Systems Approaches to Cancer Biology 2025

Poster Presentation Abstracts

Poster session A: Monday, Feb. 10, 5:00 pm - 6:30 pm

Poster #1

Unlocking Mitochondrial Dysfunction-Associated Senescence (MiDAS) with NAD+ – a Boolean Model of Mitochondrial Dynamics and Cell Cycle Control

Presenter: Erzsébet Ravasz Regan

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The steady accumulation of senescent cells with aging creates tissue environments that aid cancer evolution. Aging cell states are highly heterogeneous. 'Deep senescent' cells rely on healthy mitochondria to fuel a strong proinflammatory secretome, including cytokines, growth and transforming signals. Yet, the physiological triggers of senescence such as the reactive oxygen species (ROS) can also trigger mitochondrial dysfunction, and sufficient energy deficit to alter their secretome and cause chronic oxidative stress – a state termed Mitochondrial Dysfunction-Associated Senescence (MiDAS). Here, we offer a mechanistic hypothesis for the molecular processes leading to MiDAS, along with testable predictions. To do this we have built a Boolean regulatory network model that qualitatively captures key aspects of mitochondrial dynamics during cell cycle progression (hyper-fusion at the G1/S boundary, fission in mitosis), apoptosis (fission and dysfunction) and glucose starvation (reversible hyper- fusion), as well as MiDAS in response to SIRT3 knockdown or oxidative stress. Our model reaffirms the protective role of NAD+ and external pyruvate. We offer testable predictions about the growth factor- and glucose-dependence of MiDAS and its reversibility at different stages of reactive oxygen species (ROS)-induced senescence. Our model provides mechanistic insights into the distinct stages of DNA-damage induced senescence, the relationship between senescence and epithelial-to- mesenchymal transition in cancer and offers a foundation for building multiscale models of tissue aging.

Poster #2

Predicting Downstaging in Muscle-Invasive Bladder Cancer Using Conditional Autoencoders and SHAP-Based Feature Analysis in SWOG S1314 Data

Presenter: Sutanu Nandi

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Muscle-invasive bladder cancer (MIBC) is a highly aggressive cancer type associated with a substantial risk of progression and mortality. The standard-of-care for eligible patients with MIBC is neoadjuvant chemotherapy followed by radical cystectomy. Here, we leverage data from the Southwest Oncology Group (SWOG) trial S1314 to evaluate downstaging responses in MIBC patients after chemotherapy—a clinical marker frequently linked to improved prognosis. We applied machine learning models to analyze gene expression data, clinical variables, and treatment responses, aiming to identify molecular features associated with downstaging. Using a Conditional Autoencoder (CAE), we extracted latent representations from high-dimensional gene expression data, capturing essential molecular patterns associated with treatment outcomes. These latent features were then used to train classification algorithms, including Support Vector Machines, to predict downstaging. Our results demonstrated that deep learning models leveraging gene expression profiles achieved strong performance on the validation dataset in predicting downstaging (Balanced Accuracy: 0.8377, F1-Score: 0.8571, and AUC: 0.8961), underscoring their potential as tools for personalized therapy stratification in MIBC. SHapley Additive exPlanations (SHAP) feature importance analysis identified key genes (e.g., IRAK3, SLC18A2, ADAM28, CYP7B1) that contribute to MIBC downstaging in response to treatment.

This study highlights the value of applying the CAE framework with high-throughput molecular data in clinical oncology to identify important features relevant to MIBC treatment response. Future work will focus on validating these findings in larger, independent cohorts and exploring potential clinical applications.
Keywords: Muscle-invasive bladder cancer, Neoadjuvant chemotherapy, Downstaging, Gene expression data, Conditional Autoencoder

Poster #3

In vitro macrophage-tumor-fibroblast spheroid co-cultures model evolution and heterogeneity of tumor-associated macrophages

Presenter: Janani P. Baskaran

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Macrophage-fibroblast interactions mediated by cell-cell contact and secreted signals are critical for maintaining tissue homeostasis. During tumor progression, cancer cells alter homeostatic networks between macrophages and fibroblasts, causing macrophages to transition from an inflammatory anti-tumor state to an immunosuppressive pro-tumor state. However, we do not yet fully understand the critical signals involved in this transition. Spheroid cultures have been shown to model the physical properties of tumors, including direct cell-cell interactions and spatial orientation of cells. Therefore, we hypothesized that tumor spheroids would recapitulate interactions between macrophages, fibroblasts, and cancer cells in the early tumor microenvironment (TME) that result in the heterogeneous tumor-associated macrophage (TAM) phenotypes observed in vivo. To test this, we engineered a 3D in vitro tumor spheroid model containing macrophages, fibroblasts, and cancer cells.

We constructed spheroids with the immunogenic YUMMER1.7 murine melanoma tumor cell line, primary bone marrow-derived macrophages, and 3T3MEF fibroblasts embedded into a collagen gel. The spheroid co-cultures are viable for up to 8 days, and by day 4, they mimic the fibroblast, macrophage, and tumor cell composition of in vivo melanoma tumors. We evaluated the macrophage evolution in spheroids using a multiplex panel of surface and intracellular macrophage phenotypic markers. Over the first few days in culture, we observed increased expression of immunosuppressive markers, such as Arg1 and CD206. At later timepoints, the macrophages become more heterogeneous, with a population of mixed phenotype (iNOS+Arg1+; CD86+CD206+) macrophages. We investigated this characteristic TAM heterogeneity in both scRNA-seq and protein expression from untreated in vivo YUMMER1.7 tumors (d3, d6, d8) and found that both proinflammatory and anti-inflammatory markers become more heterogeneously expressed over time.

Similar macrophage heterogeneity could not be achieved by co-culturing cells in 2D or with tumor-conditioned media. We also assessed the functional response of spheroid macrophages to agonistic CD40 (CD40ag), an immunotherapy being tested in the clinic to reprogram immunosuppressive macrophages in solid tumors. Interestingly, while early treatment of CD40ag (day 4) showed no response, treatment on day 6, when macrophages were more immunosuppressive, elicited a pro-inflammatory response as in tumors, suggesting that the tumor spheroid model can be used to probe TAM function in some contexts. Subsequently, we are interested in understanding how communication with fibroblasts and cancer cells contributes to macrophage immunosuppression in the early TME. 3T3MEF fibroblasts from similar macrophage-melanoma cell co-cultures secrete GM-CSF, G-CSF, and CX3CL1, which may impact macrophage immunosuppression. Next steps are to block these secreted proteins in spheroids to determine if they contribute to the evolution of TAMs.

Overall, these studies suggest that the tumor spheroid co-culture recapitulates the in vivo TAM phenotype and function, which enables investigation of TAM evolution in the immunosuppressive TME and during rescue of TAM anti-tumor function with macrophage-targeted treatment.

This work is supported by the NIH (5U01CA238728-03) and Cancer Research Institute.

Poster #4

Computational alternative to cardiac biopsy in multiple myeloma: utilizing the shape of routine laboratory data for the classification of cardiac amyloidosis

Presenter: Maegan Cremer

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Multiple myeloma (MM) is characterized by the abnormal monoclonal proliferation of plasma cells with an increase in monoclonal immunoglobulins. Patients with MM are at increased risk for cardiac amyloidosis (light chain) due to high circulating levels of light chain. We are striving to identify the key clinical features which differentiate MM patients with and without cardiac amyloidosis using mathematical models. Existing approaches for sorting patients into diagnostic categories tend to ignore the temporal relationship or shape of longitudinal patient data. To utilize the potential wealth of information in longitudinal medical data, we fit regression models to laboratory data and used the parameters and characteristics of the fit as features for our machine learning models.

We built support vector machine models (SVMs) using electronic medical record data from MM patients evaluated for cardiac amyloidosis at Moffit Cancer Center using echo and/or cardiac MRI. Data includes treatment and diagnosis history, laboratory values, and scores for functional status. The regression models include linear and exponential models where the intercept is a patient's first recorded value. A classifier using raw data at a single time point proximal to the date of cardiac amyloidosis diagnostic imaging was used for comparison. SVM classifiers trained on both classical statistics and parameters of fit of the data (F1 score = 0.971, ROC AUC = 0.978) outperformed the raw data proximal to the date of diagnostic imaging (F1 score = 0.905, ROC AUC = 0.844) in identifying which patients were diagnosed with cardiac amyloidosis. The model trained on both classical statistics and parameters of fit selected features describing the temporal relationship of patient data. The most influential feature was the exponential coefficient of mean corpuscular volume. This model is likely to be the most clinically interpretable and translatable as its feature list captures a holistic view of patient physiology through measures of renal function, anemia, inflammation, and multiple myeloma markers. We will further develop this method using regression models to predict cardiac function.

Poster #5

Computational Modeling of Cancer and Immune Cell Migration in Complex Chemokine Microenvironments

Presenter: Ashlee N. Ford Versypt

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Cell migration in tumor-immune microenvironments plays a crucial role in disease progression and treatment efficacy.

Recent experimental studies reported variations in cell migration through changing microenvironments depending on phenotypes, chemokines, and collective cell structures. Here, we developed an agent-based model (ABM) with Compucell3D (a cellular Potts lattice-based model) to simulate the physiological response and identify the effects of random and directed cell migration in response to chemokines. The ABM simulates the dynamics of the transwell cell migration assay. The model shows a 3D slice space of the transwell device with 400 moving agents. Simulations are repeated for different sizes and adhesive properties to represent a set of cancer and immune phenotypes. With periodic boundary conditions applied to vertical surfaces of the domain, the model can simulate in vitro transwell experiments where cells have realistic biomechanics of neighboring cells and tissue-mimic biomaterials. The group of moving agents mimics cells with Brownian motion, located above a stationary porous plane representing a collagen-coated transwell membrane. The membrane contains randomly distributed pores that simulate the realistic structure of the transwell membrane with the same level of pore density. Chemokines are initialized from the bottom of the transwell below the membrane and can diffuse upwards to generate a concentration gradient. Several factors, including chemical concentrations, diffusion coefficients, chemotactic potential coefficient, an external potential energy term, and a contact energy term are included with a direct connection to published data. The randomized external potential energy simulates the intrinsic Brownian motion of cells and drives cells to move through membranes in the negative control group without chemokines. We observed that larger external potential energy can induce more cells to migrate through the membrane. Thus, we calibrate this energy term with negative control group data from different cell lines (e.g., Panc1, MiaPaCa2, and HPAFII).

Poster #6

Replication stress, suppressed adaptive immunity and fibroblast neighborhoods distinguish liver and lung organotropism in pancreatic cancer

Presenter: Jennifer Eng

OHSU

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Pancreatic ductal adenocarcinoma (PDAC) patients face a dismal prognosis with 5-year overall survival rates of just 13%. A minority of PDAC patients present with resectable disease; the remaining have locally advanced or metastatic disease with a median survival outlook of months. However, PDAC patients with a distant lung metastasis can survive upwards of five years following diagnosis. We compared patients with lung but not liver metastases (lung cohort, 35 patients) to those with liver metastases (liver cohort, 130 patients) to reveal lung cohort membership independently predicted overall survival while liver cohort was associated with rapid recurrence following surgery.

Transcriptomic profiling of PDAC has defined two consensus subtypes: an aggressive basal-like subtype and a relatively less aggressive but still deadly classical subtype. While liver cohort patients were enriched in the basal-like subtype relative to lung cohort, a majority were classical subtype tumors. We used two-factor modeling in DEseq2 to model subtype and liver/lung organotropism and defined a gene signature associated with liver organotropism but not basal-like subtype, termed primary organotropism (pORG). pORG scores predicted survival independent of clinical co-variates and in two external datasets (TCGA and ICGC).

The pORG signature enabled us to identify pathways associated with liver tropism but not subtype. High-pORG tumors were uniquely enriched in replication stress and interferon response while basal-like tumors had elevated glycolysis, hypoxia and EMT signatures. Tumors with high pORG scores uniquely tolerate high levels of replication stress as evidenced by tumor aggressiveness in the context of DNA damage repair deficiency. Supporting this, high pORG tumors had elevated proliferation in phosphorylated replication protein A positive cells relative to low pORG tumors.

We investigated the liver-tropic immune microenvironment with deconvolution analysis, revealing suppression of the adaptive immune response in patients with high pORG, liver tropic PDAC. These findings were supported by multiplex IHC analysis showing reduced T and B cell density and increased myeloid lineages in high pORG tumors. We further interrogated the adaptive immune response using T cell receptor (TCR) sequencing. Tumoral TCR richness and diversity were associated with good outcome generally, while clonal TCR expansion was associated with control of liver outgrowth in the metastatic setting.

To further characterize the tumor microenvironment interactions in aggressive PDAC, we generated multiplex imaging data (n=34 tumors) and matched single cell sequencing (n=7 tumors) to reveal single-cell spatial interactions associated with liver tropism. We utilized latent Dirichlet allocation to model stromal cell neighborhoods surrounding tumor. We identified two unique fibroblast neighborhoods in high-pORG, liver-tropic tumors while putative complement expressing fibroblasts surrounded tumors cells in low pORG tumors, consistent with a proposed tumor restraining role.

In summary, we interrogated deeply annotated clinical samples with genomic, transcriptomic, TCRseq, and single-cell imaging and transcriptomics methods to reveal features of metastatic organotropism in primary PDAC. Deconvolving subtype and organotropism allowed us to identify unique tumor intrinsic and microenvironmental properties, including replication stress, chronic interferon response and suppression of adaptive immunity associated with liver-avid disease. Future work will expand on single-cell, spatial analysis suggesting unique fibroblast-tumor interactions underling PDAC metastatic tropism.

Poster #7

Glutamine regulates osteoblast differentiation by modulating RUNX2 translation

Presenter: Mumtaz Shirin

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Proliferating cells require glutamine, an indispensable substrate, to build biomass for various metabolic pathways, protein synthesis, nucleotide glucosamine and non-essential amino acid biosynthesis, redox homeostasis, and uptake of other amino acids. Cancer is associated with glutamine depletion from the microenvironment. Of note, glutamine is essential for driving the differentiation of mesenchymal stem cells (MSCs) into mature bone-building osteoblasts via an unknown mechanism. The growth of cancer cells within the bone disrupts normal bone homeostasis and leads to the degeneration of bone tissue, also known as

osteolysis. However, the role of cancer-driven glutamine depletion in disrupting bone homeostasis has not been evaluated.

In this study, we demonstrate that Runt-related transcription factor 2 (RUNX2) is exquisitely sensitive to glutamine depletion. RUNX2 is a critical transcription factor that drives the commitment and differentiation of MSCs to osteoblasts and away from the alternate adipocyte fate. Our data further suggests that a unique structural domain of RUNX2, a homopolymeric polyglutamine tract, compromises RUNX2 translation and downregulates RUNX2 protein levels specifically in response to glutamine depletion. Excessive RUNX2 levels in glutamine-depleted pre-osteoblasts result in compromised cell fitness, suggesting an adaptive function of this sensing mechanism. Overall, these findings identify RUNX2 as a novel glutamine sensor and metabolic regulator that allows osteoprogenitors to make important cell survival and fate decisions upon glutamine availability. This provides a novel, metabolism-informed therapeutic strategy for combatting cancer-associated osteolysis.

Poster #8

Cell density as a determinant of de novo glutamine synthesis

Presenter: Lisa Shakachite

University of Utah

Lisa Shakachite (University of Utah, Salt Lake City, UT)
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Solid tumors are composed of a complex, heterogeneous milieu of different cell types with an environment characterized by accumulation of abundant extracellular matrix. Additionally, regions within a solid tumor have been shown to differ in cell density. The growth and proliferation of cells in the tumor microenvironment (TME) are reliant on non-cell autonomous signals like growth factors, positional cues, and cell density. Recently, the role of cell density as a modulator of glucose uptake and utilization has been demonstrated. However, whether cell density affects amino acid metabolism as well, has not been explored.

Amino acids, which are essential for building biomass, are in high demand in the TME. In response to increased demand and poor tumor vascularization, a harsh nutrient-deprived environment is established in the TME. Glutamine, particularly relevant for solid tumors, becomes largely depleted because it is necessary for many essential cell processes like growth, proliferation, TCA anaplerosis, protein translation, and nucleotide biosynthesis. Tumor cells that cannot synthesize glutamine de novo, using glutamate-ammonia ligase (GLUL), become heavily addicted to exogenous glutamine despite depriving conditions. For example, stromal fibroblasts in the ovarian cancer TME were found to synthesize glutamine de novo and supplement it to the ovarian cancer cells. Though the establishment of this metabolic dependency is well-characterized, very little is known about the signaling mechanisms that govern the switch of stromal cells towards the de novo glutamine biosynthesis. This critical knowledge gap limits our ability to develop therapeutic strategies aimed at intercepting this key source of glutamine supply to the tumor.

Our preliminary data demonstrates that the ability of mesenchymal progenitor cells to upregulate GLUL protein in response to glutamine deprivation and proliferate without glutamine in the medium is governed by high cell density. Additionally, our data indicates that high-density cultures can maintain robust protein translation in the absence of exogenous glutamine. Our findings raise the possibility that non-cell autonomous cues such as cell density can regulate glutamine synthesis capacity by the mesenchymal lineage stromal cells in the TME. Due to the emergence of an extensive body of evidence that stromal cells support tumor cells in the TME, co-targeting them has emerged as a unique way to treat cancers. Therefore, it is imperative to understand the signaling pathways linking high cell density and glutamine synthesis to identify potential novel therapeutic targets. Taken together, these findings shed light on the potential mechanisms that can replenish glutamine supply to tumor cells thereby promoting cellular processes that sustain tumor progression.

