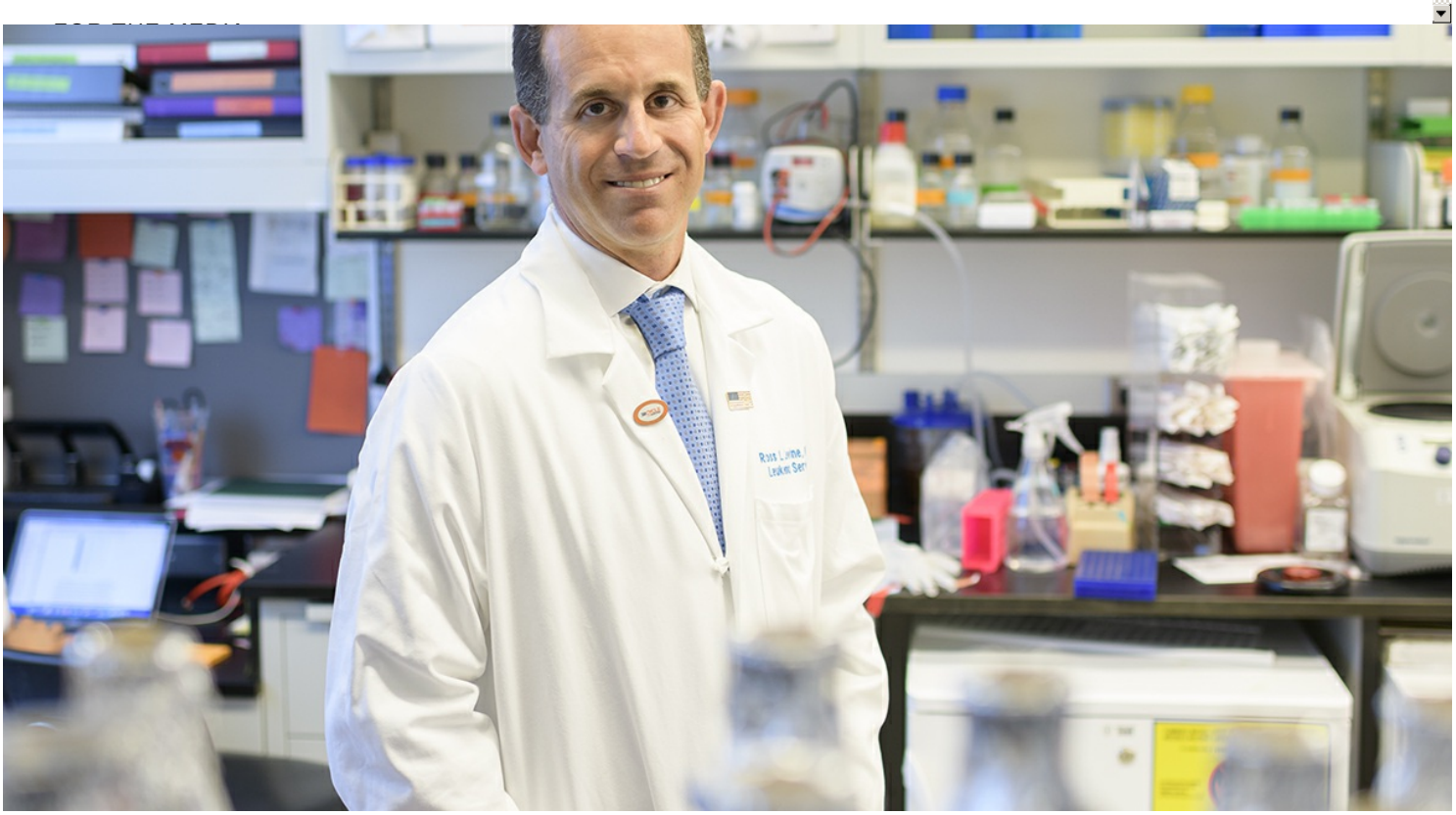


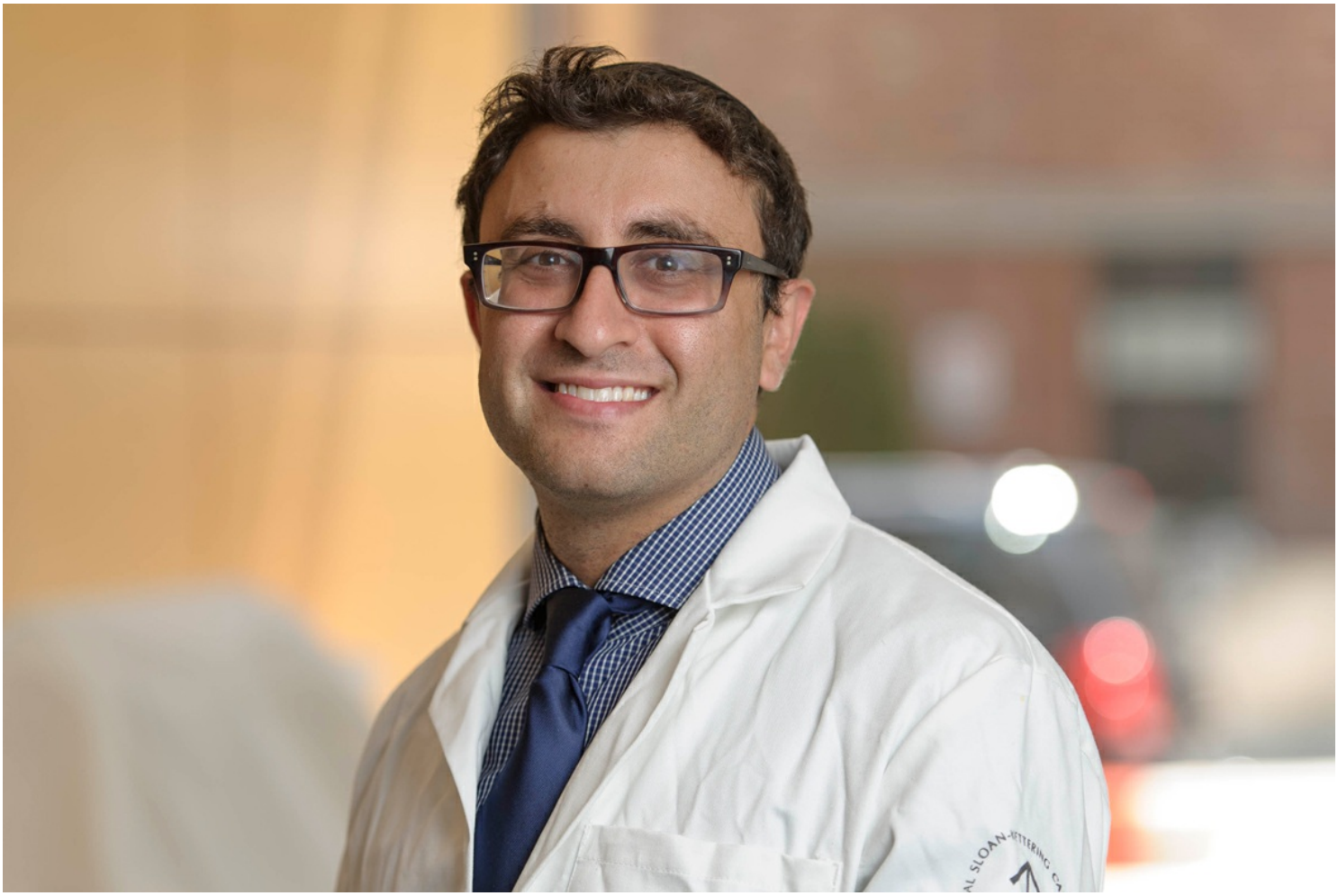
Ross Levine *(Classic Individual)*

Clonal hematopoiesis as a driver of metastatic relapse in early breast cancer

Efforts to identify genomic biomarkers to predict breast cancer outcomes have traditionally focused on tumor intrinsic factors; thus,

