# Systems Approaches to Cancer Biology 2025

## Invited Talk Abstracts

## Session #1: Tumor Heterogeneity and Metastasis
## Monday, February 10th 2025

### *Distinct Cellular States Arise from Variability in Extrachromosomal DNA Copy Number*

**Presenter: Elizabeth Brunk**

**University of North Carolina at Chapel Hill**

*Jingting Chen (UNC), Yue Wang (UNC), Oliver Cope (UNC), Dalia Fleifel (UNC), Santiago Haase (UNC), Christina Ford (UNC), William Dennis (UNC), Aarav Mehta (UNC), Nithya Gurumurthy (UNC), Jeremy Wang (UNC), Phil Spanheimer (UNC), Jeremy Purvis (UNC), Sam Wolff (UNC), Jeanette G. Cook (UNC), Elizabeth Brunk (UNC)*

Extrachromosomal DNAs (ecDNAs) are enigmatic genetic elements that amplify cancer-driving genes outside of chromosomes, fueling extensive cell-to-cell genetic heterogeneity in tumors. A key question in cancer biology is whether this genetic variability translates into functional phenotypic differences that enable tumor adaptation and resistance to therapy. Here, we present evidence that cells with varying ecDNA levels exhibit distinct transcriptional, proteomic, and cell cycle states, suggesting that ecDNA serves as a rapid mechanism to diversify cellular phenotypes and bolster cancer cell fitness under selective pressures.

To capture the full spectrum of these states, we employ an integrated approach combining single-cell sequencing, computational analysis, and advanced fluorescent imaging to profile ecDNA's impact on cellular behavior with high resolution. By isolating cell populations based on ecDNA levels using fluorescence-activated cell sorting (FACS), we uncover how ecDNA-driven differences in gene dosage generate unique cellular subpopulations with specific transcriptional programs, protein expression profiles, and growth characteristics. Our findings indicate that ecDNA-rich subpopulations exhibit adaptive advantages, such as accelerated growth rates and distinct cell cycle distributions, enhancing phenotypic diversity within the tumor and potentially supporting tumor resilience under environmental challenges, including drug treatment.

This study underscores the role of ecDNA in facilitating rapid cellular evolution, expanding our understanding of its functional consequences on tumor heterogeneity. By illuminating ecDNA as both a marker and a driver of cancer adaptability, our findings underscore its potential as a target for therapeutic intervention.

## *Single-cell proteogenomic analysis of phenotypic heterogeneity across diverse drivers of epithelial-mesenchymal transition in pancreas cancer*

**Presenter: Michelle Barbeau**

**The University of Virginia**

*Michelle C. Barbeau (The University of Virginia, Charlottesville, VA)*
*Brooke A. Brown (The University of Virginia, Charlottesville, VA)*
*Sara J. Adair (The University of Virginia, Charlottesville, VA)*
*Todd W. Bauer (The University of Virginia, Charlottesville, VA)*
*Matthew J. Lazzara (The University of Virginia, Charlottesville, VA)*

Epithelial-mesenchymal transition (EMT) is a developmental program aberrantly activated in pancreatic ductal adenocarcinoma (PDAC) to promote chemoresistance and metastasis. Antagonizing EMT may be an effective adjuvant therapy approach to promote tumor response to chemotherapy, but identifying the right drug targets is complicated by the high degree of heterogeneity with which EMT occurs in PDAC tumors. Even in PDAC cancer cells treated identically with different EMT agonists (e.g., growth factors, chemotherapeutics), EMT occurs heterogeneously, leading to problems with identifying the responsible druggable signaling pathways based on population-level measurements. We hypothesize that cell-to-cell variation of the activities of specific signaling pathways explains heterogeneity of EMT, both at baseline and in response to acute EMT induction, and that heterogeneities in signaling pathway activities among cells may be transcriptionally based. To test this, we first developed a workflow integrating: 1. iterative indirect immunofluorescence imaging of PDAC cells to quantify the activities of up to seven purported EMT-regulating pathways and two EMT phenotypic markers, and 2. a mutual information (MI) computational model to predict the cooperating pathways that are most informative of the EMT phenotype. EMT was induced in cells using growth factors found in the tumor microenvironment or with chemotherapeutics. EMT phenotypic markers and signaling pathway proteins were imaged daily for up to 96 hr. MI identified ERK as the most important signaling node explaining EMT heterogeneity across the diverse agonists investigated. Inhibition and knockdown studies confirmed the role of ERK and revealed a compensatory, EMT-driving JNK activation in the context of MEK inhibition. Importantly, a population-level model nominated JNK as more informative than ERK for determining EMT state in response to growth factors, emphasizing the need to account for heterogeneity in studying signaling-phenotype relationships. In a patient-derived xenograft mouse model of PDAC, ERK activity was similarly informative of EMT, but JNK activity became more informative in the context of strong MEK inhibition. To determine the degree to which EMT heterogeneity may be transcriptionally primed for different EMT agonists, we single-cell-sorted PDAC cells and allowed each clone to grow into its own population of highly related cells. Clonal populations were then subjected to a 90-10 split to create “parent” and “progeny” populations that were treated with EMT-promoting growth factors immediately after the split or one week later, respectively. A wide range of EMT induction was observed across the clonal populations, and the degree of EMT was significantly correlated between parent-progeny populations from the same clone. Thus, the tendency for specific clones to undergo EMT was conserved over time. We are now implementing a cell barcoding approach for lineage-traced sequencing to identify common transcriptional determinants of cellular propensity to undergo EMT. Together, our results demonstrate the pervasive importance of ERK for explaining EMT heterogeneity in response to diverse agonists and raise the possibility of durable EMT-priming within cancer cell populations.

