activation following steroid therapy. Although steroids play a significant role in regulating the amount of inflammatory damage that occurs during IBD treatment, our data suggest that they may be limiting pathways required for effective healing as well.

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Successful hand function recovery after stroke

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OBJECTIVES/SPECIFIC AIMS: Upper-extremity (UE) impairment affects 88% of stroke survivors due to dysfunctional shoulder-hand coordination. Patients may be able to grasp with the arm at rest, but unable to grasp in a functional context (eg, from a high shelf) because shoulder use elicits involuntary hand muscle activity. Further, much rehabilitation research is directed at unsuccessful stroke recovery (patients with persistent UE impairment) but very little towards patients who show successful clinical recovery (such as those with mild UE impairment) even though these patients have attained the desired rehabilitation outcome. We examined the neurophysiological trajectory of successful compared to unsuccessful post-stroke recovery in the context of functional UE movements to clearly identify what factors are necessary for successful recovery of functional UE movements after stroke. METHODS/STUDY POPULATION: We studied 3 populations: (1) mildly-impaired patients, early (at <17 d, 30 d, 90 d, and 180 d) after stroke as a model of successful post-stroke recovery, (2) moderately-impaired, chronic patients (>6-months post stroke) with persistent hand function impairment, as a model of incomplete post-stroke recovery (unsuccessful recovery), and (3) Healthy age-range matched controls. We used transcranial magnetic stimulation (TMS) in all 3 groups at the given time points to measure corticomotor excitability (motor evoked potentials, recruitment curve), corticomotor inhibition (short-interval intracortical inhibition, long-interval intracortical inhibition), and intracortical facilitation of hand muscles with the shoulder positioned in different degrees of flexion or abduction (these shoulder positions are known to elicit involuntary, undesired hand muscle activation, which leads to UE dysfunction and impairment in individuals with stroke). RESULTS/ANTICIPATED RESULTS: Data collection are in process and will be presented. Preliminary data from controls shows that corticomotor excitability of selected hand muscles is affected by changes in shoulder position. Preliminary findings in controls are consistent with clinical findings in stroke that certain shoulder positions elicit involuntary and undesired hand muscle activation, leading to UE dysfunction and disability. Findings from the stroke groups will be presented. DISCUSSION/SIGNIFICANCE OF IMPACT: We hypothesize that this centrally-facilitated coupling between shoulder and hand muscles is disrupted after stroke, which may play a central role in the inability of patients to perform functional UE movements. By comparing the TMS metrics in mildly-impaired Versus moderately-impaired chronic patients, we will be able to identify the longitudinal change in neurophysiology underlying shoulder-hand coordination that is associated with successful or unsuccessful clinical recovery of UE function after stroke. Thus, these findings will help us distinguish between the neurophysiology underlying successful from unsuccessful UE recovery leading to more mechanism-based interventions for UE dysfunction post stroke in the future.

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Enumeration of circulating tumor cells for monitoring cancer treatment response