Poster #9

Measuring interstitial fluid flow from dynamic contrast-enhanced MRI to model and optimize glioma and CAR-T cell dynamics

Presenter: Ryan Woodall

City of Hope

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Dynamic contrast-enhanced MRI is an advanced imaging modality which allows for researchers to observe the dynamics of Gadolium-based contrast agent entering into tumors. Contrast agent pools in tumors due to the enhanced perfusion and retention effect, which is caused by the leaky vasculature formed by cancer's tendency to over-express angiogenic growth factors. In the past, DCE-MRI was performed, and local perfusion rates within the tumor could be analyzed. However, perfusion dynamics only account for temporal variation in the contrast-enhancement signal within the tumor, and ignore the rich spatial information measured from this 3D imaging modality. Further, it is known that both invasive glioma cells, and therapeutic chimeric antigen receptor (CAR) T-cells preferentally migrate and invade with chemical gradients formed by interstial fluid flow in the brain and tumor interstitial space. As such, it is vital to measure this quantity for the assessment and comparison of tumors in vivo, and for use in predictive models of tumor growth and response to therapeutics. For this reason, we utilized modern machine learning techniques such as sparse identification of non-linear dynamics to develop a regression-based method for simultaneously measuring perfusion, diffusion, and advection of interstitial fluid. We call our method localized convolutional function regression. We demonstrate that LCFR is capable of measuring fluid velocity with less than 10% relative error in a hydrogel phantom, and that perfusion measured is strongly correlated with histological measurements of dye-infusions into the tumor via the blood-stream ($p<0.05$). We also demonstrate that patients with a higher standard deviation of interstital lfuid flow within their tumors demonstrate an increase in survival by 6 months when their recurrent tumors are treated with CAR-T cells. To investigate these methods, we have developed a spatio-temporal model of CAR-T cell migration, cancer killing, and exhaustion, which we use to investigate the differing effects of CAR-T cells in clinical trials. This model relies on the advection and diffusion as measured by LCFR. We anticipate that this methodology will enable us to predict patient response to many types of therapies by predicting the spatio-temporal therapeutic distribution in vivo.

Poster #10

Now or Later? Adding targeted therapies to first-line or second-line treatment produce the same survival benefits in multiple myeloma and HR+/HER2- breast cancer

Presenter: Noah Schlachter

UNC Chapel Hill

Noah Schlachter (UNC Chapel Hill), Adam Palmer (UNC Chapel Hill)

BACKGROUND:
New targeted cancer therapies are usually tested first in patients with relapsed or refractory disease, and if those therapies are approved as second-line (2L) treatments, they are often next tested in a combination therapy at the first-line (1L) of treatment. Patients who receive such drugs in first-line regimens will generally be 'on-therapy' for significantly longer, and therefore be exposed to more treatment-related adverse-effects and financial toxicities. Therefore, it is valuable to know whether the added survival time is greater when giving a drug upfront at 1L versus later at 2L. Here we investigate this question for two cancer therapies with both 1L and 2L approvals, namely CDK4/6 inhibitors for HR+/HER2- advanced breast cancer, and anti-CD38 antibody daratumumab for transplant-ineligible multiple myeloma.

METHODS:

Kaplan-Meier distributions of Progression-Free and Overall Survival were extracted from phase 3 trials evaluating 1L or 2L use of CDK4/6i for HR+/HER2- advanced breast cancer, and daratumumab for multiple myeloma, specifically CASTOR, POLLUX, MAIA, MONARCH2, MONARCH3, PALOMA2, PALOMA3, and SONIA. Different treatment sequences were simulated based on these clinically observed time-to-event distributions. For example, time to progression at first-line from "A+X" (where "X" is added drug, "A" is standard 1L treatment) was predicted from observed second-line PFS benefits (effect of "X" calculated from trial of "B" vs "B+X", where "B" is standard 2L treatment). The Overall Survival effect of daratumumab in the MAIA trial, in which 54% of control patients did not receive subsequent daratumumab, was simulated by applying observed time-to-progression benefits of subsequent daratumumab to 54% of patients.

RESULTS:
Daratumumab confers identical improvements in time to progression when added to first-line or second-line regimens for transplant-ineligible multiple myeloma. The overall survival benefit of giving Daratumumab as 1L therapy in the MAIA study was entirely explained by 54% of patients in the control group not receiving subsequent Daratumumab. Similarly, CDK4/6 inhibitors conferred identical durations of disease control in 1L and 2L HR+/HER2- advanced breast cancer. We find that the SONIA trial, which found no overall survival difference between 1L and 2L CDK4/6i treatment strategies ("A+X, then B" versus "A, then B+X"), had results that were accurately predicted using prior 1L and 2L trial data to simulate different treatment sequences.

CONCLUSION:
Cancer medicines approved for second-line treatment are often later approved in first-line combination regimens; these scenarios leave unanswered the question of when to best deploy these therapies. When clinical trials testing 1L use of drugs with 2L approval do not guarantee subsequent access to the drug for control arm patients, apparent improvements in Overall Survival do not meaningfully determine whether 1L use is optimal; they instead trivially demonstrate that it is better to receive a drug with proven survival benefits than not. For daratumumab in multiple myeloma and CDK4/6i in HR+/HER2- advanced breast cancer, these drugs confer identical durations of survival benefit at first-line or second-line, but first-line use entails markedly longer time on therapy (without longer survival benefit), increasing adverse events and costs.

Poster #11

Pol II degradation activates cell death independently from the loss of Pol II activity

Presenter: Nicholas Harper

UMASS Chan Medical School

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RNA Pol II-mediated transcription is essential for cellular life. It is commonly thought that, in the absence of transcription, mRNA pools will passively decay eventually resulting in cell death. While loss of transcription is thought to be universally lethal, the precise mechanisms of lethality remain uncharacterized largely due to the presumption that lethality in this context is not regulated. In contrast, we show that lethality of transcriptional inhibition depends on activation of a previously uncharacterized signal, which exclusively initiates apoptosis. Unexpectedly, apoptotic signaling is solely activated by the loss of inactive Pol II protein, not loss of Pol II activity. We identified unique genetic dependencies of this pathway, which we call the Pol II Degradation-dependent Apoptotic Response (PDAR). Using the genetic dependencies of PDAR, we identify common clinically used drugs that owe their efficacy to a PDAR-dependent mechanism. These findings highlight the existence of PDAR, a previously uncharacterized apoptotic response pathway that contributes to the efficacy of several anti-cancer therapies.

Poster #12

Computational Modeling in Cancer Research: Personalized Treatment Outcome Predictions

Presenter: Melike Sirlanci

University of Colorado Anschutz Medical Campus

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Computational models play a vital role in understanding biological systems, particularly in cancer research. They help elucidate time-evolving behaviors, quantify variable impacts, and support clinical predictions. These models can be categorized into two main types: data-driven and physiological knowledge-driven approaches. Data-driven models, such as machine learning (ML), focus on identifying patterns in data without prior knowledge of the system's mechanisms. While these models require large datasets for accuracy, they can incorporate diverse data types. Conversely, physiological mechanistic models are based on the underlying dynamics of the system, often represented through ordinary differential equations. These models can offer accurate predictions with smaller sample sizes but are limited to variables with known mechanistic roles. Both approaches possess unique strengths and can complement each other in research and practice.

These models can support cancer research in many ways. For instance, mechanistic models could enhance the understanding of the tumor – immune system dynamics, leading to new hypotheses, while data-driven models can identify factors influencing treatment responses. Our focus in using these models is to develop computational methodologies for personalized predictions of treatment outcomes using routine clinical data. Given the variability across cancer types and treatments, we focus specifically on predicting outcomes of chimeric antigen receptor (CAR) T-cell therapy in large B-cell lymphoma (LBCL) patients. LBCL is the most prevalent and aggressive form of non-Hodgkin lymphoma, with 60-70% of patients responding to first-line treatments, while others require additional therapies. CAR T-cell therapy has significantly increased cure rates for relapsed LBCL patients, but around 50% will still progress post-treatment.

Personalized predictions of treatment outcomes could inform the development of tailored treatment strategies. Early predictions following CAR T-cell infusion would assist clinicians in planning subsequent interventions, especially if the initial treatment appears likely to fail. However, this predictive task is complicated by challenges such as limited clinical data and the complex interactions between the immune system and cancer. We present an initial step toward our predictive goal by utilizing an existing mechanistic model of cancer cell and CAR T-cell dynamics. We estimated cancer cell counts from total metabolic tumor volume (TMTV) and used absolute lymphocyte count (ALC) measurements from the two weeks post-infusion as a surrogate for CAR T-cell counts. For each patient, we estimated the model using their routinely collected data consisting of a single TMTV measurement and 10-12 ALC measurements. Our findings suggest that mechanistic models can provide valuable insights into the personalized dynamics of cancer cells and CAR T-cells, paving the way for personalized predictions of CAR T-cell therapy outcomes. This approach may ultimately enhance treatment strategies and improve health outcomes for patients with LBCL.

Poster #13

Mechanism of Histone Deacetylase Inhibitor-Induced Lethality

Presenter: Gavin Birdsall

UMass Chan Medical School, Worcester, MA

Gavin Birdsall (Umass Chan Medical School, Worcester, MA)
Mike Lee (Umass Chan Medical School, Worcester, MA)

Post-translational modifications of histones contribute to gene regulation in cells. Perturbing histone marks by inhibiting epigenetic modifiers massively dysregulates gene expression. Histone deacetylase (HDAC) inhibitors are a class of clinically relevant drugs being explored in mono and combination therapies targeting cancer and certain neurological disorders. While HDAC inhibitor mechanism of action is well understood, their mechanism for inducing cell death remains poorly characterized. There are five classes of HDACs, localized to different compartments of the cell, with various functions. We profiled the lethality of each class of HDAC inhibitor and found that lethality is caused by a metabolic toxicity derived from acetyl-CoA depletion. Using functional genomic screening, we identified genes that regulate HDAC inhibitor induced lethality. We validated these insights with metabolomics to learn how metabolism changes following HDAC inhibitor treatment. Ongoing efforts are focused on determining what downstream metabolic pathways are disrupted following acetyl-CoA depletion that are causative for lethality, using flux balance analysis to make predictions of cells' metabolic state and measuring death kinetics with metabolite supplementation to validate contributions to lethality. Together, our results suggest HDAC inhibitor-induced lethality is caused by a collateral metabolic toxicity related to acetyl-CoA depletion.

Poster #14

Multiscale systems approach to target tumor ecosystem responses for therapeutic benefit

Presenter: Laura Heiser

OHSU

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Breast tumors arise and progress via processes that involve intrinsic deregulation of epithelial cells and that also alter the composition and function of associated stromal and immune cells. Together, these tumor-intrinsic and microenvironmental changes enable malignant epithelial cells in the tumor to acquire key cancer hallmarks, including proliferation, migration, immune evasion and further evolution. The resulting collection of cancer and stromal cells comprise a complex, adaptive tumor ecosystem. Long-term therapeutic success will require that drugs attack malignant cells, favorably activate the immune system, and create a tumor microenvironment that does not support tumor (re)growth. Creating a process for treatment selection will require an integrated systems biology approach to develop, test and refine a robust computational framework that takes into account the states, signaling relationships, and spatial organization that determine the emergent properties inherent in complex interacting systems. Toward this goal, we have developed a coordinated experimental-computational approach designed to enable identification of effective treatment strategies. To examine therapeutic responses of diverse aspects of the tumor ecosystem, we have deployed a novel drug delivery microdevice that enables rapid, high-throughput assessment of the effects of multiple therapies on tumor cells and the surrounding microenvironment. When coupled with multiplex tissue imaging, this platform provides a comprehensive assessment of the state and spatial organization of the tumor ecosystem as it adapts to therapy. Initial studies demonstrated that many drugs designed to target malignant epithelial cells strongly impact stromal and immune cells, providing new insights into the importance of considering multiple aspects of the tumor ecosystem when designing effective therapeutic strategies. The resultant data are being used to guide development of a multiscale computational model that considers the functional relationships between different tumor components and can be used to predict the effects of therapies on diverse aspects of tumor ecosystems. In total, our approach has the potential to transform cancer

treatments through rational identification of effective therapeutic strategies that lead to long-term durable control of tumors.

Poster #15

Drug Repurposing to Address Temozolomide Resistance in Glioblastoma

Presenter: Judith Landau

University of Southern California

Judith Landau (University of Southern California, Los Angeles, CA)
Nicholas Graham (University of Southern California, Los Angeles, CA)

Glioblastoma multiforme (GBM) is the most common brain cancer in adults and also the most lethal. A significant barrier to treatment is resistance to temozolomide, the standard chemotherapy for these malignancies. GBM with high levels of MGMT (O6-methylguanine-DNA methyltransferase) are even more resistant than other GBM tumors because this enzyme can repair DNA alkylation caused by temozolomide. To address this problem, we used a computational tool recently developed in our lab, drug mechanism enrichment analysis (DMEA), to predict drugs to which MGMT-high and temozolomide-resistant glioblastoma would be sensitive. DMEA is a systems biology approach that aids drug repurposing by identifying drug mechanisms of action that are likely effective for treating diseases in which they have not been explored before. We analyzed three data sets with DMEA: (1) a set of correlation coefficients between MGMT protein expression and sensitivity to 4,518 drugs across 578 cancer cell lines, (2) gene expression data from an experiment comparing patient-derived glioblastoma stem cells before and after they acquired resistance to temozolomide, and (3) an artificial gene expression data set representing a cell line with an overexpressed MGMT level. Merging the results from these three data sets demonstrated that both vitamin D receptor agonists and bromodomain inhibitors are predicted to treat MGMT-high and TMZ-resistant GBM and indicates that they may improve treatment outcomes alone or in combination with temozolomide. Experiments to validate this are ongoing. This work exhibits a framework for using systems biology approaches to overcome drug resistance in cancer.

Poster #16

Phenotypic responses of the MCF10A-BRCA wild type and MCF10A-BRCA-185delAG/+ cell lines to prioritized ligands

Presenter: Natalia Quintana Parrilla

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Keywords: cell proliferation, extracellular signaling, MCF10A, BRCA1

Abstract:
The discovery of the BRCA1 gene mutation as a significant indicator for breast and ovarian cancer risk established a genetic basis for hereditary cancer predisposition. Carriers of the BRCA1 mutation have an estimated lifetime risk of 80% of breast cancer, however the incomplete penetrance implies that additional extracellular factors are required for disease onset and progression. Epidermal growth factor (EGF), transforming growth factor β (TGFβ), and interferon γ (IFNγ) have been shown to promote or inhibit cell proliferation and found to be common in breast tissue. Further, there seems to be a clear disruption of these pathways with the development of disease. Therefore, the role of the BRCA1 in this disruption remains unclear. Here we used a functional approach to examine how these ligands alter cancer-associated phenotypes in MCF10A-BRCA-185delAG/+ as compared to MCF10A-BRCA wild type. Phenotypic differences in ligand response will provide insights into the extent of their proliferative or inhibitory control in the context of a BRCA1 mutated cell environment. On a broader scale, the identification of these microenvironmental factors could be therapeutically targeted to prevent disease onset or to treat early-stage disease.

Poster #17

Agent-Based Modeling of Cancer Cell Phenotypic Transition from Spheroid to Network Phenotype: Investigating the Role of Biophysical and Biochemical Parameters

Presenter: Temitope O. Benson

Ashlee N. Ford Versypt

Temitope O. Benson, Ashlee N. Ford Versypt (University at Buffalo, SUNY)

Understanding the phenotypic transitions of cancer cells is crucial for elucidating tumor progression mechanisms, particularly the transition from a non-invasive spheroid phenotype to an invasive network phenotype. We developed an agent-based model (ABM) using Compucell3D, an open-source biological simulation software to investigate how varying biophysical and biochemical parameters influence the phenotypic changes in cancer cells. Specifically, we focus on cell-cell contact energy, chemoattractant secretion rates, and how these parameters impact cellular behavior during the transition.

Our model simulates cancer cell's growth, division, and invasion in a 2D microenvironment and tracks their morphological changes using key metrics such as circularity and invasion. We explore how varying contact energy alters the shape and structure of the spheroid and how chemoattractant secretion rates drive the transition to a network phenotype. Our model simulations are validated using published experimental data, allowing us to compare the predicted phenotypic changes with observed behaviors in cancer cell cultures.

Our results suggest that increasing chemoattractant secretion enhances the invasiveness of the cells, promoting the transition to a network phenotype, while modulating contact energy from a strong to a weak cell-cell adhesion alters the compactness of the initial spheroid, leading to lower circularity and increased invasion. Our work advances the understanding of tumor progression and opens avenues for targeted therapeutic interventions by providing insights into the parameter-driven mechanisms behind cancer cell phenotypic transitions.