## *Targeting wtIDH1 in radiation resistant medulloblastoma*

**Presenter: Bethany Veo**

**University of Colorado Anschutz Medical Campus**

*Bethany Veo (University of Colorado Anschutz Medical Campus, Aurora, CO)*
*Dong Wang (University of Colorado Anschutz Medical Campus, Aurora, CO)*
*John DeSisto(University of Colorado Anschutz Medical Campus, Aurora, CO)*
*Rajeev Vibhakar (University of Colorado Anschutz Medical Campus, Aurora, CO)*

Medulloblastoma (MB) is the prevailing malignant brain tumor in children, accounting for 20% of all pediatric brain tumors. Medulloblastoma is molecularly characterized into 4 main subgroups consisting of WNT, SHH, Grp 3 and Grp 4, which are further divided into 13 subtypes. Medulloblastoma manifests as a clinically heterogeneous tumor with varying prognoses depending on the molecular subgroup. Group 3 tumors have the worst overall prognosis, and are associated with MYC gene amplification, higher rates of metastasis, and increased likelihood of recurrence. Treatments for these high-risk patients include craniospinal irradiation (36Gy) after surgical resection and chemotherapy. While upfront radiation therapy shows an overall survival benefit for all subgroups, Group 3 tumors with upfront radiation therapy relapse in a shorter amount of time at 1 year. Group 3 patients present at relapse with metastatic dissemination as well as local recurrences, exemplifying the challenges to retargeting treatment. Currently, there are limited therapeutic strategies that address medulloblastoma tumor recurrence which has detrimental effects on patient outcome with <5% long term survival. Subsequently, there is a critical need to investigate the molecular mechanisms underlying therapeutic resistance in medulloblastoma and identify the best strategies to combat it.

To understand what causes radiation resistance in medulloblastoma, we analyzed matched primary and relapsed patient tumors by single cell ATAC seq + gene expression. We further evaluated radiation resistance in an orthotopic mouse model and analyzed gene expression changes by single cell RNA sequencing. We identify a resistant cell population which exhibits a distinctive gene signature comprising stem and metabolic gene transcription programs. We observe elevated expression of metabolic marker genes such as wtIDH1, and stem cell markers TEAD1 and SOX6 within the recurrent cell population. Prior evidence links metabolic rewiring with the development of therapeutic resistance in breast and leukemia cancers. Accordingly, we speculate radiation resistance in group 3 medulloblastoma is facilitated by wtIDH1 metabolic reprogramming leading to epigenetic adaptation and cell persistence. Thus, we evaluate how IDH1 changes the epigenetic landscape of radiation resistant and sensitive cells by examining histone modifications (H3K27ac, H3K27me3, H3K4me3) and the broader chromatin architecture in response to IDH1 inhibition by Cut and Run and ATAC-sequencing. We observe the manipulation of wtIDH1 reduces MB cell growth, stemness characteristics, and changes the chromatin landscape. Further, we find inhibition of wtIDH1 enhances susceptibility to re-irradiation. Additionally, mass spectrometry and genetic knockdowns were utilized to validate our findings. Our results highlight evidence into the development radiation resistance and provide a potential therapeutic alternative for treatment of relapsed MB.

## *Mannose metabolism reshapes T cell differentiation to enhance anti-tumor immunity*

**Presenter: Yapeng Su**

**Fred Hutch Cancer Center**

*Yapeng Su (Fred Hutch Cancer Center, WA, USA), Yajing Qiu (Suzhou Institute of Systems Medicine, Suzhou, China), Ermei Xie (Suzhou Institute of Systems Medicine, Suzhou, China), Hongcheng Cheng (Suzhou Institute of Systems Medicine, Suzhou, China), Jing Du (Suzhou Institute of Systems Medicine, Suzhou, China), Yue Xu (Suzhou Institute of Systems Medicine, Suzhou, China), Xiaoli Pan (Suzhou Institute of Systems Medicine, Suzhou, China), Zhe Wang (Suzhou Institute of Systems Medicine, Suzhou, China), Daniel Chen (Fred Hutch Cancer Center, WA, USA), Hong Zhu (Suzhou Institute of Systems Medicine, Suzhou, China), Guideng Li (Suzhou Institute of Systems Medicine, Suzhou, China), Philip Greenberg (Fred Hutch Cancer Center, WA, USA)*

Cellular metabolic status profoundly influences T cell differentiation, persistence, and anti-tumor efficacy. Our single-cell metabolic analyses of T cells reveals that diminished mannose metabolism is a prominent feature of T cell dysfunction. Conversely, experimental augmentation/restoration of mannose metabolism in adoptively transferred T cells via D-mannose supplementation enhances anti-tumor activity and restricts exhaustion differentiation both in vitro and in vivo. Mechanistically, D-mannose treatment induces intracellular metabolic programming and increases the O-GlcNAc transferase (OGT)-mediated O-GlcNAcylation of β-catenin, which preserves Tcf7 expression and epigenetic stemness, thereby promoting stem-like programs in T cells. Furthermore, in vitro expansion with D-mannose supplementation yieldes T cell products for adoptive therapy with stemness characteristics, even after extensive long-term expansion, that exhibits enhanced anti-tumor efficacy. These findings reveal cell-intrinsic mannose metabolism as a physiological regulator of CD8+ T cell fate, decoupling proliferation/expansion from differentiation, and underscoring the therapeutic potential of mannose modulation in cancer immunotherapy.