identification of tumor extrinsic factors with prognostic and therapeutic importance is imperative to better predict patient outcomes and redefine therapeutic strategies for the prevention and treatment of metastatic breast cancer. Genomic sequencing studies have uncovered that 25% of breast cancer patients, carry somatic mutations in their leukocytes, a condition known as clonal hematopoiesis (CH). The incidence of CH increases with aging and has been recognized as a major risk factor for the development of subsequent hematologic malignancies and cardiovascular disease. CH mutations frequently occur in genes involved in myeloid malignancies, most commonly in the epigenetic modifier DNMT3A. A hallmark of breast cancer is the admixture of tumor cells and myeloid cells, which play a key role in determining therapeutic resistance and metastasis through the fine tuning of inflammatory signals. More recently, we have revealed that CH mutations are enriched in breast cancer tumor infiltrating leukocytes and that CH in putative cancer drivers (CH-PD) is associated with increased risk of solid tumor dependent morbidity and mortality. Although the most common cause of death in these patients was metastatic disease progression, the mechanisms by which CH contributes to therapeutic resistance and metastatic relapse remain unclear. Our central hypothesis is that CH alters the inflammatory responses of myeloid cells to drive therapeutic resistance and metastatic relapse via increased proliferation of disseminated cancer cells. This project will A) Determine why breast cancer cells re-emerge as metastasis by exploring the effects of CH-PD on the metastatic outcomes of patients with early breast cancer; and B) Determine how to prevent lethal metastatic recurrences by targeting inflammatory signals in mouse models of CH and breast cancer metastasis. This study represents an innovation because (1) the biological processes behind how aging and CH contribute to metastatic progression remains largely unexplored, and (2) understanding how CH mutant leukocytes contribute to the pro-metastatic process may provide a new tool to identify patients at highest risk of metastatic relapse and lead to the identification of actionable targets to limit tumor promoting inflammation and metastasis.

2022



A. Ari Hakimi (Classic Individual)

Immunometabolic Coevolution as a Determinant of Response to Immunotherapy in ccRCC

Treatments combining inhibition of PD1 with inhibition of either VEGF or CTLA4 have revolutionized outcomes for patients with metastatic clear cell renal cell carcinoma (ccRCC), but only a fraction of patients respond to these therapies. Emerging evidence implicates the metabolism of both tumor and immune cells as critical mediators of sensitivity to combination immunotherapy via at least two axes. First, immunotherapy and anti-VEGF therapy directly alter the availability of key biosynthetic/energy-rich metabolites (glucose, glutamine) and signaling molecules (kynurenine, adenosine) delivered by the circulation to the microenvironment. Second, both immunotherapy and anti-VEGF therapy remodel the individual metabolic phenotypes of tumor and immune cells, including their expression of key metabolic transporters and catabolic enzymes and therefore their ability to compete for limited nutrients in the tumor milieu. These data raise the possibility that ccRCC evolution produces unique immunometabolic niches, defined jointly by specific patterns of metabolite availability and immune composition, that shape both initial sensitivity to immunotherapy and future molecular adaptation after exposure to immunotherapy. Our research group has pioneered the analysis of multimodal metabolomic and transcriptomic data in ccRCC to study the metabolic basis of tumorigenesis, metastasis, and therapeutic sensitivity. Based on this, we have (1) discovered novel metabolite phenotypes associated with existing, prognostically significant RNA signatures of the ccRCC TME (e.g. increased T-cell infiltration and NAD+ abundance) and (2) developed novel machine learning methods (tMIRTH) to impute metabolite abundance directly from RNA sequencing data. In parallel, we have (3) developed a novel immunocompetent mouse model of metastatic ccRCC that faithfully phenocopies ccRCC tumors and demonstrates robust responses to various immunotherapeutic regimens. The overarching hypothesis of this proposal is that the metabolism and immune microenvironment of ccRCC coevolve to produce unique microenvironments with defined sensitivities to immunotherapy. To address this hypothesis we will analyze bulk, multimodal metabolomic/microenvironmental profiling of ccRCC tumors (Aim 1) and the direct effect of immunotherapy on novel, immunocompetent, mouse models of ccRCC (Aim 2). Findings from our work will lead to the identification of novel metabolomic biomarkers for sensitivity to immunotherapy that can be measured prior to therapy and will reveal the full spectrum of metabolically-defined tumor microenvironments and illuminate the basic etiology of immunometabolic evolution in ccRCC and nominate rational metabolic interventions for combination strategies.