Poster #18

Multivariate modeling uncovers differentiation state-associated epigenetic dependencies in melanoma

Presenter: Luisa Quesada

University of Virginia

Luisa Quesada (University of Virginia), Katharine Faieq (University of Virginia), Mohammad Fallahi-Sichani (University of Virginia)

Tumor heterogeneity manifests across multiple dimensions, including genetic, epigenetic, and phenotypic variations, each contributing distinctly to cancer progression. Melanoma, with its pronounced genetic diversity and varied clinical presentations, serves as an ideal model for studying these complex landscapes. Systematic analyses of high-dimensional bulk and single-cell transcriptomics data from genetically diverse melanoma tumors have revealed a critical role for differentiation states as a nongenetic source of heterogeneity in melanoma. These efforts have identified melanoma cells in four transcriptionally distinct states: melanocytic (differentiated), transitory, neural crest-like, and undifferentiated. Despite these insights, the epigenetic dependencies associated with differentiation states in melanoma cells remain poorly understood.

To uncover state-specific epigenetic dependencies, we integrated large-scale datasets from the Cancer Cell Line Encyclopedia (CCLE), including transcriptomics, mass spectrometry-based measurements of global histone post-translational modifications (PTMs), genomics, and phenotypic data. Through this approach, we identified key roles for two chromatin-modifying complexes: (1) the Set1/COMPASS complex, responsible for

H3K4 methylation, and (2) the SAGA complex, known for its histone acetyltransferase activity leading to H3K9 and H3K14 acetylation. Data-driven analysis suggested that these complexes regulate critical processes necessary for melanocytic cell growth and survival, including mTORC1 signaling, oxidative phosphorylation (OXPHOS), and cholesterol biosynthesis.

To validate the dependency of melanocytic cells on Set1/COMPASS (CXXC1 and DPY30 subunits) and SAGA (TADA1 and SGF29 subunits), we employed siRNA knockdown experiments in a panel of melanoma cell lines, including melanocytic lines (UACC62, MALME3M, SKMEL28, COLO858, SKMEL19) and undifferentiated lines (LOXIMVI, WM115). Preliminary results indicate that UACC62 and MALME3M are highly sensitive to CXXC1 knockdown resulting in significant decreases in viability (~50% in UACC62, ~25% in MALME3M) and reductions in Ki-67 high cells (~50%). Interestingly, SKMEL28 and COLO858 showed minimal sensitivity, and among undifferentiated lines, LOXIMVI exhibited significant changes (~17% decrease in cell count, ~15% reduction in Ki-67 high cells), suggesting that biological factors beyond differentiation state may determine CXXC1 dependency.

To investigate potential pathways underlying these differences, we conducted pre-ranked Gene Set Enrichment Analysis (GSEA) on RNA-seq data from cell lines treated with CXXC1 siRNA. OXPHOS emerged as one of the top enriched pathways correlating with CXXC1 dependency, particularly in highly differentiated cell lines. SKMEL28, which exhibited low OXPHOS mRNA expression, showed minimal sensitivity to CXXC1 depletion. Similarly, across 41 melanoma cell lines from CCLE, CXXC1 dependency scores were highly correlated with OXPHOS gene expression. Parallel analyses for DPY30, TADA1, and SGF29 revealed similar associations with OXPHOS regulation, while cholesterol homeostasis was specifically correlated with TADA1 and SGF29 dependency.

Future experiments will focus on validating these findings by further investigating the molecular mechanisms by which CXXC1, DPY30, TADA1, and SGF29 regulate OXPHOS and cholesterol biosynthesis in differentiated melanoma cells. These efforts will provide critical insights into the epigenetic dependencies associated with distinctive melanoma differentiation states, which may serve as potential therapeutic targets.

The funding sources of this work are: NCI grant R01-CA249229 (to MFS) and UVA Engineering Distinguished Fellowship.

Poster #19

Identifying the microenvironmental drivers of AP-1-mediated differentiation state heterogeneity in primary melanoma tissues

Presenter: Kimberly Nguyen

University of Virginia School of Medicine

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Melanoma presents remarkable heterogeneity that transitions between various cell states on a differentiation state trajectory, including: (1) a melanoc¬¬ytic (differentiated) state, (2) a neural crest-like state, and (3) an undifferentiated, mesenchymal-like state. This plasticity confers selective phenotypes with fitness advantages during both primary tumor growth and metastasis. Using single-cell imaging and transcriptomics data of genetically diverse melanoma cell lines and tumors, we previously demonstrated that a tightly-regulated balance between six key AP-1 transcription factors (cFOS, FRA1, FRA2, cJUN, JUNB, JUND), referred to as AP-1 state, predicts differentiation state heterogeneity at a single-cell level (Comandante-Lou et al., Cell Rep., 2022.). For example, cells with a higher ratio of cFOS to cJUN expression are more likely to be in a differentiated state. Furthermore, siRNA mediated depletion of JUND induces cJUN expression in these cells concomitant with their transition to an undifferentiated state. Despite this knowledge, the molecular and cellular drivers of AP-1 state heterogeneity in the tumor microenvironment (TME) remain incompletely understood. As the AP-1 transcription factors are known integrators of inflammatory, growth, and stress signaling (Bejjani et al., 2019), we hypothesize that the spatially-heterogeneous distribution of immune cells,

fibroblasts and vasculature in the TME determines the diversity of AP-1 states in primary melanomas. To systematically measure AP-1 state in melanoma cells and assign phenotypes to cells in the TME, we have utilized iterative indirect immunofluorescence imaging (4i) to perform highly-multiplexed imaging on formalin-fixed primary melanoma tissue samples (Hsu et al., 2024). Through iterative antibody stripping, 4i enables staining of 30+ markers on the same tissue slide for single-cell analysis of phenotypic states and spatial analyses at different length scales. By staining for melanoma differentiation state markers MITF, SOX10, SOX9, NGFR, and PRAME, we have captured the heterogeneous distribution of melanocytic, neural crest-like, and undifferentiated cells within and across 3 different primary melanoma tumors. Combining these differentiation state markers with AP-1 expression levels, we are assessing the predictivity of AP-1 state for differentiation state heterogeneity in primary tumors. To identify both short-range and long-range patterns of AP-1 state in the spatially-preserved melanoma TME, we are performing cellular neighborhood analysis using the MCMICRO pipeline and whole-tumor section level analysis using SpatialCells. Though heterogeneous both within and across tumors, we have found that AP-1 state often forms a gradient of high JUND and cFOS at tumor-stroma borders that decreases towards tumor cores, suggesting a stromal interaction or proliferative edge at tumor borders based on a cFOSHighJUNDHigh program in melanocytic cells. Overall, a deeper understanding of how cell-extrinsic factors in the TME drive tumor plasticity through AP-1 regulated transcriptional programs will help identify novel therapeutic targets, e.g., via blocking metastasis-initiating transitions during disease progression.

Poster #20

Identifying mechanistic regulators of the AP-1 state heterogeneity via computational modeling and multiplexed single-cell analysis

Presenter: Yonatan Degefu

University of Virginia

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Mohammad Fallahi-Sichani (University of Virginia, Charlottesville, VA)

Cellular plasticity, driven by transcription factor (TF) networks, is crucial in creating non-genetic heterogeneous tumor cell states. Among these TFs, the activator protein 1 (AP-1) family—including FOS and JUN subfamilies—predisposes cells to diverse transcriptional programs with important phenotypic consequences such as differentiation, dedifferentiation, or resistance to cancer therapies. AP-1 function is mediated through competitive homo- and heterodimeric interactions among individual AP-1 proteins, whose total abundance, stability, and activity are subject to transcriptional (auto)regulation and posttranslational phosphorylation by MAP kinases. Our previous single-cell studies uncovered a subset of key AP-1 states associated with differentiation state heterogeneity and plasticity across both genetically diverse and isogenic populations of melanoma cells. Multivariate analysis and genetic experiments showed that these AP-1 states depend on a regulated balance controlled by the abundance of five AP-1 proteins: cFOS, FRA1, FRA2, cJUN, and JUND. However, the exact mechanisms that control the emergence, stability, and diversity of AP-1 states within genetically-homogeneous or across genetically-diverse cell populations remain unclear. We hypothesized that the heterogeneity in AP-1 states is regulated by the interplay between AP-1 protein production, degradation, competitive dimerization, and feedback regulation among these five key AP-1 family members. To test this hypothesis, we constructed an ordinary differential equation (ODE) model of AP-1 regulation in melanoma cells. The model captures the emergence of a range of AP-1 states based on variations in initial conditions and parameter values. We used single-cell measurements of AP-1 proteins, acquired by highly multiplexed iterative imaging (4i), across 19 melanoma cell lines to calibrate the model. Using a partial least squares discriminant analysis (PLS-DA) classifier, we identified parameters—such as basal production rates of AP-1 monomers, and specific dimer induced feedback regulation—that explain differences in AP-1 states between genetically diverse cell lines. The model also explained how siRNA perturbation of JUND in a representative cell line (COLO858) generated new AP-1 states. Through in silico perturbations coupled with PLS-DA analysis, we showed that the emergence of these states were controlled by parameters influencing the turnover rate of cFOS in cFOSHigh COLO858 cells, and turnover rate of FRA1 in cFOSLow cells. Furthermore, our model suggests that AP-1 state heterogeneity could theoretically emerge through two distinct mechanisms: cells with similar AP-1 regulatory dynamics exhibit bi-stability, acquiring different stable states depending on

their prior AP-1 state, or transition between states due to subtle variations in their protein production and degradation parameters, providing testable hypotheses for how melanoma cells might achieve transcriptional plasticity. By linking the diversity of AP-1 states to experimentally tractable parameters at the single-cell level, our models unravel the mechanisms controlling AP-1 state dynamics and generate testable hypotheses for future experimental validation.

Poster #21

Optimal control theory as a method for designing multi-drug adaptive therapy regimens

Presenter: Afton Widdershins

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Afton Widdershins (Penn State COM, Hershey, PA), Elsa Hansen (Huck Institutes, Penn State University, State College, PA), Andrew F Read (Huck Institutes, Penn State University, State College, PA), Raymond J Hohl (Penn State Cancer Institute, Hershey, PA)

Resistance to treatment is a persistent challenge in cancer treatment, across cancer diagnosis and classes of chemotherapy. Evolutionarily informed regimen design paradigms, such as adaptive therapy, offer new approaches to cancer treatment that hope to combat the development of resistant disease. However, these regimen designs are often complicated, with multiple levels of detail to incorporate. In this work, we attempt to use optimal control theory to guide the design of a two-drug adaptive therapy regimen. We begin with an ODE model of a heterogeneous tumor driven by logistic growth, composed of four populations with different resistance statuses controlled by two drugs. The application of optimal control theory identified several rules regarding regimen creation that allowed for the design of several regimens. Using numerical simulation, these initial regimens were compared to each other over a range of population and regimen parameters, where we found that regimens that prioritized maintenance of competition tended to perform the best. With those regimens identified, we then compared them to practical and standard-of-care regimens, to predict how the optimal regimens would perform in the laboratory setting. While practical versions of the regimen do not perform as well as the initially simulated competition maintenance regimens, they still outperform the standard-of-care regimens for nearly all regimen design parameters. As such, optimal control theory and ODE modeling offers one avenue for exploring complex adaptive regimen design, with regimens that maintained competition through dose modulation performing the best. Initial in vitro tests that parallel the situations tested by the ODE model are underway to evaluate how well the model translates to actual biology.

Poster #22

Single-cell trajectory analysis reveals AP-1 dependent differentiation state heterogeneity in melanoma responses to MAPK-targeted therapies

Presenter: Audrey Kidd

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Melanoma patients with BRAF-V600E mutations often develop resistance to MAP-kinase (MAPK) pathway inhibitors despite initial treatment efficacy. Therapeutic resistance may arise through mechanisms involving cellular plasticity and drug-induced dedifferentiation, which enable cancer cells to adapt to or tolerate treatment (Fallahi-Sichani et al. Mol Syst Biol. 2017). Our previous studies have demonstrated that MAPK inhibitor treatment induces changes in AP-1 transcription factor states that predict subsequent changes in both differentiation state and MAPK pathway activity, measured by phosphorylated ERK (pERK) expression

(Comandante-Lou and Baumann et al. Cell Rep. 2022). Despite this established connection between AP-1 dynamics and treatment response, the mechanisms underlying heterogeneous cellular responses to MAPK inhibitors, particularly how initial cell states and temporal AP-1 dynamics determine treatment outcomes, remain poorly understood.

Using multiplexed immunofluorescence imaging (4i) (Hsu and Nguyen et al. Star Protocols. 2024) and computational trajectory analysis (Ko and Williams et al. Nature Protocols. 2020), we investigated single-cell responses to MAPK inhibitors in BRAF-mutant COLO858 melanoma cells. Cells were treated with varying concentrations of the BRAF inhibitor Vemurafenib (0.1-1 μM) alone or in combination with the MEK inhibitor Trametinib (0.01-0.1 μM). At eight timepoints following treatment (0, 0.5, 2, 6, 15, 24, 72, and 120 h), cells were fixed and stained iteratively for 4 melanoma differentiation state markers (SOX10, NGFR, MITF, AXL), 10 AP-1 proteins and their phosphorylation states, and various MAPK-related signaling and phenotypic markers (pERK, pp38, pS6, Ki-67, and NFkb). To map cellular transitions across time-points, we used FlowMap, an algorithm that builds force-directed graph layouts from single-cell time-course data to reveal trajectory patterns.

Through FlowMap analysis of four key differentiation markers, we identified two distinct cellular trajectories originating from populations with different initial MITF, AXL, and SOX10 expression levels. While both trajectories showed decreased MITF and SOX10 expression indicating dedifferentiation, only one trajectory exhibited increased NGFR expression after 24 hours. These trajectories displayed unique temporal patterns in AP-1 factors, with JUND expression diverging at 2 hours post-treatment and cFOS showing initial differences that converged by 120 hours. Notably, while pERK levels correlated with inhibitor dose, they did not differentiate between trajectories, suggesting MAPK pathway reactivation is not driving the divergent responses. Instead, differential expression of pp38 (a MAPK pathway member not directly related to BRAF or MEK), pS6 (a PI3K/AKT/mTORC1 pathway marker), and Ki-67 (a proliferation marker) between trajectories suggests alternative signaling mechanisms influence cell fate decisions.

To further elucidate the role of AP-1 factors in these divergent responses, we have generated a panel of isogenic COLO858 lines with single and pairwise perturbations of six AP-1 factors. These cell lines, featuring systematic knockout, knockdown, and overexpression modifications of AP-1 factors, will enable precise dissection of how AP-1 network dynamics shape therapeutic responses. This comprehensive analysis of differentiation state trajectories and AP-1 factor dynamics not only reveals mechanisms underlying heterogeneous drug responses in melanoma but also provides a framework for developing more effective therapeutic strategies that account for cellular plasticity and initial state variations.

Poster #23

Modeling Subclonal Evolution of Drug Resistance to Optimize Patient Benefit

Presenter: Matthew McCoy

Georgetown University Medical Center

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The current approach to precision oncology selects a targeted therapeutic intervention based on the biomarkers that are most prevalent in the patient sample, only changing to a new therapy once the first line treatment has become ineffective. In this way, Current Precision Medicine (CPM) fails to account for evolutionary dynamics and drug resistance in genetically distinct tumor subclones. Dynamic Precision Medicine (DPM) was developed to address this shortcoming by adapting frequently to the changing tumor

landscape, accounting for the impact of multiple therapeutic agents on the future emergence of drug resistance. Previous simulations demonstrated the DPM doubles the mean and median survival in a population of 3 million virtual patients over CPM. However, because DPM requires costly and invasive data collection every six weeks, we sought to characterize the benefit of limiting DPM to the first two windows of treatment before reverting to CPM for the remaining course of the disease. Remarkably, we found the much of the benefit from full regimen of DPM is achieved within these first two windows of treatment. Furthermore, comparing the drug selection by the CPM and DPM strategies led to the development of a straightforward classifier to identify patients likely to benefit from DPM. Those patients where drug selection during the first two moves was identical, did not receive additional benefit from the full course of DPM. Conversely, the population of patients where the drug selection for the first treatment window diverged between the CPM and DPM strategies realized most of the benefit of a full course of DPM, despite reverting to a CPM strategy. Thus, comparing the recommendations of CPM and DPM for the initial treatment windows can be used as an Evolutionary Classifier (EC), identifying patients where the increased burden associated with characterizing the subclonal tumor parameters is justified, and preventing unnecessary hardship to patients who are not likely to benefit from DPM.

Poster #24

Proteomics of sorted acute myeloid leukemia enables identification of cell type-specific resistance mechanisms

Presenter: Belinda B. Garana

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Acute Myeloid Leukemia (AML) is a hematopoietic cancer caused by a block in cell differentiation resulting in uncontrolled proliferation of immature blasts in patient blood and bone marrow. However, this block is incomplete and, therefore, leads to a heterogeneous mix of cell populations in varying stages of maturity, making it difficult to treat the entire blast population using a single targeted therapy. Specifically, patients with a large population of mature monocyte-like cells are more likely to be resistant to drugs such as venetoclax, a drug that targets the B-Cell lymphoma-2 (BCL2) protein, and azacitidine, an inhibitor of DNA methylation, that have proven to be highly effective in combination for those patients whose AML is dominated by primitive cell populations. Recent advances in single-cell RNA-seq have highlighted the population-level complexity of AML patients corresponding to poor prognosis, but how the behaviors of individual populations associate with protein abundance is poorly understood, particularly in the context of drug resistance. Here, we introduce a proteomics-based approach that sorts patient samples into distinct populations by cell surface markers and examines proteins that give rise to drug resistance within the monocytic and primitive cell populations.