## *Characterizing the immunosuppressive role of myeloid-derived suppressor cells in glioblastoma under radiotherapy*

**Presenter: John Metzcar**

**Therapy Modeling and Development Center, University of Minnesota**

*John Metzcar (Therapy Modeling and Development Center, University of Minnesota-Twin Cities, Minneapolis, MN)*
*Xuanming Zhang (Therapy Modeling and Development Center, University of Minnesota-Twin Cities, Minneapolis, MN)*
*Kamran Kaveh (Therapy Modeling and Development Center, University of Minnesota-Twin Cities, Minneapolis, MN)*
*Jasmine Foo (Therapy Modeling and Development Center, University of Minnesota-Twin Cities, Minneapolis, MN)*
*Kevin Leder (Therapy Modeling and Development Center, University of Minnesota-Twin Cities, Minneapolis, MN)*

In this work, we address the treatment of glioblastoma (GBM), a difficult-to-treat brain cancer. Upon discovery of GBM, patients are frequently treated with both surgery and chemoradiotherapy but still suffer from eventual recurrence due to unresectable microscopic disease that evades adjuvant therapy. The disease is characterized by an immunosuppressive tumor microenvironment. Immunotherapies, including immune checkpoint inhibitors, have been trialed in GBM but have so far failed to improve otherwise bleak outcomes for GBM patients. One possible way GBM tumors sustain this immune suppression, even in the face of ICI, is through recruitment and sustaining a population of myeloid-derived suppressor cells (MDSCs). These potently immunosuppressive cells decrease T-cell activity through a range of mechanisms including direct cell-cell interactions, secretion of T-cell inhibitors, and alteration of the metabolic environment. Furthermore, radiotherapy (RT), which can paradoxically lead to both immunosuppression and immune stimulation, represents an under-explored option to possibly tip the tumor in favor of immune stimulation. To understand the interplay between MDSCs, effector cells, and RT, we developed a dynamic, computational model of the tumor immune microenvironment of glioblastoma. Using ordinary differential equations, we model a growing population of GBM cells, which both stimulates immune response through antigen-presenting dendritic cells recruitment of activated T-cells and suppresses immune effector cells through recruitment of MDSCs, T-regulatory cells, and the production of TGF-beta. We model fractionated RT through the linear-quadratic formula as well as surgery through gross reduction of tumor cell count. We calibrate model parameters through fitting to clinical data including white-blood cell counts and immune panel specific flow cytometry. With the model, we recapitulate the strong suppressive effects of MDSCs on activated T-cell populations. We also use the model to explore the effects of hypofractionated radiotherapy on immune cell populations and tumor growth and present possible optimal RT dosing regimes. Finally, we use the calibrated model to produce an in silico virtual clinical trial, characterizing variability in patient response across different treatment scenarios including different fractionation regimens and the addition of the anti-inflammatory agent ibudilast.

## *A mechanistic model of curative combination therapy explains lymphoma clinical trial results*

**Presenter: Amy E. Pomeroy**

**University of North Carolina at Chapel Hill**

*Amy E Pomeroy (University of North Carolina, Chapel Hill, NC)*
*Adam C Palmer (University of North Carolina, Chapel Hill, NC)*

Introduction: Heterogeneity between patients and within tumors poses challenges to effective cancer treatment. Drug combinations can overcome heterogeneity between patients by increasing the number of patients with a response to one or more drugs and overcome heterogeneity within tumors by decreasing the number of cancer cells that resist all drugs. Precision approaches that stratify patients into subpopulations with more uniform, stronger responses to specific treatments effectively reduce patient heterogeneity within the subpopulation. These strategies can be implemented together to further improve patient outcomes. We have developed computational mechanistic models of cancer treatment in the context of both sources of heterogeneity which explain and predict clinical outcomes of treatment of Large B-cell lymphoma (LBCL) with combination chemoimmunotherapy.

Methods: We present a mathematical model of multi-drug therapy that accounts for both cell-to-cell and patient-to-patient heterogeneity. This novel population tumor kinetics (pop-TK) model simulates clinical combination therapy outcomes by implementing multidrug dose response functions in heterogeneous populations of tumor cells, within heterogeneous cohorts of patients. This approach describes kinetics of tumor growth and death in response to combination therapy and outputs Progression-Free Survival (PFS) distributions for cohorts of simulated patients. The pop-TK model can also be used to generate simulated cohorts of relapsed/refractory (r/r) patients that account for the decreased treatment sensitivity in this patient population.

Results: Our simulation reproduces multiple clinical outcomes including PFS distributions and tumor shrinkage kinetics in LBCL patients treated with the standard 5-drug combination RCHOP. We tested the prospective utility of this model by predicting the result of a clinical trial. Simulated trials accurately reproduced success or failure of nine randomized trials of new first-line combinations based on drugs' efficacies in relapsed/refractory LBCL. Most notably, informed by treatment of r/r LBCL with polatuzumab-vedotin (pola), the pop-TK predicted the success of modifying the standard treatment regimen (RCHOP) with pola before the clinical trial results were published. The pola combination improves overall survival (OS) and PFS in Activated B-Cell (ABC) LBCL (hazard ratio (HR): OS 0.3, PFS 0.4), but reduces OS (HR: 1.6) and does not improve PFS (HR: 1.0) in Germinal Center B-Cell (GCB) LBCL. The subtype specific difference in PFS was also predictable based on subtype results of r/r trials (predicted HR: ABC 0.3, GCB 0.9). Highly-sensitive liquid biopsies that use circulating tumor DNA (ctDNA) to quantify measurable residual disease (MRD) are another promising biomarker. Simulations where only patients with detectable MRD after two cycles of RCHOP receive an intensified combination predict that this approach is equally effective as intensifying treatment for all patients, suggesting the clinical utility of a biomarker-driven approach to treatment intensification.