Morgan Huse (Classic Individual)

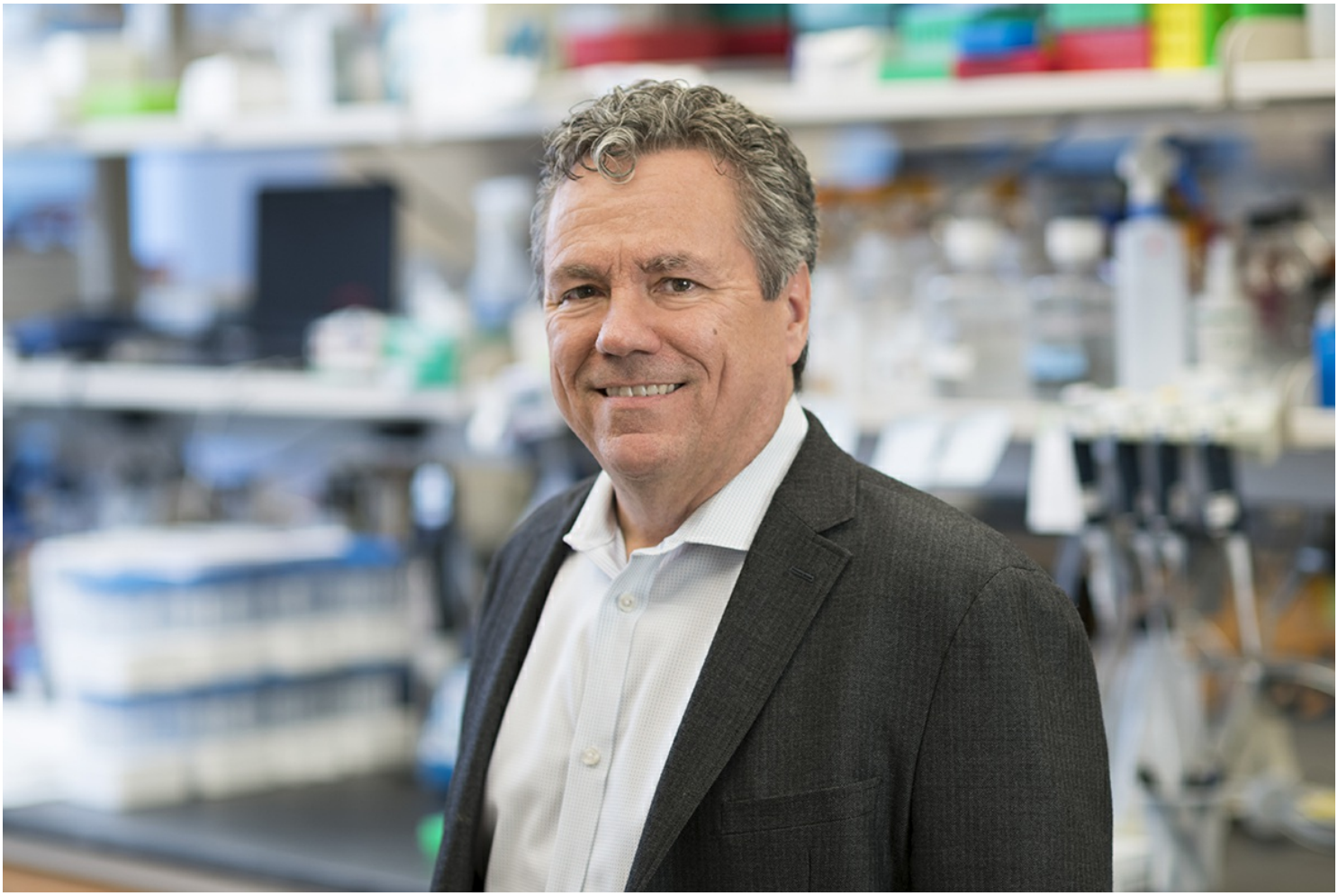
A Mechanoimmunological Basis for Metastatic Site Preference