We collected bone marrow from 10 AML patients, each with varying responses to venetoclax and azacitidine according to our ex vivo assay. We sorted these samples by expression of two cell surface markers: CD34+ to represent primitive cell populations and CD14+ to represent monocytes. Using a data independent acquisition (DIA) liquid chromatography tandem mass spectrometry (LC-MS/MS) proteomics approach, we identified 7,025 proteins, of which 2,118 were differentially expressed between CD14+ and CD34+ populations. Using

gene set enrichment analysis of 50 hallmark gene sets from the Human Molecular Signatures Database; we identified increased TNF-alpha signaling via NF-kappa B and decreased expression of MYC targets in the monocytic CD14+ samples compared to the more primitive CD34+ samples. We then used these proteins to develop a monocyte score to predict the fraction of monocyte populations in bulk measurements of AML samples. We found that our monocyte score was able to predict monocytes as effectively as single-cell RNA-seq-based approaches. Furthermore, we found that monocyte scores correlated (r=0.7, p=1E-19) with venetoclax sensitivity in our previously measured 210 patient cohort. Lastly, we compared cell populations between patients that were resistant to the combination of azacitidine plus venetoclax and those that were sensitive and observed increased TNF-alpha signaling via NF-kappa B and decreased expression of MYC targets, similar to the contrast between CD14+ and CD34+ samples. We are currently extending this work to additional patient samples and plan to evaluate our results in samples from patients currently enrolled in clinical trials for azacitidine plus venetoclax combination treatment.

Recent advances in the sensitivity of LC-MS/MS proteomics have enabled the analysis of small (~15,000 cell) patient samples with increased depth and reproducibility, enabling us to further our mechanistic understanding of drug resistance in AML. This study offers a novel protein-based approach to understand cell maturation heterogeneity in AML and may uncover new potential targets to inhibit in drug-resistant patients.

Poster #25

Optimizing Iterative Indirect Immunofluorescence Imaging (4i) for High-Resolution Multiplexed Protein Analysis in Chromosome Spreads: Implications for Cancer Research

Presenter: zeinab chitforoushzadeh

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Recent advances in highly-multiplexed protein imaging methods have significantly improved our ability to study complex biological processes at cellular and subcellular resolutions. Among these methods, iterative indirect immunofluorescence imaging (4i) in chromosome spreads enables capturing of multiplexed information on chromatin-associated proteins, their posttranslational modifications, and their spatial organization within the same sample via several iterations of indirect immunostaining, imaging, and antibody elution. Further, multiplexed single-nucleus analysis allows for the simultaneous, high-resolution detection of multiple proteins within individual nuclei at a single-chromosome level resolution.

A recently published protocol from our group has laid the groundwork to perform 4i in chromosome spreads. While the 4i method is conveniently accessible without requiring costly reagents or specialized instrumentation beyond established common fluorescent imaging assays, the application of 4i is faced with unique challenges. Some of the major challenges associated with performing 4i in chromosome spreads that we faced and were able to overcome with include, a flexible sample assembly compatible with recurring cycles of elution, staining, imaging, and relocating the same chromosome spread across rounds of imaging (etching the coverslip with a diamond scriber), and identification of an imaging buffer compatible with 0.18 mm coverslips for water-immersion objectives.

In this study, we present strategies to optimize the 4i protocol for high-resolution confocal microscopy (fluorophores compatible with laser-scanning excitation sources), increase multiplexing capacity by increasing

staining rounds, reliably locate the same nuclei across rounds of imaging, refine the sample preparation to maintain sample integrity, and prepare chromosome spreads from diverse cell lines. We further plan to use our improved 4i protocol to detect and spatially locate several epigenetic marks (H2AK118ac, H2ApT120, H3pT3) and components of the chromosomal passenger complex (CPC) including inner centromere protein (INCEP), and Aurora B kinase at single-chromosome resolution to inform the dynamics of crucial steps during early mitosis.

This accessible, scalable, and robust method enables researchers to investigate the spatial organization of multiple protein targets relative to chromatin at single-chromosome resolution. Such detailed analysis promises to elucidate subcellular mechanisms underlying chromosomal instability and mitotic fidelity, with significant implications for understanding cancer progression and developing targeted therapies.

Poster #26

Mapping the Ovarian Cancer Tumor Microenvironment: Integrating Multimodal Analysis for Understanding Treatment Resistance

Presenter: Adriana Del Pino Herrera

PhD student at the University of Florida

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Monica Kim (University of Florida, Gainesville, FL)
Meghan Ferrall-Fairbanks, PhD (University of Florida, Gainesville, FL)

Ovarian cancer is the second most common gynecological cancer accounting for 12,740 estimated US deaths in 2024. The difficulty in diagnosis is due to its nonspecific symptoms resulting in late stage detection and a 5-year survival rate of 50.9%. Standard of care treatment involves platinum-based chemotherapy, surgical resection, and in some cases targeted therapies. Traditional therapies are administered at maximum tolerated doses, which assume that the tumor is comprised of sensitive cells to the therapy. However, often there are resistant clones inherently present in the tumor population, which evade the effects of therapy and result in 80% of ovarian cancer patients will have recurrence of their disease after first line treatment. Adaptive therapy scheduling aims to delay the onsets of resistance by tailoring drug dosing to tumor composition, ultimately often reducing cumulative drug dose and allowing for treatment breaks to prolong patient sensitivity to treatment.

While researchers have considered different factors such as initial tumor density and initial resistance percentages to make accurate predictions of adaptive therapy effectiveness, there are other factors that remain understudied. The tumor microenvironment (TME) plays a crucial role in cancer progression. Tumor supporting cells such as fibroblasts and macrophages can play a role in promoting tumor progression and resistance. Multimodal approaches can be applied to interrogate the TME composition and quantify different cell types to inform treatment. We aim quantify differences in ovarian cancer TME using multiplex immunofluorescent staining of biobanked FFPE tissue from ovarian cancer patients. Sections were stained for CD163 (macrophages), CD20 (B cells), CD8 (T cells), aSMA (fibroblasts), and P2rx5 (adipocytes). Stained tissue slices were imaged, and cell type specific counts were abstracted by identifying areas with highest fluorescent areas prior to segmentation using ImageJ. Our cohort consists of 19 unique patients with tissues samples from normal, benign neoplasms, and primary cancer. Initial characterization found that the proportion of macrophages relative to total cell number was similar in both normal and benign samples but 1.2-fold changes higher in clear cell cancer samples compared to those. Additionally, macrophage counts in high-grade serous samples were 2-fold changes higher than the normal and benign samples. A similar trend was observed within proportion of B cells, which were similar in normal and benign samples but 2-fold changes higher in both clear cell and high-grade serous samples. The proportion of T cells, however, was near 0 and similar across all groups. These data suggest that macrophages and B cells may play a role in tumor progression. Specifically, CD163 macrophages are associated with an M2-like phenotype promoting cancer resistance. These findings are being validated using publicly available single-cell RNA sequencing datasets (GSE184880, GSE154600, GSE165897), which contain a variety of normal, sensitive, refractory, resistant, treatment-naïve, and post-neoadjuvant high-grade serous samples. These samples will be processed using the

Seurat package in R and singleR will be used for cell type assignment, which will provide with both presence and frequency of supporting stroma in these patient samples.

Poster #27

A spatially resolved single cell proteomic atlas of small bowel adenocarcinoma

Presenter: Zeynep Dereli

UT MD Anderson Cancer Center

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Small bowel adenocarcinoma (SBA) is a rare malignancy associated with poor prognosis. The cellular and proteomic heterogeneity within the tumor immune microenvironment (TIME) of SBA is a likely driver of prognosis, disease progression and response to therapy. There is, however, a major gap in our knowledge of tumor and immune interactions in SBA. Cyclic Immunofluorescence (CycIF), an antibody-based, highly multiplexed imaging technology for spatially resolved single cell level proteomic profiling, is well suited to map the proteomic heterogeneity and organization of TIME in SBA. Here, using CycIF, we have generated a spatially resolved single-cell proteomic atlas of TIME for 136 tumor and normal samples from clinically and genomically annotated SBA patients (N=37). The SBA TIME Atlas covers information on expression levels, spatial position, and cell morphology for > 40 proteomic markers of tumor-intrinsic processes and states, diverse immune cell types, immune checkpoints, and tumor vascularization. In total, we collected proteomics data from > 600,000 cells. Using a hierarchical decision tree of cell type markers, we have generated the cell identity annotations for > 350,000 cells, with positional information. A comprehensive computational analysis of the SBA atlas revealed various spatial and heterogeneity features that may impact the TIME organization in fine detail. Using those features, we have compared the proteomic heterogeneity in tumor vs. normal cell populations, identifying spatial and single-cell correlates of key clinical variables (e.g., patient survival, age, sex, disease stage, metastatic status). We have also performed differential analyses of spatial features involving protein markers and cell types with respect to genomic alteration states and have mapped the spatial enrichment of likely actionable immune check proteins to immune and tumor cells. Such mapping may guide future therapeutic decisions. Overall, we expect that the SBA TIME Atlas will provide a useful resource and inform future translational studies, as well as basic research for better mechanistic understanding and treatment of this disease.

Poster #28

Integrating Intracellular Apoptosis Models with Immune Response: An Agent-Based Multiscale Simulation of Cancer

Presenter: Furkan Kurtoglu

Independent Researcher

Bertan Korkmaz (Izmir Institute of Technology)

Cancer is a complex, multiscale disease involving complex interactions across molecular, cellular, and tissue levels. Understanding these interactions is essential for developing effective therapies. Apoptosis occurs at the cellular level and is a critical mechanism for eliminating damaged or unwanted cells. It is regulated by two main pathways: the extrinsic pathway, initiated by external signals, and the intrinsic pathway, triggered by intracellular stress.

In this study, we develop an agent-based multiscale simulation that integrates detailed intracellular models of both extrinsic and intrinsic apoptosis pathways into individual cancer cell agents to determine their fate. The

simulation uses PhysiCell, an open-source physics-based cell simulator designed for multicellular systems biology. By embedding these intracellular signaling networks within each agent, our model captures how cancer cells respond to different therapeutic interventions at the molecular level and how these responses influence tumor dynamics at the tissue level.

The extrinsic apoptosis pathway in our model is activated by chemical signals that represent chemotherapeutic agents, simulating the effects of chemotherapy. This involves the binding of death ligands to cell surface receptors, leading to a cascade of events resulting in caspase activation and cell death. The intrinsic pathway is triggered by radiation-induced DNA damage, representing the effects of radiotherapy. This pathway involves mitochondrial signaling in response to intracellular stress, leading to cytochrome C release and subsequent caspase activation.

As cancer cells undergo apoptosis, they transform into apoptotic bodies that need to be efficiently cleared to prevent secondary necrosis and inflammation, which can promote tumor growth. Macrophages are modeled as mobile agents that patrol the tumor microenvironment. They are attracted to apoptotic bodies by "Find Me" signals released by the dying cells. Upon arrival, macrophages recognize "Eat Me" signals displayed on the surface of apoptotic bodies, facilitating phagocytosis and clearance.

Our simulation captures the dynamic interactions between apoptotic cancer cells and macrophage-mediated clearance. By modeling the chemotactic movement of macrophages toward apoptotic bodies and their phagocytic activity, we demonstrate how the efficiency of immune clearance affects tumor development. The results highlight that enhancing macrophage recruitment and activity can significantly improve the removal of apoptotic cells, reducing inflammation and inhibiting tumor progression.

By integrating intracellular apoptosis models with extracellular immune responses in an agent-based framework, our study provides valuable insights into the complex interplay between cancer cells and the immune system. The multiscale approach allows us to explore how molecular-level alterations in apoptosis pathways can impact cellular behaviors and overall tumor dynamics. This comprehensive model serves as a powerful tool for hypothesis testing and virtual experimentation, enabling the simulation of various therapeutic scenarios, including the timing and combination of chemotherapy and radiotherapy. In conclusion, our integrative modeling approach provides a deeper understanding of the mechanisms governing cancer cell death and immune clearance. By bridging intracellular processes with cellular interactions, the agent-based multiscale simulation advances our ability to design and optimize cancer treatment strategies, ultimately aiming to improve patient outcomes.

Poster #31

Modeling gene-gene and cell-cell interactions mediating tumor microenvironment transitions during combination therapy

Presenter: Yingtong Liu

Department of Quantitative and Computational Biology, University of Southern California, Los Angeles, CA

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The tumor microenvironment (TME) is a complex ecosystem composed of various non-malignant cells that interact with and affect the tumor. Entinostat, an epigenetic modulatory drug, is a promising treatment for cancer used in combination with immune checkpoint inhibitors. However, it is as-yet unknown how these combination therapies induce cell state transitions in tumor and immune cells that are beneficial to the patient. To identify genes that promote the shift from a tumor-suppressive TME to a more immune-permissive microenvironment, we developed a method for iterative logistic regression (iLR) to identify genes from single-

cell RNA-seq data that mark for treatment-induced cell state shifts in tumor and immune cell populations. Compared to alternative methods, we found that iterative logistic regression distinguished treatment effects convoluted by technical variance on a gene level. We go on to infer key pathways mediating tumor-immune signals via cell-cell communication analysis comparatively across treatment conditions. Using motif analysis across aggregated networks of interacting cells, we can determine core network motifs (cell circuits) that are responsible for the major signaling processes occurring. Diving into the pathway and ligand-receptor interactions within the cell circuits allows us to distinguish the key interactions that change upon therapy. Overall, analysis of gene expression and cell-cell signaling changes helps us to understand cell state transitions under various combinations of therapies.

Poster #32

Unveiling the Role of Extrachromosomal DNA in Cancer Through CytoCellDB: Insights from Triple-Negative Breast Cancer

Presenter: Adesuwa Igbinigie

University of North Carolina at Chapel Hill

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Extrachromosomal DNA (ecDNA), are circular acentric DNA elements that amplify oncogenes. These elements, though atypical in healthy cells, are commonplace in cancers– present in as much as 14-20% of patient tumors. ecDNA presence is often indicative of poor prognosis and contributes significantly to treatment resistance. Its prevalence, however, stands in stark contrast to its lack of coverage in literature–only 5% of cell lines profiled by the Cancer Cell Line Encyclopedia (CCLE) have been experimentally tested for ecDNA. In response to this gap, CytoCellDB was developed, a publicly available database that characterizes 577 Dependency Map (DepMap) cell lines for cytogenetic data, including karyotyping validation of ecDNA.

CytoCellDB serves not only as a repository for experimental classification. Integrated into the database is output from predictive softwares which allows for the identification of strong candidates for ecDNA presence. Such was the case for triple-negative breast cancer (TNBC) cell lines MDAMB468 and SUM159, whose promising predictions led to their selection for experimental analysis and validation. Further investigation incorporating DepMap data on fold copy number and gene expression allowed for the identification of genes likely amplified on these ecDNA segments, which were also experimentally validated. From these findings, potential drug targets are being identified that preferentially inhibit these ecDNA-associated genes. This approach, when applied to other cancer model systems, may improve our understanding of cancer heterogeneity and inform the development of targeted drug therapies.

Poster #34

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

Presenter: David E Sanin

Johns Hopkins University

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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.

Poster #35
Model-based control of epithelial-mesenchymal transition through signaling regulation in pancreas cancer cells

Presenter: Matthew Lazzara

University of Virginia

Varghese Kurian (University of Delaware, Newark, DE)
Michelle Barbeau (University of Virginia, Charlottesville, VA)
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Epithelial–mesenchymal transition (EMT) is a normal cell developmental program that occurs aberrantly in numerous carcinomas and promotes chemoresistance. We hypothesize that various extracellular stimuli drive EMT through a conserved set of kinase-regulated signaling pathways and that the strength and duration of signaling through a critical set of those pathways dictate the degree to which EMT occurs. Based on this hypothesis, we developed a mathematical model of EMT in response to extracellular stimuli (EMT agonists) in human pancreas cancer cells. We used the mathematical model within a control problem to predict the

optimal schedules of EMT agonist treatments that minimize overall dose while still achieving near-complete EMT. To model the overall ligand–signal–phenotype system, we decomposed it into two subsystems in series: (i) a "signal-response" subsystem (f1), with ligand concentrations as inputs, and the relative abundances of downstream signaling molecules (e.g., phosphorylated ERK, STAT3) as responses, and (ii) a "phenotype-response" subsystem (f2) in which the signaling molecule abundances from f1 served as the inputs and EMT-related phenotypes (E-cadherin or vimentin expression) served as responses. Subsystem f1 was represented with a nonlinear dynamical systems model, in which the parameters were estimated from time-series data obtained from the response of a subset of measurable signaling proteins to changes in three single-ligand inputs (EGF, HGF, and TGFβ) and two ligand combinations (EGF+TGFβ and HGF+TGFβ). Subsystem f2 was represented with a partial least-squares regression model that predicted the phenotypic EMT response based on the dynamic signaling profiles. To implement our proposed approach for solving for optimized ligand trajectories, we solved the model-based optimal control problem using f1 and f2. This non-linear optimization problem was formulated with the objective of minimizing the total dosage of ligands and with the desired EMT phenotype response as a constraint. Model predictions were successfully validated in human pancreas cancer cells using immunofluorescence imaging. Our results provide a new paradigm for quantitatively predicting how multivariate signaling processes regulate complex cell phenotypes by integrating data-driven modeling approaches with dynamic control system models. The framework enables deployment of the model in reverse to determine how to manipulate process inputs optimally to achieve desired phenotypic responses. Future implementations of this modeling approach will be used to predict how best to schedule combinations of small molecule drugs that antagonize EMT to promote PDAC chemoresponse with minimal dose-related toxicities.

