECTIVES/GOALS: Familial Hypercholesterolemia (FH) is a common disorder that is vastly underdiagnosed and causes an increased risk for sudden cardiac death. Cardiology providers (CHCPs) are in an ideal position to care for patients with FH. This research aimed to understand the practice behaviors of CHCPs in the screening, diagnosis, and management of FH. METHODS/STUDY POPULATION: An explanatory mixed methods design was utilized for this study. Adaptation of an existing FH knowledge tool guided survey development. The results of the quantitative survey, along with the Knowledge to Action framework and Theory of Planned Behavior, guided development of the interview protocol. Convenience and snowball sampling recruited CHCPs in the Division of Cardiology at Columbia University Irving Medical Center (CUIMC). Descriptive statistical analysis was performed on survey data. Qualitative interviews were conducted with survey respondents who volunteered to participate. Interviews were audio recorded, transcribed, and analyzed thematically. A descriptive review of the educational materials offered by the Division of Cardiology was conducted to identify FH knowledge domains presented. RESULTS/ANTICIPATED RESULTS: CHCPs with MDs, at CUIMC for 6-10 years, in clinical practice for 1-5 years, and in Inpatient Services had the highest average total FH knowledge scores. CHCPs with RNs, at CUIMC for less than 1 year, in clinical practice for 6-10 years, and in Cath Lab had the lowest average FH knowledge scores. Twenty interviews were completed, and four themes emerged- variability in FH care; issues related to addressing FH at institutional, practice setting and individual levels; importance of identifying FH early; and intervention approaches to overcome barriers to caring for FH patients in cardiology. CHCPs with MDs or with experiential FH knowledge were the only CHCPs to describe FH care beyond the point of screening. The document review revealed that only MDs were provided four lectures over the course of 4 years pertaining to FH. DISCUSSION/SIGNIFICANCE: CHCPs with didactic or experiential FH knowledge provided care beyond screening. Future interventions should increase didactic and experiential FH knowledge by incorporating institutional, local, and national FH resources. Improving the FH care CHCPs provide, can reduce FH-related morbidity and mortality as well as improve FH health outcomes.

258

Analysis of the Hepatic Microenvironment Before and After Direct-Acting Antiviral (DAA) Therapy for Viral Hepatitis C

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OBJECTIVES/GOALS: The effect of the Direct-Acting Antiviral (DAA) on hepatic histopathological features from patients treated for HCV has not been thoroughly evaluated. The goals of this retrospective study were to determines differences between the liver biopsies collected before and after DAA treatment and correlated the histopathology with clinical outcome. METHODS/STUDY POPULATION: Spectral imaging was used to evaluate differences in intrahepatic macrophage (CD68, CD14, CD16, MAC 387, and CD163) and T cell (CD3, CD4, CD8, CD45, and FoxP3) phenotypes in paired liver biopsies collected from the same patient before (n=10) and after (n=10) achieving SVR (Figure 1). Imaging analysis and machine learning algorithms were used to evaluate changes in these key immune cells. We also compared differential gene expression of over 700 genes using RNA isolated from liver biopsies with NanoString. RESULTS/ANTICIPATED RESULTS: Multispectral imaging analysis showed a significant increase of proinflammatory/M1-like (e.g., CD14+) and anti-inflammatory/M2-like macrophage (e.g., CD163+) phenotypes in pre-treatment versus post-treatment biopsies, respectively. Gene expression analysis revealed enrichment of inflammatory (HLA-B, STAT1, CXCL10) and interferon induced-antiviral (ISG15, OAS3, MX1 and IFIT1) genes in the pre-treatment vs the post-treatment group. Cell deconvolution analysis also showed a significant increase of M1-like macrophages in the pre-treatment group when compared to the post-treatment group or controls. Upregulation of genes associated with cell proliferation and differentiation (c-KIT and Fos) was observed in the post-treatment biopsies of patients with persistent inflammatory infiltrates. DISCUSSION/SIGNIFICANCE: Protein and gene expression

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profiles observed in patients before DAA therapy showed a strong macrophage-mediated inflammatory response against HCV infection in the liver that shifted significantly to a tissue remodeling microenvironment after treatment.