Conclusions: The pop-TK model supports LBCL cell-of-origin subtype as a biomarker for pola performance and suggests that MRD status based on ctDNA is a promising biomarker for future intensifications of the RCHOP regimen. These examples of model-driven precision trial design have the potential to improve outcomes using readily-available biomarkers and existing therapies.

## *A Translation-Oriented Pipeline for Analyzing Drug Combination Screens*

**Presenter: Christian Meyer**

**University of Colorado Boulder**

*Christian T. Meyer (University of Colorado Boulder, Boulder, CO; Duet BioSystems, Nashville, TN)*
*Brianna N. Smith (Vanderbilt University, Nashville, TN)*
*Stephen W. Fesik (Vanderbilt University, Nashville, TN)*
*William P. Tansey (Vanderbilt University, Nashville, TN)*
*Vito Quaranta (Northeastern University, Boston, MA; Duet BioSystems, Nashville, TN)*

For over a century, debates have persisted over the merits of various drug synergy frameworks, yet their impact on advancing clinical drug combinations remains limited. This disconnect exists even as combination therapies form the backbone of treatments for numerous diseases, highlighting the need for rigorous, quantitative methodologies that promote clinical translation of drug combinations. Here, we introduce an adaptive discovery pipeline based on the MuSyC synergy framework, which emphasizes absolute efficacy in evaluating drug combinations. We apply this framework to a screen of clinically approved therapies in combination with a novel drug targeting the WIN site of WDR5 (WINi), a Myc-interacting, epigentic regulator. In hematologic cancers, WINi induces apoptosis by selectively reducing ribosomal protein synthesis, leading to p53 activation. Our pipeline identifies venetoclax as a efficacious WINi adjuvant, especially in leukemia cell lines, with the combination demonstrating a bi-directional increase in potency. Subsequently, we use MuSyC to find the Pareto-optimal solution (termed the MuSyC isobole) for all dose pairs of venetoclax and WINi resulting in a 90% growth inhibition. Guided by this isobole, we select concentrations for in vivo validation, finding that the combination significantly reduces leukemia burden in a disseminated leukemia model across several organ compartments. Our approach shifts the traditional focus of drug combination studies on synergy to a clinically-oriented mindset focused on the potency and efficacy of drug combinations.

## *Altered lipid metabolism across AML cell differentiation states indicates distinct therapeutic vulnerabilities*

**Presenter: Raghav Jain**

**Pacific Northwest National Lab**

*Raghav Jain (Pacific Northwest National Lab, Richland, WA)*
*Dylan Ross (Pacific Northwest National Lab, Richland, WA)*
*Hyeyoon Kim (Pacific Northwest National Lab, Richland, WA)*
*Soumyadeep Sarkar (Pacific Northwest National Lab, Richland, WA)*
*Josie Eder (Pacific Northwest National Lab, Richland, WA)*
*Athena Schepmoes (Pacific Northwest National Lab, Richland, WA)*
*Tao Liu (Pacific Northwest National Lab, Richland, WA)*
*Sara Gosline (Pacific Northwest National Lab, Richland, WA)*
*Jennifer Kyle (Pacific Northwest National Lab, Richland, WA)*

Acute myeloid leukemia (AML) is cancer of white blood cells. As of 2022, the NCI reported a 5-year survival rate of 31.9%, indicating a need for better treatments. Recent studies have used genomic and proteomic data to interrogate molecular processes as therapeutic targets. Characterization of unique proteogenomic alterations in AML has revealed disease heterogeneity that affects cellular differentiation and confounds treatments. For example, mature, monocytic AML cells are more resistant to the drug venetoclax than undifferentiated AML cells. While transcript and protein abundances provide insight into potential pathways that are active in cancerous cells, they may not provide a clear phenotypic characterization of cell maturation state. In contrast, measuring lipids provides a direct readout of cellular metabolism, including energy homeostasis, which is altered in cell differentiation and dysregulated in cancer. Lipids are also signaling molecules intra- and intercellularly, providing context regarding the broader tumor environment. We employed a systems approach that integrates transcript, protein, phosphosite, and lipid measurements from the CPTAC4 AML cohort of 87 patient samples with an explicit focus on changes in lipid metabolism between monocyte-like committed and primitive populations. Transcriptomics was used to classify patients as primitive (n=44) or committed (n=43) subtype using MuSiC, a tumor deconvolution method that leverages single-cell RNA-seq to predict bulk AML tumor composition. Through LC-MS/MS, we identified 10,957 proteins, 49,999 phosphosites, and 1365 lipids expressed across samples. Primitive samples had significantly ($q<0.05$) more phosphatidylcholines (PC), ceramides (CERs), and arachidonic acid (AA)-containing lipids, whereas committed AML was enriched in triglycerides and phosphatidylinositols (PIs). PCs are associated with cellular growth and expansion, whereas AA, CERs, and PIs are important for signaling pathways. For further insight, we compared total proteomic and phosphoproteomic abundance changes, focusing on 718 protein groups related to lipid metabolism. Of 318 lipid-proteins with significantly ($q<0.05$) altered abundances, 200 were increased in committed. 63 of these proteins also had significantly different phosphorylation status, the majority (n=41) being higher in committed. Pathway analysis revealed PI3K and downstream PKC signaling proteins were highly enriched in committed but not primitive AML. PI3K signaling is activated by PI lipids, which were higher in committed, and negatively regulated by CERs, which were higher in primitive. The lipoprotein and lipid remodeling proteins APOBR and FASN were also significantly higher in committed AML, in line with increased triglycerides in those cells. These results indicate agreement in lipidomic and proteomic changes between committed and primitive AML.