Poster #36

Role of cancer associated fibroblasts in the tumor microenvironment: mathematical modeling

Presenter: Junho Lee

Korea Institute of Science and Technology, Gangneung, Korea

Eunjung Kim (Korea Institute of Science and Technology, Gangneung, Korea)

The heterogeneity of cancer-associated fibroblasts (CAFs) within the tumor microenvironment (TME) plays a pivotal role in the progression and treatment of cancer. Understanding the distinct behaviors and effects of various CAF phenotypes is crucial for the development of more effective cancer treatment strategies. This study is driven by the purpose of elucidating the heterogeneity of CAFs within the TME and evaluating how different CAF phenotypes influence tumor progression and immune response dynamics. However, the exact role and mechanism of CAFs within the TME remains to be elucidated. This study highlights the need to dissect the complex roles of different CAF phenotypes that affect cancer progression and immune regulation and proposes mathematical models to explore these interactions. Utilizing method that combine an agent-based model with differential equations, we simulate the complex interactions between CAFs and T cells and analyze spatial effect. The agent-based modeling allows for the simulation of individual cellular behaviors within their microenvironments. This method provides nuanced insights into how cells interact with and influence their surroundings, which is critical for understanding the complexity of the TME. The results of our simulations indicate that the anti-immune CAF phenotype contributes to the inhibition of T cell activation, thereby enhancing tumor survival, while the prophylactic immune phenotype may support T cell activity and thus disrupt tumor growth. The conclusion of our study suggests that strategic manipulation of the CAF population may significantly alter the immune environment of TME, suggesting new avenues for treatment of target cancer. Adjusting CAF phenotypes can improve the effectiveness of immunotherapy strategies and lead to more personalized approaches in cancer treatment.

This study not only advances our understanding of the TME's complexity but also opens new avenues for the development of personalized cancer therapies that are finely tuned to the specific characteristics of the tumor environment.

Poster #37

scGCA: A single cell global composition analysis of tumor heterogeneity and drug responses in triple-negative breast cancer

Presenter: Yue Wang

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Understanding molecular drug responses from a global viewpoint is very important to identify pathways/cellular states that are relevant to drug adaptation, especially in triple-negative breast cancer (TNBC), a type of cancer with intensive level of tumor heterogeneity. Here, we present scGCA, a bioinformatic framework to characterize TNBC into different cellular states and furthermore unveil cellular state changes upon drug treatments on the single-cell level globally. Applying our framework on many TNBC cell lines and tumor samples, we find that switching to a luminal-like state seems to be common in TNBC in response to treatments from different categories of drugs, including chemotherapy and immunotherapy. Further, we found that in the tumor microenvironment, cancer cellular state changes are not uniformly reflected in every cancer cell cluster, but are rather driven by certain cancer cell clusters within the tumor. To validate this, we used an in-house built TNBC organoid model and found that a considerable amount of single cells switched into NF-kappaB state from their original partial-epithelial-to-mesenchymal-transition (EMT) state after treated with MS-177, an EZH2 PROTAC degrader. And more importantly, this cellular state shift is contributed more from a stem cell-like cluster in the organoid, which indicates that intra-tumoral heterogeneity has potentially great impact on drug adaptations. Overall, our framework provides a pipelined workflow of characterizing cellular composition changes to understand drug adaptations in triple-negative breast cancer, which may ultimately provide insights on combination therapies.

Poster #38

An evolutionary-conserved molecular signature of cell senescence

Presenter: Prasanna Vaddi

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Cell senescence is a cell phenotype characterized by a durable exit from the cell cycle. Senescence is initiated in response to external cellular stress and internal DNA damage stimuli or carcinogen activation. The INK4 genomic locus on human chromosome 9p21 is activated when senescence is triggered and is a hot spot genomic region that undergoes frequent inactivation or deletion in a wide spectrum of human cancers. The locus encodes three cell cycle inhibitory proteins: p15INK4b encoded by CDKN2b, p16INK4a encoded by CDKN2a, and p14ARF (p19Arf in mice) encoded by an alternative reading frame (ARF) of CDKN2a. We identified a novel single evolutionally-conserved vertebrate homolog, among the oldest Vertebrata root species (~550 million years), with conserved repeats spanning Lamprey, Hagfish, and zebrafish to vertebrates with lower cancer

incidence such as the naked mole and including mouse and human INK4 genes. Functional studies demonstrated that this single ink4ab gene is activated in response to oxidative- and oncogenic-stress-induced senescence in zebrafish animals and mouse and human cells. Conditional oncogenic transformation of mouse and human cells induced a cell cycle phenotype associated with senescence and/or apoptosis that was rescued with the single ink4a transcript. Animals deficient in ink4ab expression failed to activate cellular senescence and developed multiple spontaneous cancers, including metastatic melanoma, osteosarcoma, colon cancer, pancreatic cancer, hepatocellular adenoma, and leukemias. Moreover, monitoring of cells and animals deficient in both ink4ab and p53 demonstrated a reciprocal regulation of senescence and apoptosis, controlling cell fate, accelerating cancer latency, and enhancing cancer formation, indicating that ink4ab haploinsufficiency promotes tumorigenesis, and combined ink4ab/p53 deficiency could reverse the protective tumor suppressive role of cell senescence. In a conditional mouse model targeting hematopoietic stem cells, single-cell spatial RNA sequencing (scSpRNA-seq) identified various immune cell types and the trajectories of cell differentiation associated with modulated senescence signals. We identified 30 clusters and 9 major cell subtypes including B-cells, T-cells, natural killer (NK) cells, dendritic cells (DCs), monocytes, macrophages, granulocytes, and stem cells forming the splenic red and white pulp architecture. Moreover, we evaluated gene expression differences among cell subtypes and performed enrichment analyses of cell transcriptomes associated with INK4 loss and senescence deregulation. Supervised cell typing revealed a scSpRNA-seq signature of altered immunocyte development with the interleukin IL6 and the chemokines Cxcl13 and Ccl5 most enriched in the senescence associated secretory profile. Our studies identify for the first time a highly conserved vertebrate genomic repeats involved in cell cycle regulation, cell fate, tumor suppression, and cell senescence phenotypes. The identified cell and spatial signatures of senescence provide targets for use to develop new strategies for cancer prevention and therapy.

Poster #39

Recon8D: A metabolic regulome network from oct-omics and machine learning

Presenter: Ryan Schildcrout

University of Michigan, Ann Arbor

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The complexity and incompleteness of metabolic regulatory networks make it challenging to predict metabolomes from other omics. Furthermore, cellular metabolite levels are controlled at the transcriptional, post-transcriptional, translational, and post-translational levels, but the interactions between these mechanisms and their relative influence is unknown. To explore multiomic regulation of the metabolome, we used machine learning to predict metabolomic variation across ~1000 different cancer cell lines from matched oct-omics data: genomics, epigenomics (histone post-translational modifications (PTMs) and DNA-methylation), transcriptomics, RNA splicing, miRNA-omics, proteomics, and phosphoproteomics.

Overall, the metabolome is tightly associated with the transcriptome, while miRNAs, phosphoproteins and histone PTMs have the highest metabolic information per feature. Metabolites in peripheral metabolism are predictable via levels of corresponding enzymes, while those in central metabolism require combinatorial predictors in signaling and redox pathways, and may not reflect corresponding pathway expression. We reconstruct multiomic interaction subnetworks for highly predictable metabolites, and YAP1 signaling emerged as a top global predictor across 4 omic layers. Top predictors were enriched for synthetic-lethal interactions and synergistic combination therapies that target compensatory metabolic modulators. This

integrated approach can help mechanistically combine cellular networks into models capable of elucidating the underlying hierarchy of metabolic control in cancer.

Poster #40

High-throughput cross-microscope imaging reveals poor capacity of telomere features to predict senescence induction in single cells

Presenter: Victor Passanisi

University of Colorado, Boulder

Victor Passanisi (University of Colorado, Boulder)
Sabrina Spencer (University of Colorado, Boulder)

Replicative senescence is the primary barrier to malignant transformation in normal human cells. Current textbook models posit that senescence is induced either when telomeres become too short or when a threshold number of telomeres elicit a DNA damage response (DDR), possibly triggered by telomere oxidation. However, senescence is defined by prolonged cell-cycle withdrawal, and technical challenges have barred the measurement of cell-cycle dynamics and telomere features in the same cell. Here, we use long-term time-lapse imaging and single-cell tracking to describe the cell-cycle behaviors of primary human dermal fibroblasts as they approach replicative senescence. We then develop a strategy to link high-throughput long-term time-lapse imaging with high-resolution 3D confocal imaging, enabling the quantification of cell-cycle dynamics and telomere features in the same cell. By leveraging natural heterogeneity in cell's proximities to senescence, we assess the importance of telomere length, DDR association, and oxidation in driving cells toward senescence. Our data indicate that the capacity of telomere length, telomeric DDR foci, and telomeric 8-oxoguanine (8oxoG) damage to predict senescence induction in primary human fibroblasts is poor compared to other canonical biomarkers of senescence. Rather than clearly triggering senescence, telomere-associated DDR foci and telomere length are weakly correlated with a cell's proximity to senescence, and, unexpectedly, telomeric 8oxoG levels are higher in cycling vs. non-cycling cells regardless of cellular age. Our results depict replicative senescence as a complex tumor-suppressive cellular state transition driven largely by telomere-independent processes.

Poster #41

MC2 Center and Cancer Complexity Knowledge Portal: Expanding Tools for Equitable Data and Tool Reuse

Presenter: Angela Bowen

Sage Bionetworks - MC2 Center

Angie Bowen, Ashley Clayton, Verena Chung, Orion Banks, Amber Nelson, Jay Hodgson, Jineta Banerjee, and Susheel Varma on behalf of the MC2 Center
Sage Bionetworks, Seattle, WA

Data and tool reuse to improve equity in research is a key area of growth in biomedical research. Data and tool repositories offer a vital infrastructure for centralizing, storing, and sharing such resources with the global research community of cross-disciplinary scientists. The Cancer Complexity Knowledge Portal (CCKP) is a NIH-listed domain-specific repository that makes oncology data findable and accessible. The CCKP is developed and maintained by the Multi-Consortia Coordinating (MC2) Center, led by Sage Bionetworks. The MC2 Center supports resource coordination among six cancer-focused interdisciplinary research consortia funded by the National Cancer Institute (NCI). Through this work, the CCKP has emerged as a significant repository for oncology data and tool sharing.

On the CCKP we host data models for diverse data modalities including genomics and imaging, and are developing new models for emerging multimodal data types like spatial transcriptomics. The data models undergo iterative development based on emerging community needs, with versioned releases maintained in a

public GitHub repository. These data models power established data management tools developed by Sage Bionetworks, including the Schematic Python package and Data Curator App, to support annotation of data according to FAIR standards. The data models are designed to help researchers link research outputs (e.g., publications, datasets) and develop training and education tools to empower resuse, thus helping the CCKP synthesize and highlight the activities and outputs of the NCI-funded cancer research programs in a meaningful way while improving data access equity.

As of November 2024, the portal hosts curated information on 3,786 publications, 904 datasets, and 292 computational tools emerging from over 140 research grants. Our data models also incorporate elements of the Cancer Research Data Commons (CRDC) Data Hub data model to support integration and reuse of datasets within the CRDC ecosystem.

The recent advances in computational oncology showcase the strength of secondary use of research data and highlight an unmet need to ensure that data and tools available through repositories are reliable, and reusable. We are engaging with scientists, clinicians, and patient advocates, developing innovative education and training modules and leveraging user-centered design approaches and structured data models to make cancer data and tools more findable, accessible, and reusable.

Poster session B: Tuesday, Feb. 11, 10:50 am – 12:00 pm

Poster #42

PhysiNN: Deep Neural Networks Add-on for PhysiCell

Presenter: Aneequa Sundus

Indiana University Bloomington

Aneequa Sundus (Indiana University Bloomington), Furkan Kurtoglu, Randy Heiland(Indiana University Bloomington) and Paul Macklin(Indiana University Bloomington)

PhysiCell is a multiscale biological modeling software that is used to create tissue scale models. Due to the multiscale design of the software, it can add different types of intracellular models in each cell agent. One possible way to model intracellular models is to use data-driven approaches like deep neural networks. These machine leaning algorithms are usually written in Python frameworks while PhysiCell core is written in C++. We have developed this PhysiNN add-on that augments the capability of PhysiCell to take python trained model and uses it inside each cell in PhysiCell framework. This add-on maintains the PhysiCell ecosystem and provides more options for users to add intracellular capabilities.

Poster #43

High-grade serous ovarian cancer autoantibodies interact poorly with cytotoxicity-inducing Fc receptors

Presenter: Michelle Loui

University of California, Los Angeles

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Scott Taylor (University of California, Los Angeles, CA)
Crystal Xiao (University of California, Los Angeles, CA)
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In high-grade serous ovarian cancer (HGSOC) patients, malignant epithelial cells arise from the fallopian tube and ovarian surface epitheliums. Endogenous antibodies (tumor-reactive autoantibodies) target these cells and should promote recognition by the immune system. Patient-derived tumors have been found to be frequently coated in IgG, and tumor-reactive autoantibodies are present both in the tumor mass and in the fluid that builds up in the peritoneum surrounding the tumor microenvironment. They are derived from B cells that have undergone somatic hypermutation, indicating an active immune response. However, despite their widespread abundance in HGSOC, these patient tumor-reactive autoantibodies fail to eliminate the tumor cells. We hypothesized that the tumor-reactive autoantibodies are unable to eliminate tumors due to the dysregulation of immune interactions via their Fc region. Therefore, we applied a quantitative, multiplexed assay for profiling the Fc properties and immune receptor interactions of patient tumor-reactive autoantibodies. Our data demonstrate that HGSOC patient tumor-reactive autoantibodies interact poorly with FcγRIIIa—a potent activating receptor for antibody-dependent cellular cytotoxicity (ADCC) found on natural killer cells—due to fucosylation. Understanding the mechanisms of humoral immunity evasion will help with the prediction of therapeutic responses in cancer patients and uncover how immunotherapies might reactivate effective humoral immunity.

Poster #44

Cell cycle inhibitors (CCI) help promote immunogenicity and T cell cytotoxicity in the addition of Interleukin-15 (IL-15) in Estrogen Receptor Positive (ER+) breast cancer

Presenter: Jiyeon Park

The University of Utah

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Frederick R. Adler (University of Utah, Salt Lake City, UT)
Jason I. Griffiths (City of Hope National Medical Center, Duarte, CA)
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Estrogen receptor positive (ER+) breast cancers are typically treated with cell cycle inhibitors (CCI), however, about 20% of ER+ breast cancers unfortunately recur due to resistance to treatments. Interleukin-15 (IL-15), a potent immunostimulatory cytokine, stimulates and activates CD8+ cytotoxic T cells, and could enhance immune capacity to regulate and control cancer growth. In order to overcome the hindrance to development of new T-cell based immunotherapy regimens due to cancer heterogeneity in genotypes and phenotypes, progression rate, and the response to immune-activating signals or treatments, we leverage mathematical modeling in collaborations with In vivo experimental works.

We develop a nonlinear ordinary differential equation mathematical model that describes the tumor ecosystem through interactions of host-tumor cells (C) and immune-activating cytotoxic T cells (T) linked with pharmacokinetics by ribociclib (R; CCI) or IL-15(I). This model, developed based on mixed treatment of co-cultured 3D spheroids using CCI-sensitive and resistant cancer cell lines and patient-derived cytotoxic T cells, not only provide excellent fits to the experimental data but also allow pharmacokinetic to appear in the intrinsic growth rates, with both cancers and T cells suppressed by ribociclib and T cells more effectively activated by IL-15.

The key parameters of our model are two interactions: promotions of T cell recruitment and activation by cancer cell antigens and the subsequent killing of cancer cells. Analyzing these parameters under various dose drug combinations drives to two key findings: (1) cell cycle inhibitors, shown to slow the growth of immune T cells as well as cancer cells, in fact help promote immunoogenicity and immune cell toxicity in the addition of IL-15, and (2) the enhanced immune capacity under this combination treatment is observed higher in ribociclib resistant cancer cell lines compared to sensitive cell lines.

These findings, that would have not been obtainable without exploiting the mathematical modeling, support that combining immune-activating treatment with ribociclib outperforms in controlling cancers and overcomes recurrence, due to drug resistance, by strengthening immune surveillance in ER+ breast cancers. Furthermore, the fact that the resistant cancers to cell cycle inhibitors are more targeted for this combination therapy draws attention to be proven at the translational study level.