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OBJECTIVES/SPECIFIC AIMS: The goal of this research is to use circulating tumor cells (CTC) enumeration and characterization to monitor anticancer treatment response. Emerging evidence strongly suggests the implications that epithelial-to-mesenchymal transition may have in cancer metastasis. Consequently, we hope to elucidate the significance of mesenchymal and stem-like CTCs in the peripheral blood of metastatic pancreatic cancer patients by analyzing the prevalence and frequency trends of CD133 + CTCs, as they relate to clinical events. We also hope to determine if there is a correlation between EpCAM + CTCs and CD133 + CTCs numbers with tumor size, disease stage, and patient clinical outcome. METHODS/STUDY POPULATION: Blood samples of patients with metastatic pancreatic cancer (stage IV) were obtained from the University of Florida Health Cancer Center after informed consent through an IRB-approved protocol. CTC capture, characterization, and enumeration was performed on the blood of these cancer patients during their anticancer treatment. Patients had blood drawn for this purpose at time points aligned with clinical phlebotomy (every 2 weeks). CTC capture was performed by introducing treated patient blood samples into antibody-functionalized microdevices. The PDMS devices were functionalized by immobilizing either anti-EpCAM or anti-CD133, through an avidin-biotin complex. After capture, cells were fixated and permeabilized with 4% paraformaldehyde and 0.2% Triton X-100, respectively. Three-color immunocytochemistry (anti-cytokeratin-FITC, anti-CD45-PE, and DAPI) was performed to identify CTCs from nonspecifically captured blood cells. To be counted as a CTC, based on the FDA-approved technical definition, a cell with the appropriate cell size and morphology must be nucleated (DAPI+), express cytokeratin (CK+), and lack the leukocytic CD45 marker (CD45−). RESULTS/ANTICIPATED RESULTS: We tested the clinical utility of the device for monitoring the response of patients with advanced pancreatic cancer to a chemotherapy treatment consisting of anticancer drugs including 5-fluorouracil, leucovorin, oxaliplatin, and dasatinib. We have detected EpCAM + CTCs in 47/47 (100%) and CD133 + CTCs in 41/47 (87.2%) of blood samples, coming from a cohort of 16 patients. We studied the correlation between the CTC numbers and the clinical result of patients in the study. We found that the size and changes in the size of the primary tumor (confirmed by CT scans) correlated with the frequency and increase/decrease trends in the number of CTCs detected. We expect to find some relationship between the number of detected CD133 + CTCs, or rather stem-like CTCs, and the clinical outcome of patients (eg, disease progression leading to withdrawal from study). DISCUSSION/SIGNIFICANCE OF IMPACT: Enumeration of patient CTCs and stem-like CTCs at different stages of a patient's treatment may correlate with disease stage and prognosis, and prove useful in monitoring early recurrence, patient-specific treatment response, and newly acquired resistances; all of which would aid in providing guidance for the next step in clinical intervention. This type of liquid biopsy technology has great potential to make an impact in the future of personalized medicine and point-of-care diagnostics, as well as become a sturdy tool for translational research.

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Defining critical features of the immune microenvironment in melanoma using multiplex immunohistochemistry and spatial analysis

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OBJECTIVES/SPECIFIC AIMS: Precise biomarkers are urgently needed to characterize the tumor immune microenvironment in primary melanoma tumors both for prognostication and to predict the benefit of immunotherapeutic intervention. The goal of this work is to define spatial relationships between CD8 + T cells, CD68 + macrophages and Sox10 + melanoma cells in order to define features correlating with prolonged survival METHODS/STUDY POPULATION: Five micrometer slides from either the primary biopsy or subsequent wide local excision procedure were stained using Opal multiplex IHC for DAPI, CD3 (LN10, Leica), CD8 (4B11, Leica), CD68 (KP1, Biogenex), SOX10 (BC34, Biocare), HLA-DR (LN-3, Abcam), and Ki67 (MIB1, Abcam). Cell phenotypes within representative fields preselected by a trained dermatopathologist and were visualized using the Mantra quantitative pathology workstation (PerkinElmer), and analysis of spatial distribution of CD3+ CD8+ cells analyzed using inForm® image analysis software (PerkinElmer), and Spotfire software (TIBCO). In order to test whether mIHC can better characterize the tumor immune microenvironment, we screened databases at the Herbert Irving Cancer Center (HICC) at Columbia University for stage II/III melanoma patients diagnosed between 2000 and 2012, with available FFPE of primary melanoma tissue and documented clinical follow-up. We identified a preliminary population of 57 patients to begin our analysis. Clinical follow-up was available on 35 patients of whom 21 patients were alive with no evidence of recurrence or died with no evidence of recurrence and 14 had died of melanoma. Twenty-four patients had more than 24 months of survival information available but no detailed clinical information to determine cause of death. RESULTS/ANTICIPATED RESULTS: First, we evaluated whether density of immune cells in tumor and stroma predicted prognosis in 35 patients with disease specific survival information. We find that high number of CD3 + CD8 + cells in tumor correlates with Disease Specific Survival (DSS) ($p = 0.0323$*) and CD3 + CD8 + cells in stroma may also correlate with DSS ($p = 0.0671$). This is consistent with what is known in the literature regarding tumor infiltrating lymphocytes (TILs). We also found that CD68 + cells in stroma predict poor prognosis (0.0259*). This is consist with the proposed