Our findings suggest that targeting PI3K and/or PKC signaling may be more effective in committed than primitive AML. Alternatively, disrupting lipid processing might interfere with the metabolic needs of committed AML. Primitive AML, an undifferentiated cell state associated with more adverse outcomes than committed, might become more responsive to PI3K inhibition through dual ceramide synthesis inhibition. This agrees with a previous finding that maintenance of a primitive cellular status is associated with muted PI3K/mTORC1 activity. Together, these results provide novel insight into lipidomic differences between primitive and committed AML, and rationalize the targeting of specific lipid-associated metabolic pathways for AML therapeutics.

## *Personalized cancer treatment strategies incorporating irreversible and reversible drug resistance mechanisms*

**Presenter: Wei He**

**Georgetown university**

*Wei He (Georgetown University, Washington, DC), Matthew D. McCoy (Georgetown University, Washington, DC), Rebecca B. Riggins (Georgetown University, Washington, DC), Robert A. Beckman (Georgetown University, Washington, DC), Chen-Hsiang Yeang (Academia Sinica, Taipei, Taiwan)*

Despite recent advances in targeted cancer therapy, the promise of precision medicine has been limited by resistance to these treatments. Intratumoral genetic heterogeneity and non-genetic plasticity in cancer cells are two major factors of cancer treatment resistance, and are widely associated with poor outcomes and reduced response to therapies. Previously we proposed a personalized treatment strategy involving two drugs that designed individualized treatment sequences by simulations of irreversible genetic evolutionary dynamics in a heterogeneous tumor. We termed the strategy Dynamic Precision Medicine (DPM), as the conventional precision medicine approach attempts to match a drug (or a combination of drugs) to the molecular profile of a patient but does not address the complex relations between the patient's molecular profile, possible treatment sequences, and the dynamic response of the tumor. The treatment strategies can be summarized as follows:

Current personalized medicine strategy: treats the virtual patient with the most effective drug on the most abundant cellular population and continues until one of the following events occurs: (i) the total cell number reaches twice the minimum of the total cell number among the time-series profile or (ii) the total cell number reemerges from a level below the detection threshold. Upon discontinuation of one drug, the process is repeated.

DPM strategy 1: At each time step, select the drug or reduced dose simultaneous combination that minimizes the predicted total cell number in the next time step. This strategy is intuitive but also myopic.

DPM strategy 2: At each time step, minimize the predicted cell number of the doubly irreversible resistant cell state unless immediate mortality is imminent, and switch to minimizing the total cell number if the latter occurs.

DPM strategies capture the population dynamics of tumor subclones as they acquire resistance to two non-cross resistant drugs through independent mutations, and proposes a treatment selection strategy to design the treatment sequence to balance the immediate goal of shrinking tumor size and the long-term goal of preventing the emergence of an incurable subclone resistant to both drugs.

We now report a single, integrated mathematical model incorporating cellular heterogeneity, genetic evolutionary dynamics, and non-genetic plasticity, accounting for both irreversible and reversible drug resistance. The unified framework encompasses both irreversible and reversible drug resistance for two non-cross resistant drugs and we apply DPM to the joint model that simultaneously tackles irreversible and reversible drug resistance mechanisms. We evaluate the effectiveness of nine treatment strategies by stimulating the dynamics of cancer cell populations. We conduct a clinical trial simulation over 6 million virtual patients over 5 years and demonstrate that the DPM-based personalized treatment strategies result in superior patient outcomes compared with the current personalized medicine treatment approach. Furthermore, DPM strategies incorporating periodic treatment sequences that cycle between therapies over a shorter treatment window, designed to combat reversible resistance, are marginally superior to those without such options. Our results provide insights into cancer treatment strategies for heterogeneous tumors with genetic evolutionary dynamics and non-genetic cellular plasticity, potentially leading to improvements in survival time for cancer patients.

## *Dynamic rewiring of cell-cell interactions in the metastatic tumor microenvironment primes response to checkpoint inhibition*

**Presenter: Adam MacLean**

**USC**

*Edgar Gonzalez (University of Southern California, Los Angeles, CA)*
*Jesse Kreger (University of Southern California, Los Angeles, CA)*
*Yingtong Liu (University of Southern California, Los Angeles, CA)*
*Sarah M. Shin (Johns Hopkins University, Baltimore, MD)*
*Arianna Barbetta (University of Southern California, Los Angeles, CA)*
*Sarah-Elisa B. Bangerth (University of Southern California, Los Angeles, CA)*
*Julie Jang (University of Southern California, Los Angeles, CA)*
*Matthew Jacobo (University of Southern California, Los Angeles, CA)*
*Batul Al-Zubeidy (University of Southern California, Los Angeles, CA)*
*Aaron G. Baugh (University of Southern California, Los Angeles, CA)*
*Vered Stearns (Cornell University, Ithaca NY)*
*Roisin M. Connolly (University College Cork, Cork, Ireland)*
*Won Ho (Johns Hopkins University, Baltimore, MD)*
*Juliet Emamaullee (University of Southern California, Los Angeles, CA)*
*Adam L. MacLean (University of Southern California, Los Angeles, CA)*
*Evanthia T. Roussos Torres (University of Southern California, Los Angeles, CA)*