We will extend the model structure to include additional cell states or types of immune cells by leveraging single cell RNA sequencing (scRNAseq) to unravel the diminished immune-activation signals, resulting in reduced T cell capacity to kill cancer cells, in resistant cancers and identify the key mechanisms of T cell recruitment and activation. Then the extended predictive mathematical model of mechanism will provide the basis to propose optimal adaptive therapy regimens that minimize the total dosage of the two drugs and maximize treatment response, assessed against the objectives of minimizing cancer cell numbers and resistance, and avoiding toxicity and other harmful side effects.

Poster #45

Purine biosynthesis tunes estrogen responses in breast cancer

Presenter: Dina Hany

Plevritis lab, School of Medicine, Stanford University

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Vasiliki Vafeiadou (University of Geneva, Switzerland)
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Two-thirds of breast cancers express estrogen receptor α (ERα), on which they are dependent for growth and survival. Targeting ERα with specific antagonists, such as tamoxifen and fulvestrant have substantially improved the survival of patients. However, about 40% of ERα+ tumors acquire resistance to therapy, which represents a major clinical problem. A switch of growth towards estrogen-independence remains poorly understood. We performed a genome-wide CRISPR/Cas9 knockout screen to discover new genetic determinants of the response of breast cancer to endocrine therapy. PAICS, an enzyme involved in the de novo biosynthesis of purines, appeared as one of the top hits whose loss of function sensitizes the cells to tamoxifen. We found that increased expression of PAICS can shift the growth of ERα-dependent breast cancer to be estrogen-independent and tamoxifen-resistant. Some mechanistic insights revealed that this can be mediated by increased cAMP-activated protein kinase A and mammalian target of rapamycin activities. Finally, we propose PAICS as a novel drug target in combination with ERα for the efficient and potentially safe treatment of ERα+ breast cancer.

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Poster #46

Starving Glioblastoma: Proteomic Characterization of the Response to Arginine Deprivation

Presenter: Ali Basirattalab

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Background: Glioblastoma multiforme (GBM) is a highly aggressive brain tumor with limited treatment options. Targeting metabolic vulnerabilities, such as arginine dependency, has emerged as a promising therapeutic approach due to arginine's critical role in tumor growth. Utilizing systems biology approaches, this study investigates the effects of arginine deprivation in GBM using mass spectrometry-based proteomics to 1) better understand mechanisms of resistance to arginine deprivation and 2) identify possible therapies that synergize with arginine deprivation.

Methods: Four GBM cell lines (A172, LN18, T98, and U87) were subjected to arginine deprivation for 48 hours. Viability assays, flow cytometry, and bottom-up proteomics were conducted to analyze changes in cell cycle progression, and global protein expression, respectively, focusing on cellular stress responses, metabolic reprogramming, and adaptive changes in protein synthesis.

Results: Arginine deprivation led to cell cycle arrest, with GBM cells entering a quiescent state. Integrative analysis of the 2400 proteins identified in all four cell lines by mass spectrometry-proteomics indicated activation of the PERK–EIF2–ATF4 pathway, a key component of the unfolded protein response (UPR), suggesting an adaptive response to endoplasmic reticulum (ER) stress. Additionally, there was upregulation of specific tRNA ligases, including YARS1, GARS1, WARS1, SARS1, AARS1, and NARS1, suggesting an enhanced protein synthesis capacity. Beyond protein synthesis, arginine deprivation induced metabolic reprogramming, evidenced by increased expression of enzymes in the serine biosynthesis pathway, which channels glycolytic intermediates into folate-mediated one-carbon (1C) metabolism. This shift supports biosynthetic and bioenergetic needs under nutrient stress and may help sustain anabolic growth despite metabolic limitations.

Hypotheses and Future Directions: We hypothesize that combining arginine deprivation with inhibitors of the PERK pathway or RNA biosynthesis (e.g., RNA polymerase I inhibitors) will further disrupt the adaptive mechanisms in GBM cells, enhancing the anti-tumor effects of arginine deprivation. Future experiments will assess the efficacy of these combinatorial strategies.

Conclusion: This study reveals that arginine deprivation in GBM cells activates the PERK–EIF2–ATF4 pathway and induces metabolic reprogramming, particularly through the serine biosynthesis and one-carbon metabolism pathways. Targeting these adaptive responses, in combination with arginine deprivation, may represent a novel approach for GBM therapy.

Poster #47

Investigating the role of NPEPPS in regulating High-Grade Serous Ovarian Cancer response to platinum chemotherapy

Presenter: Lily Elizabeth Feldman

University of Colorado Anschutz Medical Campus

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Ovarian cancer is a deadly gynecologic malignancy that caused an estimated 207,000 deaths in 2020. This disease is categorized into histological subtypes, of which High-Grade Serous Carcinoma (HGSC) is the most prevalent and lethal (average 5-year cause-specific survival: 43%). The standard of care for HGSC is platinum chemotherapy. Despite new therapeutic approaches, such as PARP-inhibitor therapy, up to 80% of patients have residual, platinum-resistant disease. There is an urgent unmet need for new approaches to prevent, bypass, or overcome platinum resistance in HGSC. Prior work in the Costello lab discovered puromycin-sensitive aminopeptidase, NPEPPS, as a novel driver of platinum resistance by interacting with a Volume Regulated Anion Channel, SWELL1, to block cisplatin (Cis) import in cancer cells. NPEPPS knockout in Cis-resistant HGSC cells rescues Cis sensitivity and intracellular accumulation of platinum. Cis dose-escalation in murine ID8 HGSC cells results in stable resistance and increased NPEPPS expression. ATAC-Seq data show increased chromatin accessibility in Cis-resistant HGSC cells. These findings point to NPEPPS as a novel driver of platinum chemotherapy resistance in HGSC, but we have yet to establish the clinical relevance of NPEPPS

in HGSC, and the mechanism by which NPEPPS is upregulated to drive resistance is unknown. Our ongoing investigations explore the role of NPEPPS in syngeneic mouse models of cisplatin resistance in HGSC and the role of chromatin reorganization in platinum drug resistance both at the NPEPPS locus and across the genome. This work explores a novel mechanism of cisplatin resistance in HGSC, providing a molecular foundation for developing new therapeutic strategies. I will generate new syngeneic, orthotopic models of platinum-resistant HGSC and develop a tool for investigating the chromatin architecture of HGSC cisplatin resistance.

Poster #48

ProstaMine: A bioinformatics tool for identifying subtype-specific co-alterations associated with aggressiveness in prostate cancer

Presenter: Michael Orman

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Prostate cancer is a leading cause of cancer-related deaths among men, marked by heterogeneous clinical and molecular characteristics. The complexity of the molecular landscape necessitates tools for identifying multi-gene co-alteration patterns that are associated with aggressive disease. The identification of such gene sets will allow for deeper characterization of the processes underlying prostate cancer progression and potentially lead to novel strategies for treatment. We developed ProstaMine to systematically identify co-alterations associated with aggressiveness in prostate cancer molecular subtypes defined by high-fidelity alterations in primary prostate cancer. ProstaMine integrates genomic, transcriptomic, and clinical data from five primary and one metastatic prostate cancer cohorts to prioritize co-alterations enriched in metastatic disease and associated with disease progression. Integrated analysis of primary tumors defined a set of 17 prostate cancer alterations associated with aggressive characteristics. We applied ProstaMine to NKX3-1-loss and RB1-loss tumors and identified subtype-specific co-alterations associated with metastasis and biochemical relapse in these molecular subtypes. In NKX3-1-loss prostate cancer, ProstaMine identified novel subtype-specific co-alterations known to regulate prostate cancer signaling pathways including MAPK, NF-kB, p53, PI3K, and Sonic hedgehog. In RB1-loss prostate cancer, ProstaMine identified novel subtype-specific co-alterations involved in p53, STAT6, and MHC class I antigen presentation. Co-alterations impacting autophagy were noted in both molecular subtypes. ProstaMine is a method to systematically identify novel subtype-specific co-alterations associated with aggressive characteristics in prostate cancer. The results from ProstaMine provide insights into potential subtype-specific mechanisms of prostate cancer progression which can be formed into testable experimental hypotheses.

Poster #49

Personalizing Care: Leveraging clinical patient data to predict patient responses to frontline pharmacological interventions in metastatic prostate cancer

Presenter: Ralf Philipe Dagdag

University of Colorado Anschutz Medical Campus

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Prostate cancer (PCa) has the highest rate of incidence among all cancer types in men and is the second leading cause of cancer-related death. Typically, most men diagnosed with PCa will not require immediate treatment and will undergo active surveillance. A fraction of those individuals, however, will develop a metastatic form of the disease (mPCa), likely due to: 1) the inability of clinicians to identify whether a patient will have a prolonged response to androgen receptor signaling inhibitors (ARSi) such as abiraterone (ABI) and enzalutamide (ENZ), and 2) germline single nucleotide polymorphisms (SNPs) that act as heritable risk factors for resistance to treatment. Information regarding the treatment of mPCa is lacking compared to localized disease, represents in a critical need in the field – a better understanding of late-stage mPCa at the clinical and molecular levels pertaining to drug response will improve patient outcomes. This study propose two aims to study the hypothesis that patient responses rates to ARSi treatment can be effectively understood through patient health history, as well as heritable influencers of response.

Specific Aim 01: Determine the clinical variables that are most predictive of patient response to treatment. A meta-analysis is proposed, using de-identified clinical data from mPCa patient EHRs within various healthcare organizations within the UCHealth Global Research Network and the Flatiron Institute. These data represent a comprehensive assessment of clinical variables based on ICD-10-CM, CPT, LOINC, and RxNorm codes. Patients are stratified based on whether they received ABI or ENZ as a frontline ARSi, and cases and control are defined as non-response and response, respectively. Elastic net, XGBoost, and dimensionality reduction methods will be used to: 1) identify clinical variables that are pertinent for predicting response, and 2) build classification models to predict response based on health history. The anticipated outcomes are that clinical variables are present in EHRs that are predictive of patient response to ABI or ENZ and capable of training effective ML models.

Specific Aim 02: Identify germline variants that contribute to the pharmacogenomics of non-response. A GWAS for ARSi non-response in mPCa will be generated by applying a linear mixed model on a retrospective meta-analysis of human germline genomic data from the Colorado Center for Personalized Medicine, as well as the NIH All of Us research program. Functional mapping and annotation (FUMA) will be used to identify genes and loci dysregulated by germline variants. The most significant genes are considered candidates for drug repurposing, ranked by CADD score, eQTL analysis, and MAGMA analysis. The overall anticipated outcomes will determine whether germline variants exist that impact functional genes, contributing to ARSi non-response.

The findings of this study will provide clinically significant findings for improving clinical gestalt in mPCa through precision care. ML models built from patient EHR data alone can be used to suggest best treatment options based on clinical information, while pharmacogenomics approaches can enhance care by elucidating heritable drivers of molecular dysregulation and highlight gene candidates with a potential for drug repurposing efforts against end-stage mPCa.

Poster #50

High-content microscopy for characterizing and predicting drug response in NF1-/- Schwann cell cultures and NF1 patient-derived tumor organoids

Presenter: Gregory P. Way

University of Colorado Anschutz, Aurora, CO

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Neurofibromatosis type 1 (NF1) is a multi-system, autosomal dominant genetic disorder driven by the systemic loss of the NF1 protein neurofibromin. Loss of neurofibromin in Schwann cells is particularly detrimental, as the acquisition of a 'second-hit' (e.g., complete loss of NF1) can lead to the development of neurofibromas, including cutaneous neurofibromas (cNFs) and plexiform neurofibroma tumors (pNFs). pNFs are painful, disfiguring tumors with an approximately 1 in 10 chance of sarcoma transition into malignant peripheral nerve sheath tumors (MPNSTs). Selumetinib is currently the only drug approved by the U.S. Food and Drug Administration (FDA) for NF1, and it is only indicated for a subset of NF1 patients with pNFs. Neurofibromas are highly heterogeneous, containing fibroblasts, mast cells, and Schwann cells, which contribute to NF1 deficient tumor microenvironments. Lack of treatment options motivates the need to develop new therapies, and neurofibroma heterogeneity motivates the need for complex, patient-derived models to identify effective therapies. We are developing a precision medicine framework to rapidly identify candidate therapeutic agents in high-throughput and to test them in NF1 patient-derived organoids (Nguyen et al, 2024).

We have identified a high-content microscopy signature of neurofibromin loss in Schwann cells, which will enable us to pursue large-scale drug screens in NF1-deficient Schwann cells to identify therapeutic agents that return NF1 patient Schwann cells to phenocopy the NF1 wildtype and healthier phenotype. We applied Cell Painting, a multiplexed high-content fluorescence microscopy assay, to mark nuclei, endoplasmic reticulum, mitochondria, and F-actin of two isogenic Schwann cell lines: an NF1+/+ wildtype genotype and an NF1 null genotype (NF1-/-). We used CellProfiler to perform quality control, illumination correction, segmentation, and cell morphology feature extraction. We segmented 22,585 NF1 wildtype and null cells, utilized 907 significant cell morphology features representing organelle shapes and intensity patterns, and trained a logistic regression machine learning model to identify an NF1-/- genotype signature of single Schwann cells. The machine learning model had high performance, with training and testing data yielding a balanced accuracy of 0.85 and 0.80, respectively (Tomkinson et al. 2024).

Given that patient-derived NF1 tumor organoids are clinically-relevant models of disease that faithfully recapitulate the heterogeneity and complexity of these tumors (Nguyen et al 2024, Al Shihabi et al 2024), they can be leveraged for drug discovery studies to identify effective regimens that can be rapidly translated to the clinic. We adapted our platform for rapid establishment and screening of patient-derived NF1 tumor organoids (Nguyen et al 2024, Al Shihabi et al 2024) to incorporate a Cell Painting assay compatible with our 3D mini-ring culture system (Phan et al, 2019). We performed proof-of-principle screenings utilizing Cell Painting as well as cell type-specific markers on patient-derived tumor organoids from cNFs, pNFs, and MPNSTs. We are developing an advanced image analysis framework for processing phenotypic information from high-resolution, single-organoid resolution images and determining whether extracted features are predictive of drug responses.

Poster #51

Mechanisms of Parthanatotic Cell Death

Presenter: Kelly Ward

UMass Chan Medical School

Kelly Ward (UMass Chan Medical School, Worcester, MA)
Mike Lee (UMass Chan Medical School, Worcester, MA)

Cancer treatments can induce either growth inhibition, death, or both in cancer cells, though cell death is required for the shrinkage of a tumor. There are fourteen known types of cell death, yet the mechanisms of many of these death types remain unresolved. Elucidating these mechanisms could provide valuable insights into which treatments may induce which death types and how these death mechanisms can be leveraged for therapeutic value in patients. Parthanatos is a poly(ADP-ribose) polymerase 1 (PARP1)-mediated form of cell death and occurs following hyperactivation of PARP1 and excessive buildup of PAR, though it remains unclear how the death results. Importantly, parthanatos occurs following treatment with cytarabine and idarubicin for acute myeloid leukemia, showing its clinical importance. To fully characterize the mechanism, I performed genome wide chemo-genetic profiling with methylnitronitrosoguanidine (MNNG), the canonical activator of parthanatos. The screen was then analyzed with the Method for Evaluating Death Using a Simulation-assisted Approach (MEDUSA) to specifically characterize how each gene knockout impacted the death rate. Preliminary data suggests that previously identified mechanisms are not universally required for parthanatos. My poster will detail the setup utilized to optimize studying parthanatotic death, the analysis techniques performed, and new details about the mechanisms of parthanatos.

Poster #52

p16 expression confers sensitivity to CDK2 inhibitors in CCNE1-amplified ovarian cancers

Presenter: Chance Sine

CU Boulder

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Blocking the cell cycle is a promising avenue for cancer therapy, with Cyclin-Dependent Kinase 2 (CDK2) emerging as a key target. However, in multiple cell types, CDK4/6 activity compensates for CDK2 inhibition and sustains the proliferative program, enabling CDK2 reactivation. Thus, we hypothesized that sensitivity to CDK2 inhibition is linked to the absence of this CDK4/6-mediated compensatory mechanism. Here we show that Cyclin E1-driven ovarian cancers often co-express the tumor suppressor p16, which inhibits CDK4/6. We show that ovarian cancer cells expressing p16 exhibit heightened sensitivity to CDK2 inhibitors and that depletion of p16 renders them significantly more resistant. Multiplexed immunofluorescence of 225 ovarian patient tumors reveals that at least 18% of tumors express high Cyclin E1 and high p16, a group that we expect to be particularly sensitive to CDK2 inhibition. Thus, p16 may be a useful biomarker for identifying the patients most likely to benefit from CDK2 inhibitors.

Poster #53

Causes and consequences of off-target activation of the GCN2-eIF2a-ATF4 axis in targeted cancer therapy

Presenter: Riley Ill

Spencer Lab, CU Boulder

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Nasreen Marikar (University of Colorado Boulder, CO)
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Alicia Darnell (Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA)
Stacey Bagby (University of Colorado Anschutz School of Medicine, CO)
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Matthew G. Vander Heiden (Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA)
Philip Reigan (Skaggs School of Pharmacy, University of Colorado Anschutz, CO)
Sabrina L. Spencer (University of Colorado Boulder, CO)

Kinase inhibitors are notorious for their promiscuity and off-target effects. We discovered that multiple clinical inhibitors used in the treatment of cancer, including dabrafenib and erlotinib, activate the GCN2 kinase in an off-target manner via direct interaction. GCN2 is an activating kinase of the integrated stress response (ISR) that leads up induction of the transcription factor ATF4. Cotreatment with a GCN2 inhibitor in and EGFR19-/- lung adenocarcinoma significantly increases the rate of drug resistance development and cell outgrowth. We thus propose that off-target activation of GCN2 limits the development of drug-resistant and metastatic phenotypes. Finally, we perform an in silico inhibitor screen, identifying dozens of FDA-approved, clinically utilized cancer inhibitors that appear to share this off-target GCN2 interaction.