Over the past decade, cytotoxic lymphocytes, comprising cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, have emerged as critical players in the control of metastatic outgrowth. Immunosurveillance by cytotoxic lymphocytes is generally conceived as a biochemical process in which tumor specific markers are recognized by activating receptors on the lymphocyte membrane. We have found, however, that immune cells also respond to the characteristic biophysical properties of cancer cells during metastasis. To colonize target organs, disseminated cancer cells activate a mechanotransduction pathway that enables them to spread and migrate along the abluminal surface of blood vessels. While this pathway is necessary for effective invasion of the metastatic niche, it also renders cancer cells more stimulatory to cytotoxic lymphocytes by rigidifying cell surface immunoreceptor ligands. Hence, the biophysical properties required for efficient colonization also sensitize metastatic cells to destruction by the immune system. Here, we will explore how this mechanical form of immunosurveillance, which we call mechanosurveillance, influences where metastatic growth occurs. Cellular mechanics are modulated continuously by cell-extrinsic biophysical signals. A particularly important manifestation of this crosstalk, called mechanoreciprocity, induces cells to adopt the mechanical properties of their immediate surroundings. Consistent with this principle, we and others have shown that cancer cells grown on stiffer substrates become stiffer themselves, whereas those grown on softer surfaces become softer. Whether environmentally-induced stiffening might sensitize cancer cells to mechanosurveillance in vivo, however, has not been explored. This an interesting question because metastatic microenvironments vary widely in their physical properties, ranging from very rigid (e.g. bone) to very soft (e.g. lung). Enhanced mechanosurveillance in rigid microenvironments would establish a regime in which cytotoxic lymphocytes control the spectrum of metastatic site preference by disproportionately suppressing outgrowth in organs like the bone. Using a murine model of metastasis, we have found that cancer cells colonizing the bone are significantly stiffer than cancer cells colonizing the lung, and that the in vivo expansion of bone metastasis is exquisitely sensitive to cytotoxic

lymphocytes. Building on these preliminary observations, we propose that microenvironmental stiffness dictates the efficacy of mechanosurveillance and that this relationship shapes both metastatic site preference and the power of anti-tumor immunotherapy. We will investigate this hypothesis by applying materials science, functional assays, and single cell transcriptomics to mouse models of metastasis and patient samples. The successful completion of our Specific Aims could identify biomarkers for guiding antitumor immunotherapy and aid development of strategies for treating metastatic growth in specific target organs.