Tumors grow, evolve, and metastasize as a result of an intricate set of interactions between numerous signals and cell types that compose the tumor microenvironment (TME). Many of these interactions remain poorly understood, even in the absence of new therapeutic regimes. Immune checkpoint inhibition (ICI) has been shown to promote durable responses in a minority of patients with metastatic, triple negative breast cancer. Evaluation of changes in the breast TME revealed that decreased infiltration or suppression of myeloid derived suppressor cells (MDSCs) by entinostat is a potential mechanism of action, but the effects of entinostat on the TME have not been studied at metastatic sites, predicted to be most responsive to treatment. Here, we measured the molecular properties of the metastatic TME in high resolution via scRNA-seq, quantifying 39 cell states across six treatment arms. We observed significant shifts in composition of C1q macrophages, metabolically activated macrophages, classic dendritic cells, activated/primed Th2 T cells, and terminally differentiated T regulatory cells. Entinostat treatment led to an increase in stemness in tumor cells and decreased mesenchymal gene expression. We then developed methods to reveal crucial cell-cell interactions in the data, by inferring cell circuits that are over-represented in their signaling probability. The highest ranked cell circuits (out of 9,139 possible three-state networks) were composed of myeloid and T cell subtypes; some interactions of which were dramatically affected by combination therapy. Top pathways contributing to these interactions included the chemokine, galectin, and ICAM pathways: we identified specific ligand-receptor pairs for each of these pathways, and tested them using functional suppression assays. From these we can determine the contribution of signaling axes to the improved outcomes (decreased immunosuppression) observed within the TME. We also simulate simple mathematical models to predict the maximal possible therapeutic response for a given treatment intervention. Beyond the clinical impact of our work, these methods offer a framework with which to decompose large, complex TMEs to infer multiscale networks mediating treatment effects and model their dynamics.

## *Myeloid Cell Regulation in Patients with Advanced Prostate Cancer treated with Bipolar Androgen Therapy*

**Presenter: David E. Sanin**

**Johns Hopkins University**

*David E. Sanin (Johns Hopkins University, Baltimore, MD, USA)*
*Laura A. Sena (Johns Hopkins University, Baltimore, MD, USA)*
*Elizabeth A. Thompson (Johns Hopkins University, Baltimore, MD, USA)*
*Rajendra Kumar (Johns Hopkins University, Baltimore, MD, USA)*
*Tracy Jones (Johns Hopkins University, Baltimore, MD, USA)*
*Carolina Gomes Alexandre (Johns Hopkins University, Baltimore, MD, USA)*
*Mark C. Markowski (Johns Hopkins University, Baltimore, MD, USA)*
*Angelo M. De Marzo (Johns Hopkins University, Baltimore, MD, USA)*
*Samuel R. Denmeade (Johns Hopkins University, Baltimore, MD, USA)*

Prostate cancer patients acquire resistance to standard-of-care strategies progressing to advanced disease and resulting in 350,000 yearly deaths. As acquired resistance is mediated by increased androgen receptor (AR) expression, “Bipolar Androgen” therapy (BAT) is being developed to cycle serum testosterone from supraphysiological to near-castrate levels, maximizing toxicity to high and low AR-expressing cells respectively. BAT is a clinically effective, safe and unique approach to treat castration-resistant prostate cancer (CRPC) patients that improves quality of life, produces biochemical and objective responses, and re-sensitizes tumors to AR inhibitors. Data from a recent clinical trial (NCT03554317) shows that prostate tumor cells produce inflammatory cytokines following BAT, and patients who benefited most from this therapy have an enriched inflammatory transcriptional signature in tumors. Thus, despite its conception as a targeted therapy, consideration for BAT’s effects on the immune system appears critical for success. To capitalize on this unappreciated potential and bridge the gap between patients who benefitted or not from this novel strategy, we set out to define the changes in immune cells from patient peripheral blood mononuclear cells (PBMCs) and in tumor biopsies before and after treatment with BAT. We used a combination of high-resolution high-throughput techniques including spectral flow cytometry, single cell RNA sequencing and spatial transcriptomics, then applied state of the art computational methods to extract meaningful insights from these samples. Our observations indicate that BAT skews the development of classical and non-classical monocytes in peripheral blood, which in turn impacts the resulting infiltration and differentiation of these cells into macrophages in the tumors. Indeed, patients that failed to respond to treatment displayed unique myeloid populations that went onto differentiate into macrophages with a tumorigenic phenotype. The precedent in the literature that testosterone dampens the pro-inflammatory phenotype of macrophages, plus the critical role of the inflammatory response in controlling tumor growth following BAT, lead us to the hypothesis that these changes in the myeloid compartment induced by BAT may restrict antitumor immunity leading to reduced therapeutic efficacy. We are furhter investigating transcriptional signatures in myeloid cells that are associated with therapeutic response and modeling monocyte tumor engraftment as these cells contribute to the immunosuppressive tumor microenvironment. This effort across the disciplines of computational biology, oncology, and myeloid cell biology, will build a detailed understanding of how BAT reprograms tumor immunity and determine if myeloid cell remodelling underpins resistance to BAT, thus providing a target to improve therapeutic efficacy in the design of future clinical trials.