Poster #54

An Evolutionary Approach to Reproductive Cancers

Presenter: Walker Mellon

Arizona Cancer Evolution Center

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Reproductive cancers constitute a significant portion of cancer incidence and mortality in humans. Unique insights may be obtained by adopting an evolutionary approach when studying reproductive cancers. Drawing from a cross-species data set, these findings have the potential to provide a novel understanding of the diseases under study. Leveraging Phylogenetic Comparative Methods enables us to estimate the evolution and suppression of reproductive cancers. Using necropsy data for 16,406 individuals spanning 307 species, a significant positive correlation was observed for malignant reproductive cancers and the average adult weight of the species ($p = 0.0039$), suggesting that larger species tend to exhibit higher rates of reproductive cancers. Additionally, there was a significant positive correlation between neoplasm development ($p = 0.0052$) and malignancy development ($p = 0.0176$) and litters per year, indicating that species with larger litter sizes may have higher rates of tumor progression. By applying an evolutionary approach to analyzing reproductive cancer prevalence, we may better uncover patterns in tumor progression that deepen our understanding of cancer mechanisms and inform the development of potential treatments.

Poster #55

A Computational Pipeline for Evaluating Agreement Between Large-Scale Models and Diverse Datasets

Presenter: Jonah R. Huggins

Dept. of Chemical and Biomolecular Engineering, Clemson University

Jonah R Huggins (Clemson University, Clemson, SC)
Isabel Leal (Clemson University, Clemson, SC)
Benjamin Childs (Clemson University, Clemson, SC)
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Computational models capturing the function of every gene within a cell, known as Whole-Cell Models (WCMs), can predict complex, multi-gene phenotypes while reconciling discrepancies in current understanding1,2. Constructing such models requires the integration of diverse datasets of varying sizes from different labs and assay types. However, aggregating these datasets into a model-readable format to scalably identify model-data mismatch (i.e. knowledge gaps) pose a large challenge for model construction3. We are creating a computational pipeline to rapidly evaluate agreement of a large-scale mechanistic model of a human epithelial cell (the SPARCED model4) with a compendium of data spanning multiple sources and modalities. Conditions, duration, and results of wet-lab experiments are converted into a machine readable format based in-part on PEtab guidelines5. To ensure this pipeline covers a broad range of potential use case scenarios, we constructed 13 benchmarks SPARCED has previously been validated against, comprising various biological conditions, perturbations, and measurement techniques. Initial deployment (i.e. creating new benchmarks) on the LINCS Microenvironment (ME) perturbation dataset6 indicates mixed agreement with Reverse Phase Protein Array (RPPA) data. Further model agreement is being evaluated with RNAseq, ATACseq, and highly multiplexed immunofluorescence perturbation data. This pipeline will provide a means to rapidly evaluate how diverse datasets collectively compare to model variants, thereby improving the accuracy and scalability of SPARCED and contributing to the creation of a human Whole-Cell Model.

Poster #56

Histopathology and RNA-sequencing to Inform Immune-Related Adverse Events Following Checkpoint Inhibitor Treatment

Presenter: Melanie Joy

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Background and Purpose: Immune checkpoint inhibitors (ICIs) have advanced cancer treatment by restoring the ability of the immune system to remove tumor cells. However, use of ICIs can be haltered by immune-related adverse events (irAEs). The purpose of this study was to evaluate the ability of a novel tumor-bearing, Human Immune System (HIS) mouse model to recapitulate the histopathological changes associated with ICI-mediated irAEs and use bulk RNA-sequencing to identify gene pathways dysregulated during irAEs.

Methods: Non-humanized BALB/c-Rag2nullIl2rgnullSIRPaNOD (BRGS) and human immune system (HIS-BRGS) mice, humanized with human CD34+ hematopoietic cells, were implanted with MDA-MB-231 triple-negative breast cancer cells and treated with vehicle (PBS) or two ICIs (anti-PD-1 nivolumab, anti-CTLA-4 ipilimumab, 20mg/kg and 10mg/kg respectively weekly, ip) for 4 weeks. Histomorphological evaluation was conducted on all major tissues. Transmission electron microscopy (TEM) was performed on fixed kidney tissues. Total RNA was isolated from the lungs, kidneys, and livers of vehicle- and ICI-treated HIS-BRGS mice

and subjected to bulk RNA Sequencing for identification of differentially expressed human genes and pathway analysis using KEGG.

Results: No significant pathological findings were observed in the skin, heart, muscle, thyroid, and intestines of BRGS and HIS-BRGS mice, regardless of treatment. Livers of BRGS mice had healthy parenchyma with occasional tumor cells and extramedullary hematopoiesis. By comparison, HIS-BRGS mice exhibited mononuclear cell inflammation and hepatocyte necrosis that was more severe following ICI treatment. The lung parenchyma of BRGS mice was histologically normal and exhibited tumors that were reduced in size by ICI treatment. By comparison, lungs of HIS-BRGS mice had little to no tumors but exhibited mild-to-moderate perivascular inflammation following ICI treatment. Similarly, kidneys of ICI-treated HIS-BRGS mice exhibited minimal-to-mild periarterial accumulations of lymphocytes and multifocal, minimal interstitial mononuclear cell aggregates. TEM revealed podocyte changes in HIS-BRGS mice that were more extensive following ICI treatment. RNA sequencing revealed human transcriptomic pathways that were associated with pathologically confirmed ICI irAEs in HIS-BRGS mice. For lung irAEs, the 'cytokine-cytokine receptor interaction' and 'Hippo cancer signaling' pathways were significantly enriched. For liver irAEs, the 'hematopoietic cell lineage' pathway was most altered. By comparison, no human transcriptomic pathways were significantly associated with kidney irAEs in HIS-BRGS mice.

Conclusions: Human Immune System-BRGS mice recapitulate some clinically observed ICI toxicities, particularly in the lung, kidney, and liver, following treatment with ICIs. Furthermore, profiling of the human transcriptome in these mice can be used to identify differentially altered pathways associated with ICI-induced irAEs. This research was supported by R01CA277313, NJ Health Foundation, R25ES020721, P30CA072720, and P30CA046934.

Poster #57

Interrogation of Mechanisms of Kidney Injury in HIS-BRGS Mice Treated with Immune Checkpoint Inhibitors

Presenter: Sarah Asby

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Background and Purpose: Immune checkpoint inhibitors (ICIs) are monoclonal antibodies blocking surface receptors on immune cells, including PD-1 and CTLA-4, to enhance their antitumor responses. Overactivation of the immune response can trigger systemic toxicity to multiple organs, with at least 5% of cancer patients receiving ICIs developing acute kidney injury. In the present study, we sought to 1) profile the expression of protein biomarkers of kidney disease and 2) interrogate potential mechanisms of kidney injury in mice treated with ICIs.

Methods: To reconstitute mice with a humanized immune system (HIS-BRGS), BRGS (BALB/c-Rag2nullIl2rynullSirpαNOD) mice were irradiated and repopulated with human CD34+ hematopoietic cells. BRGS and HIS-BRGS mice were implanted with human tumor cells and treated with vehicle control or ICIs (nivolumab/ipilimumab combo, 10 mg/kg weekly, ip) for 4 weeks. Tumor growth was monitored during the treatment. After treatment, mouse plasma, urine, and kidneys were collected for: 1) histopathology scoring; 2)

clinical chemistry; 3) immune cell populations by flow cytometry; 4) novel kidney injury biomarkers in urine and kidney tissue including KIM-1, IP-10/CXCL10, renin, B2M, VEGF, TIMP-1, EGF, NGAL, OPN, and clusterin; and 5) human cytokine and chemokine biomarkers in plasma and kidneys. Biomarkers were assessed using multiplex ELISAs.

Results: Compared to vehicle-treated HIS-BRGS mice, the volume and weight of tumors were decreased in ICI-treated HIS-BRGS mice by ~50% and ~30%, respectively. A humanized immune system in BRGS mice increased BUN levels by 2- to 3-fold, with no further change in response to ICI treatment. Notably, vasculitis and interstitial nephritis were observed in ICI-treated HIS-BRGS mice, but not in vehicle-treated counterparts. The kidneys of ICI-treated HIS-BRGS mice had a 50% reduction in infiltration by PD-1+/CD4+ and PD-1+/CD8 cells and an overall shift towards CD4+ cell enrichment compared to CD8+ cells. This was accompanied by increased circulating and renal concentrations of numerous human cytokines and chemokines including C-C motif chemokine ligands (CCL1/3/4/17/22), interleukins (IL15/16), macrophage migration inhibitory factor (MIF), C-X-C motif chemokine ligands (CXCL10/13), and soluble receptors including CD30, B cell-activating factor (BAFF), and TNF receptor superfamily member 1B (TNF-RII). ICI treatment also preferentially increased serine proteases, granzyme A and B, as well as nerve growth factor beta (NGF-beta) in the kidneys, but not the plasma, of HIS-BRGS mice. Significant correlations were observed between more extensive vasculitis/interstitial nephritis and elevated kidney levels of BAFF, granzyme A/B, IL16, MIF, and CCL4 proteins in ICI-treated HIS-BRGS mice. In addition, NGF-beta concentrations negatively correlated ($p<0.05$) with PD-1+/CD4+ (r: -0.7020) and PD-1+/CD8+ cells (r: -0.7125), respectively. Notably, ICI treatment did not significantly change the concentrations of emerging kidney injury biomarkers, including KIM-1 and NGAL, among others in the mouse model.

Conclusions: Our study suggests that shifts in human T lymphocyte populations and increased secretion of certain immune proteins are potential mechanisms of ICI-induced nephrotoxicity. The cytokines and chemokines identified in this study could serve as promising biomarkers and potential targets for counteracting ICI-induced nephrotoxicity. This research was supported by R01CA277313, NJ Health Foundation, R25ES020721, P30CA072720, and P30CA046934.

Poster #58

High plex single cell spatial signatures of lung cancer resistance to therapy

Presenter: Hatim Sabaawy

University of Colorado Anschutz

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(University of Colorado Anschutz Medical Campus)

Lung cancer (LC) is the leading cause of cancer-induced mortality. Tyrosine kinase inhibitors (TKI) and targeted therapy against oncogenic drivers (KRAS, EGFR, or ALK fusions, others) are effective initial therapies; however, nearly all patients treated with standard of care (SOC) eventually develop resistance to therapy, likely due to the heterogeneity and different tumor and tumor microenvironment (TME) phenotypic cell states among and between patients. Here, we developed a novel approach utilizing small LC biopsies from primary and metastatic sites of patients, including paired diagnostic and resistance tissues when available, dividing tissues between single cell spatial profiling (scSP) and patient derived organoid (PDO) 3D cultures to perform: 1) SOC clinical NCCN-guided CLIA targeted genomic sequencing panel, 2) Comprehensive molecular profiling of transcriptome (bulk/single cell scRNAseq), scSP using spacial molecular imager (SMI) with CosMx (980 transcripts and 78 proteins, including custom phospho proteomes) to assess lineage plasticity in pre- and post-TKI resistance sections, and 3) primary multi-region PDOs (~1,000 organoid/region) to validate cell and

lineage phenotypes and drug responses, and 4) patient derived xenografts (PDXs) in mice for confirming in vivo responses to therapy. Tumor and TME cell types were identified using high-plex multiscale single cell imaging and scSP of the full transcriptome and tumor/TME proteome. Pathology annotations of H&E images were overlaid, cell typing were performed, and AI-based spatial analyses were done with Seurat and Giotto in R. Spatial profiles are used to determine the key molecular bypass mechanism for resistance to TKI for each patient, including on-target variants, driver bypass, lineage plasticity and acquired resistance. Functional assays for acquired resistance such as PI3K/AKT signaling, Src kinase, BRAF fusion and MET hyperactivity are validated in PDOs and allowed the identification of potential novel therapies for each patient. Our approach provides a comprehensive molecular and drug sensitivity profiles for each patient and could create a feasible path for precision medicine-guided impact on patient care.

Poster #59

Transcriptional Regulation of Protein Synthesis by Mediator Kinase Represents a Therapeutic Vulnerability in MYC-driven Medulloblastoma

Presenter: Dong Wang

University of Colorado Anschutz Medical Campus

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MYC-driven medulloblastoma (MB) is a highly aggressive cancer type with poor prognosis and limited treatment options. Through CRISPR-Cas9 screening of MB cell lines, we identified the Mediator-associated kinase CDK8 as a critical regulator of MYC-driven MB. Loss of CDK8 substantially reduces MYC expression, induces pronounced transcriptional changes, suppresses monosome assembly, and decreases ribosome biogenesis and protein synthesis, consequently inhibiting MB growth. Mechanistically, CDK8 regulates the occupancy of RNA polymerase II at specific chromatin loci, facilitating an epigenetic alteration that promotes the transcriptional regulation of ribosomal genes. Targeting CDK8 effectively diminishes the stem-like neoplastic cells characterized by hyperactive ribosome biogenesis. Furthermore, we demonstrated that the combined inhibition of CDK8 and mTOR synergizes to optimize therapeutic outcomes in vivo and in vivo. Overall, our findings establish a connection between CDK8-mediated transcriptional regulation and mRNA translation, suggesting a promising new therapeutic approach that targets the protein synthesis for MYC-driven MB.

Poster #60

Assessing Drug-Response Dynamics of Glioblastoma Stem-like Cell Populations

Presenter: James Park

Tulane University

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Tumor cell plasticity and heterogeneity represent major challenges to achieving effective treatment in cancers. In the context of glioblastoma (GBM), the most prominent and lethal primary brain tumor in adults, tumor

cell plasticity and resulting phenotypic shifts can result from epigenetic mechanisms that lead to the emergence of resistance, i.e., acquired resistance, in a subpopulation of tumor cells. We have previously observed drug-induced phenotypic changes in GBM stem-like cells (GSCs), a rare subpopulation of tumorigenic cells that undergo a proneural-to-mesenchymal transition (PMT) as a mode of therapy evasion. Here, we examined the dynamics of PMT in two patient-derived GSC populations (PD-GSCs) that either acquired resistance or were inherently resistant to pitavastatin, a drug that has anti-proliferative effects on glioma cells. We applied a combination of methodologies from network inference, machine learning, and optimal transport theory to assess longitudinal single-cell RNA sequence (scRNA-seq) characterization of drug responses for these two PD-GSC populations. Our results revealed a subset of responsive PD-GSCs, along with corresponding gene expression markers and regulatory network states that were more "poised" to respond to treatment and undergo PMT, leading to the development of sustained resistance. In contrast, non-responsive PD-GSCs exhibited an incoherent population structure with no discernable presence of cells that were more or less poised to undergo a phenotypic transition. The current work highlights a systems biology approach to evaluate the causative mechanisms underlying GSC dynamics that result in acquired resistance.

Poster #61

Stacked, Conditional, Variational Autoencoder to Capture Cancer Type and Subtype Heterogeneity Using TCGA Data

Presenter: Weishan Li

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Biological systems are inherently hierarchical, yet this structure is often not reflected in mathematical models. To take advantage of this hierarchical structure, we present a stacked, conditional, variational autoencoder (VAE) to capture hierarchical dependencies for cancer type and subtype classification. Model inputs include a transcriptome matrix A (n samples x m genes) and k categorical variables y1, ..., yk (e.g., cancer type, subtype). A primary encoder e0 processes A to yield an initial latent space l0. Each encoder ei (for 1 <= i <= k) then encodes the previous latent l_{i-1}, conditioned on yi, producing a progressively refined latent li. A shared decoder reconstructs A and the categorical labels y1, ..., yk from each latent space.

We applied our model to RNA expression data from TCGA to classify both cancer types and subtypes. Accordingly, our model encodes RNA expression, A, one-hot-encoded cancer types ycancer and subtypes ysubtype progressively with three layers of encoders e_RNA, e_cancer, e_subtype, which generate latent spaces l_RNA, l_cancer, l_subtype. The shared decoder reconstructs A', y'cancer, and y'subtype from each latent space. Predictions can be made directly from l_RNA = e_RNA(A); with cancer type information, l_cancer = e_cancer(l_RNA | y_cancer) offers more refined subtype predictions.

We hypothesized that this stacked, progressively conditioned model (hereon referred to as stacked model), in which latent spaces incorporates increasingly specific label information (RNA → cancer type → subtype), produces a structured latent space from the deepest encoder e_subtype that achieves near-perfect classification performance. The shared decoder setting would then allow the model to transfer the learned optimal encoding scheme to shallower encoders for improved predictive performance for subtype from RNA expression and/or cancer type over a simple unconditional or unconditional VAE.

