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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