# Session #5: Systems Pharmacology & Translational Systems Biology
# Tuesday, February 11th 2025

## *A Mathematical Model of Tumor Cell Interactions with Bone-Resident Cells Predicts Tumor-Type-Specific Responses to Perturbations*

**Presenter: Leonard A. Harris**

**University of Arkansas**

*Natalie E. Bennett (Vanderbilt University, Nashville, TN)*
*Alexandra Gutierrez Vega (University of Arkansas, Fayetteville, AR)*
*Erik P. Beadle (Vanderbilt University, Nashville, TN)*
*Saja Alshafeay (University of Arkansas, Fayetteville, AR)*
*Ramcharan Chitturi (University of Arkansas, Fayetteville, AR)*
*Anishka Nagarimadugu (University of Arkansas, Fayetteville, AR)*
*Julie A. Rhoades (Vanderbilt University, Nashville, TN)*
*Leonard A. Harris (University of Arkansas, Fayetteville, AR)*

Bone metastases cause severe skeletal-related events--including pain, fractures, and hypercalcemia--a condition known as tumor-induced bone disease (TIBD). Current treatments for TIBD, based on the so-called "vicious cycle" model, address bone resorption but offer limited survival benefits and may cause adverse side effects. The lack of effectiveness of these therapies is likely due to limited understanding of the complex interactions among cells within the bone-metastatic microenvironment. A major challenge in studying these cell-cell interactions is the reliance on murine models, which, while informative, are limited by interspecies differences and the complexity of in vivo experimentation, highlighting the need for complementary approaches. In this study, we present a computational model of the interactions among tumor cells, osteoclasts, osteoblasts, and the bone matrix. Expanding on an established model of osteoclast-osteoblast dynamics, we incorporate tumor-bone crosstalk and simulate responses to pharmacological interventions, including zoledronic acid (ZA), an osteoclast inhibitor. Using Bayesian Monte Carlo parameter estimation, we calibrate the model to experimental data from two murine tumor types under untreated and ZA-treated conditions. This ensemble approach enables robust predictions across diverse experimental conditions, allowing us to explore tumor-type-specific responses to therapeutic interventions. Our findings predict that untreated mice with mildly-aggressive tumors will experience a modest increase in osteoclasts and little to no change in osteoblasts, in sharp contrast to observations in untreated mice with highly-aggressive tumors, which exhibit a significant reduction in osteoblasts. For ZA-treated mice with highly-aggressive tumors, the model predicts an initial spike in bone density followed by a decline, ranging from gradual to rapid, and minimal effects on osteoblasts and tumor growth. This differs from observations in ZA-treated mice with mildly-aggressive tumors, where bone density stabilizes after an initial spike. Taken together, these findings underscore tumor-type-specific differences in treatment responses, highlighting the potential need for tailored therapeutic strategies against TIBD. Our model provides a flexible in silico platform for investigating therapeutic interventions within the bone-metastatic microenvironment and represents an essential first step toward a comprehensive computational model of TIBD that can complement physical models and serve as a resource for the research community. Ultimately, by integrating in silico predictions with in vitro and in vivo data, this approach can guide the development of therapies that reduce tumor burden, prevent bone destruction, and improve clinical outcomes for patients with metastatic cancer.

## *Network topology explains drug synergistic effects*

**Presenter: Emily Bozich**

**University of California, Los Angeles**

*Emily R. Bozich (University of California, Los Angeles, CA)*
*Jennifer L. Wilson (University of California, Los Angeles, CA)*

Gene and drug combinations are attractive for effective disease management, yet predicting their synergistic effects remains challenging as it requires an intricate understanding of their targets' connections to signaling pathways. For cancer drug combinations, even machine learning models trained on single perturbation and cell line information report model-experiment correlations of ~0.24-0.48. Further, while protein-protein interaction network (interactome) methods have been useful in ranking and predicting binary drug combination effects, few have predicted continuous drug synergy solely from interactome topology.

Given this gap, we developed cell signaling-informed, diffusion-based topological distance metrics to predict continuous experimental synergy. Specifically, we first used a network diffusion algorithm to model how a single target perturbation can propagate and affect the activity of nearby proteins. Expanding this to consider the effects of a dual target perturbation, we measured the relative topological positions (i.e., distance) of a combination's target neighborhoods within the interactome. Given the interactome's complex topology, we exhaustively tested 36 diffusion-based distance metrics, each encoded by a unique set of network features that quantified various assumptions of local vs. global diffusion, the importance of target neighborhoods, and signal propagation directionality.

First, we applied our interactome distances to target pairs screened in a pairwise CRISPR knockout experiment conducted in a chronic myeloid leukemia (CML) cell line. Across distance metrics, we found that the screen's most toxic and synergistic target pairs (n = 57) are significantly closer than background target pairs (t-test, $P < 0.05$), suggesting that target closeness may sufficiently explain strong experimental effects. Additionally, we found that for these 57 target pairs, there exists a moderately negative correlation between network distance and experimental synergy (Pearson's correlation coefficient = ~-0.40).

To determine if this distance-synergy relationship extended to combinations with weaker effects, we turned to five breast cancer cell line validation sets derived from a multi-target small molecule combination screen. Because multiple target pair distances corresponded to a single small molecule combination, we employed a curve fitting framework to identify the single target pair that best explained a combination's cell line-specific experimental effects. Using the most accurate and robust distance metric across cell line models, we found synergy correlations of -0.35 – -0.58, which corroborate our findings in the CML cell line model. Separately, as each model was fit per cell line, we identified unique target pair interaction(s) that we speculate may drive a combination's cell line-specific pathway mechanisms.

We further used sensitivity analyses to understand performance differences between distance metrics encoded by varied network features. In doing so, we identified features of intracellular signaling – namely the importance of local diffusion and inter-target neighborhood interactions in recapitulating single and combined perturbation effects – that are most predictive of continuous synergy effects.