Past Projects

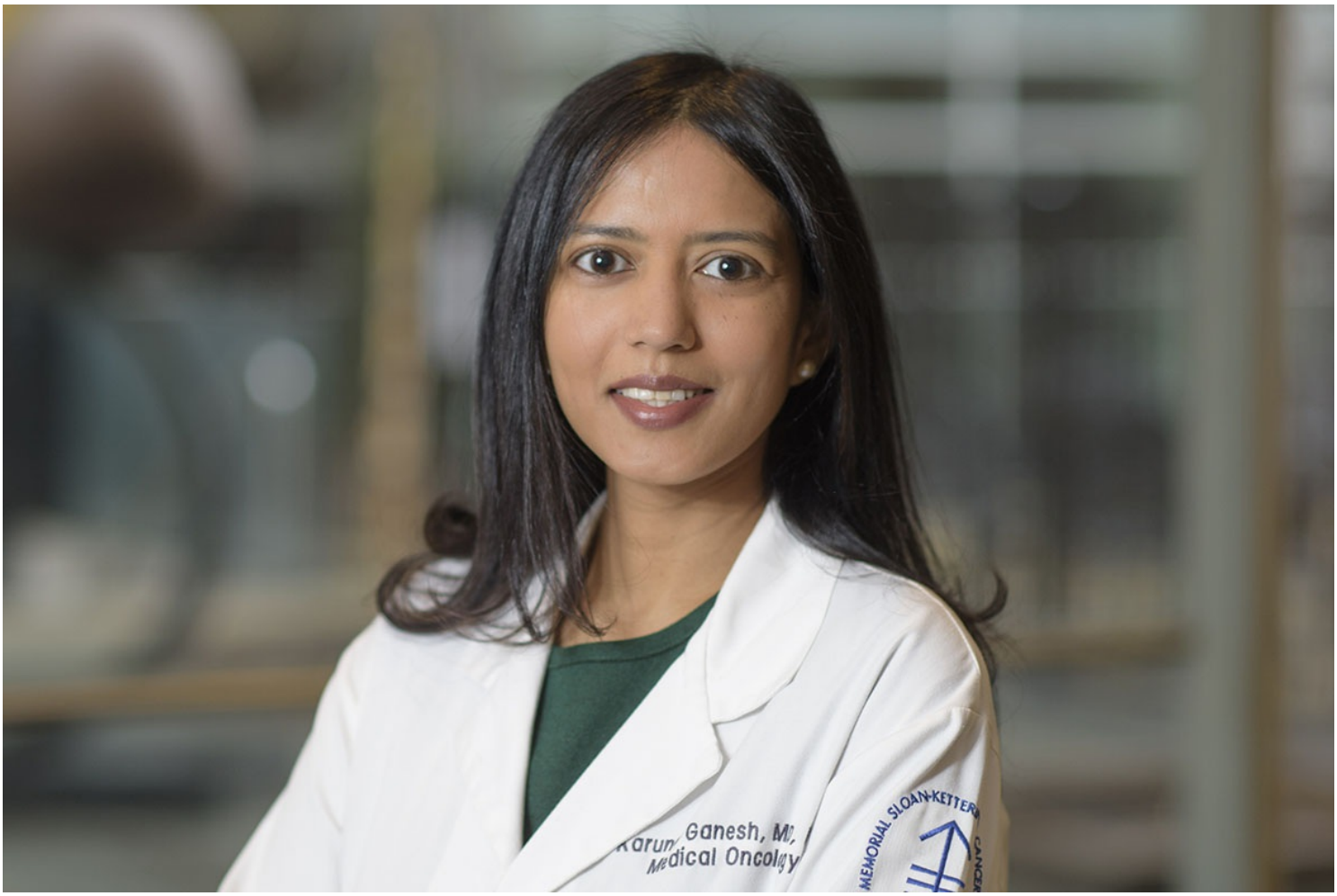
2021



Scott Lowe (Classic Individual)
Somatic Deletions of type I IFNs in Immune Evasion and Metastasis

Deletions of chromosome 9p21.3 are pervasive across human cancers, and strongly associated with poor prognosis, altered immune infiltration, and resistance to immune checkpoint blockade. While the contributions of these deletions to cancer biology have been broadly ascribed to the disruption of the cell cycle inhibitors CDKN2A/B, approximately half of 9p21.3 deletions include a neighboring cluster of 16 type I interferon (IFN) genes. To determine whether IFN co-deletion contributes to the phenotypes associated with 9p21.3 loss, we leveraged a new genome engineering approach developed in our group termed Molecular Alteration of Chromosomes with Engineered Tandem Elements (MACHETE), which enables the rapid and flexiblegeneration of megabase-sized chromosomal deletions. By applying MACHETE to an immunocompetent mouse model of pancreatic ductal adenocarcinoma (PDAC), we found that tumors bearing concomitant loss of Cdkn2a/b and the IFN cluster exhibit reduced immune

surveillance and enhanced metastatic spread due to evasion of CD8+ T cell surveillance. Motivated by these observations, this project aims to molecularly and functionally dissect the impact of IFN signaling and its disruption on the PDAC ecosystem in tumor progression and upon immunotherapy. We propose to refine our understanding of relevant activities in the 9p21.3 locus and to interrogate the immune environment and epithelial-immune cell interactions using latest-generation single cell methods. These studies will provide novel insights and broadly applicable knowledge on what may be the most frequent genetic mechanism of immune evasion in human cancer.



Karuna Ganesh (Classic Individual)
Molecular Mediators of Dynamic Cell State Plasticity in Colorectal Cancer Metastasis

Metastasis is the principal cause of cancer death. Recent studies have reveal that phenotypic plasticity, the dynamic adaptation of cancer cells to the stresses of dissemination and tumor regeneration in distant sites, is an overarching hallmark of metastasis. However, the molecular mechanisms that underpin the dynamic diversification and selection of cell states during tumor progression, and the ultimate endpoint cell states that are selected for during metastasis in advanced human solid tumors remain poorly understood. We have pioneered an integrated approach for single cell profiling and organoid derivation matched trios of synchronously resected normal colon, primary colorectal cancer (CRC), and CRC liver metastasis from patients undergoing cancer surgery at MSK. Our preliminary single cell analysis reveals distinct lineage trajectories that are selected for and against in the progression from normal epithelium to primary tumor and metastasis in the same patients. In this proposal, we will leverage our patient-derived organoid and orthotopic mouse models and cutting-edge single cell, live imaging, and computational approaches to dissect the transcription factors and lineage programs that underpin metastatic plasticity. Our work will illuminate fundamental mechanisms and clinically actionable signaling pathways underlying dynamic cell state transitions and lineage plasticity in metastasis, poised for clinical translation to improve cancer outcomes.