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deleterious role for macrophages in tumor progression. Next, using nearest neighbor analysis we examined the effect of HLA-DR and Ki67 expression on spatial distribution of CD3+ CD8+ T cells. We find that CD8+ T cells are closer to myeloid (CD68+) cells expressing HLA-DR. This is consistent with the potential of HLA-DR expressing cells to present antigens to T cells, and suggests that T cells may preferentially interact with HLA-DR expressing myeloid cells. Conversely, we find that Ki67 expression on tumor (SOX10+) cells correlates with increased distance from CD3+ CD8+ T cells relative to SOX10+ Ki67- tumor cells. This finding is consistent with the observation that more advanced tumors with higher mitotic rates have decreased T cell infiltrates, and suggests that dividing melanoma cells are less likely to interact with T cells. In addition, we performed analysis to determine whether spatial relationships defined above impact prognosis. Clinical oncology follow-up was available on 35 of the 57 patients evaluated above. We compared proximity of CD3+ CD8+ cells to both myeloid (CD68+) and tumor (SOX10+) cells in patients who recurred and those with no evidence of recurrence. We found that CD3+ CD8+ cells in patients who had recurrence were closer to CD68+ HLA-DR− cells than in patients who had no recurrence (*t*-test, $p = 0.0377$), this correlated with DSS ($p = 0.003$). Conversely, distance from CD3+ CD8+ to CD68+ HLA-DR+ in relationship to recurrence was not significant with a trend towards CD3+ CD8+ T cells being closer in nonrecurrent patients (*t*-test, $p = 0.1362$). DISCUSSION/SIGNIFICANCE OF IMPACT: Consistent with the literature, we find that densities of CD8+ T cells correlates with favorable outcomes in early stage melanoma. We also find that density of CD68+ macrophages in stroma correlates with poor outcome. If proximity is a surrogate for interaction, these data indicate that dividing, Ki67+, melanoma cells interact less with CD8+ T cells than do Ki67+ melanoma cells. Further, HLA-DR expression on CD68+ infiltrating cells likely enhances their interaction with T cells. Interestingly, on further analysis, CD3+ CD8+ cells were significantly closer to CD68+ HLA-DR− cells in patients who recurred, implying that interactions between these cell types may not be favorable. This analysis demonstrates that spatial analysis may be useful in predicting prognosis in early stage melanoma, and this is the first report of this type of analysis predicting outcomes in primary tumor specimens to our knowledge. Further staining and analysis of the complete patient cohort (n = 120) is ongoing.

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Understanding epicardial fat biology by imaging

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OBJECTIVES/SPECIFIC AIMS: The goal is to understand the underlying mechanism of epicaridial fat biology and its response to cardiometabolic disease by using quantitative multi-echo Dixon (mDixon) of water and lipid sequence, T2* blood-oxygen-level-dependent (BOLD) sequence of iron content, and data analysis methods to determine the quantity of brown Versus white fat. To accomplish this goal, we propose to define the histological, genetic, and metabolite state of epicardial fat and to confirm the relationship between fat phenotype and magnetic resonance (MR) characteristics. We will then investigate whether MR is more effective in identifying patients with lower cardiovascular disease risk than computed tomography (CT). METHODS/STUDY POPULATION: We will recruit 100 patients undergoing open-heart surgery and will quantify mDixon (proton density fat fraction), BOLD (T2*), and T2/T1 maps of epicardial, extrapericardial, and subcutaneous fat before their surgery. We will then (a) validate MR findings by direct depot-specific tissue analysis for lipid content, histological, and genetic markers of inflammation and brown and white fat, (b) develop plasma and fat depot specific metabolite profiling of cardiovascular disease risk and correlate with imaging characteristics. We will categorize cardiovascular risk score (Cardiovascular Health Status) of our 100 patients on quartiles. We will then build models where the categorized cardiovascular risk score are regressed on MR measures (epicardial fat fraction, T2*, and T2/T1 maps) and CT measures (epicardial fat volume and coronary calcium score). RESULTS/ANTICIPATED RESULTS: We anticipate to learn about epicardial fat biology and the role of inflammation in cardiometabolic disease. We will validate proton density fat fraction, T2* and T2 map against histology of epicardial fat for lipid content, established markers of brown and white fat and inflammation, respectively, to help us translate imaging technique to clinical practice. In respect to our second aim we anticipate that MR identifies patients at lower cardiovascular risk quartile than CT. DISCUSSION/SIGNIFICANCE OF IMPACT: Interest in epicardial fat as a visceral fat of the heart and coronary arteries is rapidly growing as the scientific based evidence indicates that the anatomic specificity is an important contributor to the cardiovascular diseases. The transformation of epicardial fat from a cardioprotective phenotype to a pro-inflammatory, atherosclerosis-promoting state triggers inflammation that is coincident with the expansion of epicardial fat volume detected by anatomic imaging. This study will impact the management of patients at risk for cardiovascular disease because it will demonstrate that quantification of epicardial fat status by MR identifies fat tissue changes validated by histology at lower cardiovascular disease risk quartile than CT.

