Colorectal cancer (CRC) is the second leading cause of cancer deaths in the United States. Metastases are the main cause of cancer-related death, and the most frequent metastatic sites in patients with CRCs are liver and lung. To confirm the diagnosis of metastatic CRC (mCRCs) and to classify mCRCs, tumor biopsy of a suspected metastasis is often required. The consensus molecular subtype (CMS) classification which is based on gene expression profiles of CRC tumor specimens, is a predictive factor for treatment outcomes of standard chemotherapies for mCRCs. The practicality of repeated tumor biopsies for disease monitoring in mCRC patients is often limited. Liquid biopsy utilizing extracellular vesicles (EVs) is an alternative approach for obtaining insights into molecular landscapes of mCRC. Cancer-derived EVs play a critical role in cancer cell-to-cell communication and carry surface proteins and cell-specific cargos of nucleic acids. For this reason, we aimed to characterize ribonucleic acid (RNA) cargoes and surface proteins on EVs disseminated from CRC cancer cells to longitudinally monitor mCRC patients and identify immune modulatory signaling molecules that potentially play a role in the progression and metastasis of CRC. Using novel bioinformatics deconvolution pipelines applied to RNA sequencing data of circulating EVs, we demonstrate that transcriptomic profiling of EVs can be used for the detection, classification, and longitudinal monitoring of mCRC. In the context of both conventional and targeted therapies, assessing the transcriptomic cargo of circulating EVs can provide pivotal insights into molecular pathways that are altered under treatment selection pressure, and which likely contribute to the emergence of resistance. Additionally, we were able to characterize cancer-related protein composition of EVs originating from colorectal cancer cells. We demonstrate the immunomodulatory protein CD276/B7H3 as a prominent surface protein on CRC-associated EVs. This study highlights the potential of EVs in both detection and therapeutic monitoring mCRC, offering valuable insights into cancer-derived protein and RNA cargo, and
emphasizing the role of longitudinal sampling via liquid biopsy in the clinical care of patients with advanced cancer.

**Advisory Committee:**
Anirban Maitra, MBBS, Chair
Michael Green, PhD
E. Scott Kopetz, MD, PhD
Pierre McCrea, PhD
John Paul Shen, MD
Ryan Sun, PhD
Jody Vykoukal, PhD