Overall, our work demonstrates that topological distance is as performant as monotherapy and molecular information while still providing interpretable explanations for combination pathway mechanisms. Our model can provide an exciting opportunity to more efficiently design resource-deprived combination screens and ultimately facilitate the transition of more efficacious drug combinations to the clinical setting.

## *Rapid non-genetic drug adaptation to MAPK pathway inhibitors in melanoma*

**Presenter: Varuna Nangia**

**University of Colorado Boulder**

*Varuna Nangia (University of Colorado Boulder, CO)*
*Humza Ashraf (University of Colorado Boulder, CO)*
*Victor Passanisi (University of Colorado Boulder, CO)*
*Nasreen Marikar (University of Colorado Boulder, CO)*
*Christopher Ill (University of Colorado Boulder, CO)*
*Timothy Hoffman (University of Colorado Boulder, CO)*
*Sabrina Spencer (University of Colorado Boulder, CO)*

Over 50% of melanomas are driven by BRAFV600 mutations. Clinically, targeted inhibitors of mutant BRAF and MEK are the primary treatment for the >50% of melanoma patients refractory to standard-of-care immunotherapy. While targeted therapies are initially clinically effective, a subset of residual cancer cells persist and inevitably drive tumor recurrence and tumor-wide drug resistance. While some cells persist due to pre-existing mechanisms (e.g. genetic mutations), others can non-genetically adapt to tolerate drug by rewiring their internal signaling cascades. Importantly, a key feature of these non-genetically adapted persister cells is that their drug-tolerant phenotype is acquired in response to drug pressure, making them extremely challenging to identify and characterize. By using cutting-edge long-term time-lapse microscopy and an automated single-cell tracking pipeline, we studied the behaviors of persister cells in drug-treated BRAFV600E melanoma populations. Notably, we found significant heterogeneity within persister populations. While all persister cells initially enter a state of drug-induced quiescence, a subset of these cells—termed “escapees”—can escape drug action and resume proliferation. By computationally reconstructing single-cell lineages from long-term time-lapse imaging experiments, we monitored key signaling pathways before, during, and after escape from drug. Using this approach, we identified a significant increase in mTorC1 signaling activity in cells that would go on to escape drug. Additionally, mTorC1 signaling caused increases in protein translation and cell growth rates during the quiescent periods of these cells. The abundance of Cyclin D1, a key driver of proliferation, is extremely sensitive to cellular translation rates due to its short half-life. In order to determine if the observed increase in mTorC1-mediated growth and translation is driving the upregulation of Cyclin D1 levels past a threshold necessary for escape, we used CRISPR to tag Cyclin D1 at the endogenous locus. Importantly, we found that escapees significantly upregulate Cyclin D1 levels prior to their escape in comparison to non-escapees. These findings have significant clinical implications because they suggest that Cyclin D1 protein levels are the bottleneck for cell-cycle re-entry under MAPK inhibition. Additionally, these findings implicate Cyclin D1 as one of the first markers that can distinguish future escapees from non-escapees in the quiescence period before these distinct cell-cycle fates emerge.

## *Systems modeling of mitotic signaling in triple-negative breast cancer*

**Presenter: Todd Stukenberg**

**University of Virginia**

*P. Todd Stukenberg (University of Virginia, Charlottesville, VA)*
*A. Catalina Alvarez-Yela (University of Virginia, Charlottesville, VA)*
*Sarah M. Groves (University of Virginia, Charlottesville, VA)*
*Monserrat Gerardo-Ramírez (University of Virginia, Charlottesville, VA)*
*Kevin A. Janes (University of Virginia, Charlottesville, VA)*

Abstract:
Chromosome missegregation causes whole-chromosome aberrations and aneuploidy characterizing most solid tumors and virtually all breast cancers of the triple-negative subtype. The origins of chromosome instability (CIN) are poorly understood because genes involved in the mitotic spindle are almost never mutated in cancer, although abundances are highly variable. We have found that the overexpression of the G2 transcriptional regulator FoxM1 correlates with aneuploidy in breast tumors and its overexpression is sufficient to cause CIN in nontransformed vertebrate tissues. However, it is not clear which of the proteins that are transcriptionally regulated by FoxM1 lower the fidelity of mitosis after its overexpression. We hypothesize that FoxM1 lowers the fidelity of mitosis by altering the abundances of mitotic signaling proteins. Mitotic signaling protein abundances are highly altered in tumors that have high FoxM1 expression. To test the hypothesis, we have built a reaction diffusion model of mitotic signaling on a prototypical metaphase chromosome that includes proteins regulating the spindle checkpoint, sister chromosome cohesion and correcting improper kinetochore-microtubule attachments. Initially we are focusing on how the network employs epigenetic signals to assemble a signaling center between kinetochores to generate chromosome autonomous regulation. Specifically, we are following the localization of the Chromosome Passenger Complex (CPC) to the inner centromere, which is a chromosome location between kinetochores. The network includes protein kinases and histone modifications that recruit the CPC to chromatin within minutes after prophase. Upstream kinases are localized to kinetochores and by interactions with cohesin, which is lies between sister chromatids, and positive feedback of the networks allows rapid and robust recruitment of the CPC to inner centromeres at the overlap of these locations. Our model recapitulates the dynamics of CPC localization and is highly constrained by biochemical parameters from the literature and protein concentrations measured in metaphase-arrested triple-negative breast cancer cells and controls. This first model of mitotic signaling explains how cells can use the histone code to epigenetically build a single signaling center on each mitotic chromosome and enables future studies to determine how mitotic events are coordinated and how the overexpression of FoxM1 generates CIN.