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

### 2326 Successful hand function recovery after stroke

Shashwati Geed, Peter S. Lum, Michelle L. Harris-Love, Jessica Barth, Peter E. Turkeltaub and Alexander W. Dromerick

**Georgetown - Howard Universities, Washington, DC, USA**

**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.

**METHODOLOGY/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 corticomoctor 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 progress and will be presented. Preliminary data from controls shows that corticomoctor 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. 

### 2367 Defining critical features of the immune microenvironment in melanoma using multiplex immunohistochemistry and spatial analysis

Robyn Gartrell, Douglas Marks, Thomas Har, Yan Lu, Ed Stack, Camden Esancy, Basil Horst, Yvonne Saenger, Camille Gerard, Dan Tong Jia, Paul Armenta, Dai sku Izaki and Kristen Beck

**Irving Institute for Clinical, Columbia University, New York, NY, USA**

**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, CD8+ 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, CD8+ Leica), CD68 (KP1, Biogenex), 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.

### 2343 Enumeration of circulating tumor cells for monitoring cancer treatment response

Jose Ignacio Varillas, Jinling Zhang, Weian Sheng, Kangfu Chen, Isis Barnes, Thomas George, Chen Liu and Hugh Fan

**OBJECTIVES/SPECIFIC AIMS:** The goal of this research is to use circulating tumor cells (CTC) enumeration and characterization to monitor anticanine treatment response. Emerging evidence strongly suggests the implications that epithelial-to-clinical outcome. **METHODOLOGY/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 anticanine 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 non-specifically adherent 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 chemotherapeutic 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.