260

Breast Cancer Biopsy Triage with Ex-Vivo Microscopy for Downstream Analysis*

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OBJECTIVES/GOALS: In this study, the ability of a pathologist to detect malignancy on digital pseudo-H&E slides obtained via structured illumination microscopy (SIM) imaging of fresh diagnostic breast biopsies was assessed. The speed of imaging and processing was also assessed for potential clinical implementation. METHODS/STUDY POPULATION: This study was conducted in accordance with an Ochsner Medical Center of New Orleans IRB. 200 patients undergoing either stereotactic or ultrasound-guided diagnostic breast biopsies were consented and an additional core from the suspicious lesion was collected for research use. Research biopsies were transported to the lab and stained with DRAQ5 and Eosin-Y and imaged with SIM before being submitted for histology processing. Imaging and digital processing times were recorded. The resulting SIM images and histology slides were given to a pathologist for blind review to assess accuracy. RESULTS/ANTICIPATED RESULTS: The ex-vivo structured illumination microscopy images and subsequent histology slides from 79 research cores have been assessed to date. Some samples were excluded from the total data set and not included in the final assessment due to technical failures of the imaging protocol. Of the current set, the pathologist has a specificity of 88% and a sensitivity of 65%, as well as an NPV of 88% and a PPV of 65%. Staining time for each biopsy was completed within 3 and a half minutes and imaging at 20x magnification took between 4 and 12 minutes, depending on size and implementation of autofocus to the imaging system. Image processing took approximately 5 minutes per biopsy and is a direct function of biopsy size. DISCUSSION/SIGNIFICANCE: Decreased time between cancer suspicion and treatment will improve the prognosis of breast cancer patients. SIM imaging of fresh breast biopsies could ultimately allow primary and secondary histology to be performed simultaneously and minimize histopathology time, thus allowing clinicians and patients to implement treatment course more quickly.

261

Characterization of Glycosylation Patterns of Single IgA Molecules Using Single-Molecule Fluorescence Microscopy

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OBJECTIVES/GOALS: IgA1 nephropathy, which can lead to kidney failure, is caused by complexes formed between aberrant galactose-deficient IgA1 and antibodies directed against it. Our goals are to characterize shifting glycosylation patterns at the level of single IgA1 molecules and to apply this to patient samples for early detection and understanding of the disease. METHODS/STUDY POPULATION: To characterize glycosylation patterns on single IgA1 molecules, labelled IgA1 in low concentration was physisorbed to borosilicate glass in a fluidic cell and labelled Jacalin was flowed in to bind with Glycans on IgA1. The samples were observed with a Nikon TiE epi-fluorescence microscope. FRET images were created by exciting the Jacalin dye with a blue laser and recording the red emission of the IgA1 dye with an EMCCD camera. FRET emission intensities of individual IgA1 molecules over time were analyzed to determine how frequent and how long Jacalin binds to each of them. The rate of binding is roughly inversely proportional to the amount of abnormal glycans on a given IgA1 molecule. After the method is perfected, we intend to compare the glycosylation patterns of healthy and diseased patient samples. RESULTS/ANTICIPATED RESULTS: Addition of the competitive binder Galactose to the solution led to an increase in the off times of Jacalin and IgA1 and a decrease in the on times in a concentration-dependent manner, yielding an increase in the dissociation constant. Dissociation constants of individual molecules within a single experiment vary by 3 orders of magnitude, which cannot be attributed to stochastic fluctuations but rather reflects differences in the adsorption geometry. Nevertheless, the unaffected dissociation constant can be identified. We expect that when this method is applied to samples from healthy and IgA1 Nephropathy patients, specific IgA1 molecules from patients will have higher dissociation constants for Jacalin compared to those from healthy patients. DISCUSSION/SIGNIFICANCE: The binding rates of Jacalin to single IgA1 vary by 3 orders of magnitude. The observed heterogeneity shows the Jacalin probe can differentiate between different IgA1 populations. An understanding of which IgA1 molecules in patient samples are problematic and what their distribution of Glycans is can lead to discovering biomarkers and treatments.

265

Connecting Electronic Medical Record Sub-phenotypes of Obstructive Sleep Apnea to Adverse Outcome Risks

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OBJECTIVES/GOALS: Prior work has established subtypes of OSA and linked them to risks of future adverse events but rarely with the longitudinality and richness of data available in the EMR. Our goal is to leverage EMR data identify clinically meaningful sub-phenotypes of OSA and better study how they affect risks of adverse outcomes. METHODS/STUDY POPULATION: Vanderbilt's EMR database has over 61,000 adult patients with a literature-validated EMR definition of OSA with a median EMR follow-up period of 4 years after OSA diagnosis. Of these patients, 12,516 have fully recorded sleep study data in addition to EMR variables such as age at study and most recent BMI. We applied several clustering methods including to identify natural sub-phenotypes of OSA and assessed cluster quality. We also applied techniques which allow a single patient to belong to multiple clusters in various degrees. After selecting final clusters, we plan to analyze the associations between OSA sub-phenotypes and risks using statistical tools like logistic regression and Cox proportional hazards regression, with and without adjusting for factors such as age, gender, and certain medications. RESULTS/ANTICIPATED RESULTS: Preliminary clustering with primarily sleep study data has shown overlap with literature-described patient clusters, including a severe, high non-REM stage 1 sleep, high BMI cluster and a high

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