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Perception- and behavior-related attention systems distinguished by phase amplitude coupling and high-gamma power

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OBJECTIVES/SPECIFIC AIMS: Attention is a cognitive function that binds perception and behavior. Recent evidence suggests that attention involves phase-amplitude coupling (PAC) of neural signals. PAC occurs when the amplitude of one frequency (frequency for amplitude) is maximal at particular phases of another frequency (frequency for phase). However, some studies suggest PAC improves attention, while others maintain that PAC inhibits attention. The present study seeks to determine whether PAC promotes or inhibits neural signals that underlie attention. METHODS/STUDY POPULATION: Six adult epilepsy patients with implanted electrodes participated in a cued attention task. Subjects participated in a cued attention task where they oriented attention to one side of the screen at a time and discriminated between stimuli as fast as possible with mouse clicks. Perception-related electrodes discriminated the location and/or shape of the target. These were determined with a cluster-based permutation test. Behavior-related electrodes predicted reaction time (RT) with neural activity prior to target appearance. These were determined with correlations between PAC and RT. PAC was calculated using the modulation index (MI). RESULTS/ANTICIPATED RESULTS: We found 47 perception-related electrodes that discriminated location and/or shape of target ($p < 0.05$, FDR corrected). We found 27 behavior-related electrodes where PAC prior to the target predicted RT ($p < 0.05$ FDR corrected). There was little overlap between the perception-related and behavior-related electrodes (3%). PAC also did not discriminate left-sided and right-sided cues. In addition, behavior-related electrodes had less local neural activity and higher PAC during the period of cued attention than perception-related electrodes. DISCUSSION/SIGNIFICANCE OF IMPACT: PAC minimally facilitates perceptual aspect of visual attention. However, PAC facilitate response speed. We suggest that PAC might improve response speed by "quieting" task irrelevant neural activity. For the same reason, PAC is absent in electrodes that are actively processing meaningful streams of visual data. These findings highlight separable aspects of the human attention system and how PAC contributes to both. Future directions include determining differences in PAC for attentional disorders like ADHD and neurological neglect.

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Metabolite and biomarker predictors of WTC-lung injury: An integrated multiplatform pilot analysis

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OBJECTIVES/SPECIFIC AIMS: In this pilot case-control study, the metabolome was quantified in subjects with previously measured serum and clinical biomarkers. The serum metabolome was then integrated with existing serum and clinical biomarkers of WTC-exposed firefighters to identify pathways significant to loss of lung function following acute PM-exposure. This robust subset of metabolite and serum biomarkers may be clinically relevant to predicting progression to lung disease in a larger cohort. METHODS/STUDY POPULATION: Serum drawn within 6 months of 9/11 was analyzed in this pilot. Clinical measures were obtained from electronic medical records. Never-smoking, male, WTC-exposed firefighters with normal pre-9/11 lung function were segregated based on FEV1 percent predicted (FEV1 %Pred) at symptomatic presentation. Cases of WTC-LI (FEV1 %Pred < LLN, n = 15) and controls (n = 15) were identified from previous cohorts. Ultrahigh performance liquid chromatography tandem mass spectroscopy quantified the metabolomic fingerprints of a group with previously assessed (by multiplex panels; ELISA and Luminex) serum chemokines and cytokines. High-dimensional data analysis and dimension reduction techniques integrated metabolites, cytokines, chemokines, and clinical data to identify pathways of

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