Benchmarking against a base VAE and a flat conditional VAE (encoding A | y1, ..., yk), we trained all models on TCGA data covering major cancer types (BRCA, OV, Pan-GI, Pan-Glioma, THCA, UCEC, KIRC, HNSC) and their subtypes. The stacked model achieved higher balanced accuracy (validation = 61.1%) across 20 bootstraps versus the base model (59.6%), with stronger type-specific performance in 7 out of the 8 cancer types.

To evaluate biological relevance, we assessed the hierarchical cluster of genes based on gene expression-to-

latent variable correlation matrix from models trained on single cancer-type datasets and measured the degree to which the resulting groupings of genes resembled previously established cancer-specific gene expression signatures. When trained on the bootstrapped BRCA dataset, hierarchical clustering of the stacked model gene expression-to-latent variable correlation matrix grouped PAM50 genes similar to KMeans clustering obtained from PAM50 gene expression centroids, resulting in a mean Adjusted Rand Index (ARI) of 0.39 (base model = 0.35). In models trained on OV only dataset, clustering aligned with ovarian cancer subtype signatures by Verhaak et al. (PMID: 23257362), with a mean ARI of 0.61 (base model = 0.47).

These findings suggest that the stacked model consistently outperforms reference models and effectively organizes RNA features in line with established subtype expression signatures. Future work will extend this architecture to hierarchical dependencies beyond categorical variables, including treatment response prediction.

Poster #62

Exploring uneven redistribution of extrachromosomal DNA after population heterogeneity loss

Presenter: Oliver Cope

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Cancer cell population heterogeneity drives the evolution of the disease and presents a difficult challenge when developing cancer treatments. One factor contributing to population heterogeneity is extrachromosomal DNA (ecDNA), or amplifications of key driver genes found outside of chromosomes. Because ecDNAs are not located on the chromosomes and lack centromeres, they segregate randomly into daughter cells leading to unequal inheritance. Understanding how this process happens can help us better understand how cells may exploit ecDNA for fast-tracking evolution and population fitness.

In this study, we used fluorescence-assisted cell sorting (FACS) to divide a primary cancer cell line into two subpopulations based on high and low protein expression of a specific cell surface marker. Previous work has shown that this cell line harbors a large and variable amount of ecDNA, with copy numbers ranging from less than a hundred to more than a thousand per cell, making it ideal for studying the evolution of intratumoral heterogeneity. The ecDNA in these cells contains specific oncogenes colocalized on the same structure, so sorting based on one marker allows us to control for both these targets. The cell surface protein, detectable with fluorescent antibodies, enables FACS sorting on live cells that can be cultured post-sorting. Samples of these subpopulations were collected every 48 hours to measure ecDNA levels through fluorescence in situ hybridization imaging and protein expression using flow cytometry.

Our results show that after just 48 hours, the two subpopulations had become remarkably similar. The difference in average total ecDNA between the high and low protein expression groups decreased significantly, and the difference in mean fluorescence intensity of protein expression between the two groups also showed a substantial reduction. Computer simulations modeling a purely random division of ecDNA into daughter cells predict that the average total amount of ecDNA between these subpopulations would remain relatively stable over time, unlikely to redistribute significantly within one or two generations. The rapid shift in ecDNA distribution observed experimentally suggests additional selective pressures acting on cancer cells, independent of chemotherapy. This change points to differences in growth rate and cellular behavior that likely correlate with ecDNA levels in cancer cells. These findings support the idea that ecDNA affords cancer cells remarkable adaptability and enables cancer cells to rapidly develop intratumoral heterogeneity, contributing to distinct cellular phenotypes and growth dynamics.

Poster #63

Defining the relationship between chemotherapy-induced quiescence and senescence

Presenter: Brianna Fernandez

University of Colorado Boulder

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Quiescence, a reversible cell-cycle arrest, and senescence, an irreversible cell-cycle arrest, are difficult to distinguish from one another. Additionally, both quiescent and senescent cells arise side by side following chemotherapy or IR treatment. While senescent cancer cells are tumor suppressive as they limit the proliferation of damaged cells and recruit the immune system, quiescent or dormant cancer cells are a source of drug resistance as they evade chemotherapeutic treatments targeted at cycling cells and may proliferate again in the future. Thus, there is a critical need for a quantitative molecular characterization of the relationship between these two states.

Using eight canonical senescent biomarkers, we previously showed that quiescence and senescence are not binary distinct states, but rather exist on a continuum. Here we used single-cell time-lapse imaging of CDK2 activity of thousands of single cells after chemotherapeutic treatment to measure cell-cycle progression and cell-cycle withdrawal, and paired this with scRNA-sequencing. We found that cells that cycle after chemotherapy retain a 'memory' of drug treatment even though they appear to cycle normally, suggesting they did not completely evade the effects of chemotherapy. We also found two paths to senescence: a canonical Mitosis-->cell-cycle exit, and a non-canonical G2-->cell-cycle exit where mitosis is skipped, resulting in large arrested cells with 4N DNA content. We further found that cells take on different senescence states depending on which route they take, leading to multiple types of senescent cells with distinct expression patterns and novel biomarkers. Finally, we characterized the transcriptional changes cells undergo as they move gradually from reversible arrest (quiescence) to irreversible arrest (senescence).

Poster #64

The colocatome as a spatial omic: a quantitative framework enabling spatial cell-cell colocalizations comparisons between in vitro patient-derived models and pathological specimens

Presenter: Gina Bouchard

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The emergence of highly multiplexed spatial omics technologies is advancing translational oncology research by enabling a more comprehensive understanding of the cellular organization within the tumor microenvironment (TME). While these diverse -omic technologies can capture spatially resolved molecular information, often at single cell resolution, analysis and comparison of spatial data across studies poses several challenges. In parallel, translational researchers are increasingly relying on patient-derived co-cultures as high-throughput experimental platforms to model human diseases. While these in vitro models do not perfectly replicate human tissue architecture, cell heterogeneity, or the effects of environmental stimuli, their usage continues to rise due to their accessibility, ease of gene editing, cost advantages, and advocacy for alternatives to animal testing in research.

For these reasons, a standardized, quantitative way to compare and analyze spatial data between in vitro patient-derived models and human tumor samples is an important step toward gaining clinically relevant information from in vitro models.

In this work, we build on those concepts and propose a quantitative framework, termed colocatome analysis, for comparing spatial features across conditions and studies. We demonstrate how the colocatome framework allows for the direct comparison of cell-cell colocalizations between three-dimensional in vitro models and patient specimens, enabling the identification of conserved spatial features between different types of samples. Our quantitative framework uses the colocation quotient (CLQ) spatial metric for identifying cell subpopulation pairs in close proximity (positive colocalization) versus those that are distant (negative colocalization). We apply spatial randomization to assess the significance of each colocalization compared to a null distribution established by permuting the cell types. Lastly, we apply normalization to the CLQs under a given condition to enable comparisons of statistically significant colocalizations to each other and across different conditions, including between in-vitro assays and clinical samples.

We apply colocatome analysis to tumor-stroma assembloids generated with lung adenocarcinoma (LUAD) epithelial organoids and cancer-associated fibroblasts (CAFs) harvested from spatially distinct tumor sites (edge: Tumor-adjacent fibroblast (TAFs); vs. core: Tumor core fibroblasts (TCFs)) and correlate these spatial features with specific LUAD histopathological growth patterns in clinical samples, a well-accepted strategy for investigating common tumor-stroma interactions. Further, we interrogate fibroblast-induced spatial rearrangements related to drug resistance in both assembloids and treatment-naïve human tumor samples.

Using the colocatome framework, we identify which drug-resistant and drug-sensitive cell-pairs colocalizations from the in-vitro model are present in treatment-naïve clinical samples. Our findings demonstrate that spatially distinct fibroblasts exert a unique influence on the spatial organization of cancer cells and primarily promote treatment resistance through spatial reorganization rather than specific subpopulation selection or depletion. In addition, we show that the reference colocatome inferred from our assembloids accurately recapitulates the cell-cell colocalizations associated with specific histopathological growth patterns (lepidic, acinar and solid) of a small cohort of LUAD clinical specimens. In conclusion, our quantitative framework enables direct comparison of specimens across various conditions or assays with the potential to enable the spatial biology community to advance toward a common goal of cataloging and comparing spatial features involved in biological processes and human diseases.

Poster #65

Towards Digital Twins: Simulating in vitro Experiments to Model the Tumor Microenvironment in Pancreatic Ductal Adenocarcinoma

Presenter: Dorothy N. Beck

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by a highly fibrotic and hypovascular stroma that inhibits treatment delivery. As a result, PDAC tumors exhibit remarkable chemoresistance and poor prognosis for patients. Current in vitro model systems fail to faithfully recapitulate the dynamic spatial and temporal heterogeneities of the tumor microenvironment (TME) during disease progression, necessitating innovative platforms that capture the cellular interactions within the TME. We present a novel agent-based model (ABM) for designing advanced biomaterials that can be used as sophisticated 3D culture models to investigate the TME. Engineered biomaterial systems allow for variables to be independently perturbed irrespective of the entire system, providing a broad opportunity for univariate analysis within the TME. ABM complements this as a powerful computational tool that models heterogeneous cells in their environment and uses their interactions with other cells to deduce emergent phenomena.

Our ABM simulates the crosstalk among endothelial cells, PDAC cells, inflammatory cancer-associated fibroblasts (iCAFs), and myofibroblastic cancer-associated fibroblasts (myCAFs) across 3D experimental co-culture assays. ABM predictions inform the design of two mechanically tunable hydrogel scaffolds to recapitulate distinct stages of PDAC: 1) a highly porous, granular hydrogel scaffold and 2) a bulk hydrogel. The granular hydrogel scaffold is more representative of the early PDAC stroma because it emulates the heterogeneous structural features in both the healthy pancreas and the early stage/PanIN stage, such as acini, islets, interlobular ducts, and blood vessels. In contrast, bulk hydrogels are more representative of late PDAC, which appears to be more structurally homogeneous and lacks the variety of structural features present in early PanIN and healthy pancreas. ABM simulations were also used to investigate how changing the total number of CAFs and ratios of iCAFs to myCAFs impact TME neovascularization during disease progression, where changes to vascularization conserved across different hydrogel systems and in silico models are likely to be conserved in the complex and heterogeneous in vivo TME. Specifically, we modeled an endothelial cell spheroid implanted within each hydrogel that is also seeded with myCAFs and iCAFs. In both models for granular and bulk hydrogels, we found that an increased abundance of myCAFs and iCAFs at a 1:1 ratio correlated to a decrease in vessel density and a less intricate vessel network due to cytokine secretion from iCAFs and matrix stiffening from myCAFs. In the same models, we also determined that iCAFs have a more significant impact on vessel network patterning and angiogenic growth than myCAFs. Overall, the porous granular hydrogel scaffold had increased vessel density compared to a bulk hydrogel model. Integrating 3D culture systems with ABMs enables a multi-scale exploration of the TME in vitro and in silico through modeling vascularity and fibrosis throughout the disease time course. Future work will use this framework to explore how chemotherapeutic agents can target endothelial cells and CAFs to modulate the fibrotic and hypovascular PDAC stroma through CAF-medicated effects.

Poster #66

Single-cell transcriptomes identify patienttailored therapies for selective co-inhibition of cancer clones

Presenter: Kristen Nader

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Intratumoral cellular heterogeneity necessitates multi-targeting therapies for improved clinical benefits in advanced malignancies. However, systematic identification of patient-specific treatments that selectively co-inhibit cancerous cell populations poses a combinatorial challenge, since the number of possible drug-dose combinations vastly exceeds what could be tested in patient cells. Here, we describe a machine learning approach, scTherapy, which leverages single-cell transcriptomic profiles to prioritize multi-targeting treatment options for individual patients with hematological cancers or solid tumors. Patient-specific treatments reveal a wide spectrum of co-inhibitors of multiple biological pathways predicted for primary cells from heterogenous cohorts of patients with acute myeloid leukemia and high-grade serous ovarian carcinoma, each with unique resistance patterns and synergy mechanisms. Experimental validations confirm that 96% of the multi-targeting treatments exhibit selective efficacy or synergy, and 83% demonstrate low toxicity to normal cells, highlighting their potential for therapeutic efficacy and safety. In a pan-cancer analysis across five cancer types, 25% of the predicted treatments are shared among the patients of the same tumor type, while 19% of the treatments are patient-specific. Our approach provides a widely-applicable strategy to identify personalized treatment regimens that selectively co-inhibit malignant cells and avoid inhibition of non-cancerous cells, thereby increasing their likelihood for clinical success.

Poster #67

Single cell spatial proteomics analysis and computational evaluation pipeline:

Presenter: Behnaz Bozorgui

MD Anderson Cancer center

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Resolving the spatial heterogeneity of the tumor-immune microenvironment (TIME) is critical for understanding key cancer phenotypes as well as therapy response. Such understanding requires profiling tumor and immune cell features with spatial resolution at the single-cell level. Although spatially resolved methods and datasets are becoming increasingly available, analytical methods that quantify and extract the highly complex features and interactions within TIME are lacking.

Here, we introduce the Spatial Proteomics Analysis and Computational Evaluation (SPACE) pipeline, which integrates informatics, visualization, image analysis, and machine learning for processing, quantification, quality control, downstream analyses, and interpretation of spatial omics data. The easy-to-use pipeline generates and interprets biomarker expression and positional information using algorithms for image indexing, image registration, quality control, cell segmentation, identification and removal of non-specific signals, data normalization, and automatic identification of missing data and lost tissue. The accurate intensity measurements at the single-cell level are then used to calculate proposed spatial features representing cellular interactions within TIME. The integration with the NAPARI viewer module enables interactive visualization and annotation of the complex multi-dimensional data in parallel to molecular quantification. A user-modifiable hierarchical decision tree of cell lineage markers annotates types and identities of individual cells enabling characterization of immune landscape within TIME.

A novel expression-weighted proximity score, termed the SPACE score, is defined for single cells and protein markers to inform on spatial enrichment, cellular neighborhoods, and ligand-receptor interactions in close proximity. Normalized by the structural components of tissue, our spatial enrichment score makes spatial enrichment comparable across tissues with different intrinsic structures such as tumor and normal tissue. SPACE enrichment scores thus provide an ideal tool for differential analysis of spatial transcriptomic profiles of structurally distinct microenvironments such as in tumor and normal tissues, tissues undergoing drug-induced structural changes or different cancer types. Finally, a machine learning process that combines spatial feature selection and classification algorithms based on SPACE scores enables the identification of TIME domains, enabling unbiased selection of composite spatial markers, resulting in the discovery of tissue niches and states shared across diverse sample cohorts. We expect SPACE to be a widely used tool that will accelerate major discoveries through mining spatial omics data for TIME composition, organization, and heterogeneity.

Poster #68

Fibroblasts modulate targeted therapy response dynamics in HER2+ breast cancer

Presenter: Matthew Poskus

University of Pittsburgh

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Introduction:
HER2-overexpressing (HER2+) breast cancer accounts for 15-20% of breast cancer diagnoses. Disease recurrence and resistance to HER2-targeted therapies such as lapatinib pose treatment challenges. Stromal fibroblasts in the tumor microenvironment are associated with poor survival outcomes and contribute to HER2-targeted therapy resistance. Cancer cells dynamically adapt to therapeutic stress through cell-intrinsic (e.g. compensatory pathway activation) or cell-extrinsic (e.g. microenvironment-mediated) mechanisms. However, existing studies investigating stroma-mediated therapy resistance have focused on endpoint cell death, thereby overlooking temporal changes to drug sensitivity. We evaluated the growth and death dynamics of HER2+ breast cancer cells cultured with fibroblasts under lapatinib treatment and applied an ordinary differential equation (ODE) mathematical model to these data to decouple intrinsic and extrinsic mechanisms of lapatinib resistance and characterize tumor death dynamics. Understanding therapy-induced tumor death kinetics may yield insight into the impact of the tumor microenvironment on residual disease and adaptation to therapy.

Methods:
A panel of 11 HER2+ breast cancer cell lines (AU565, BT474, EFM192, HCC1419, HCC1569, HCC1954, HCC202, MDA361, SUM225, UACC812, UACC893) were seeded in monoculture (2000 cells/well) or coculture with AR22 fibroblasts (2000 fibroblasts and 2000 cancer cells/well) in a 96-well plate and treated with 0-3μM lapatinib for four days. Tumor cells expressed nuclear H2B-GFP and were incubated with ethidium homodimer to distinguish live from dead cells. Cell count measurements were performed every four hours using time-lapse microscopy. Drug response dynamics (maximum death rate and death delay) and two endpoint viability metrics, relative viability (ratio of live cell in treated versus untreated conditions) and fractional viability (ratio of viable cells to total cells), were compared between monoculture and coculture. Nine cell lines exhibited death at the highest dose (viability <90%, HCC1569 and HCC202 excluded) and were modeled using ODEs of growth and death dynamics. Four models of time-dependent death rates (constant, increasing, decreasing, or transient death rate) were fit separately to monoculture and coculture drug response data for each cell line (18 conditions each fit to four models) and the best model was selected using the Akaike Information Criterion. Drug response parameters were compared across conditions.

Results:
Lapatinib induced growth inhibition or cytotoxicity in tumor cells. BT474 and EFM192 exhibited the lowest relative and fractional viability, indicating cytotoxic responses, whereas HCC1419, HCC1954, and HCC202 cells were growth inhibited without significant cell death. Coculture with fibroblasts differentially impacted relative and fractional viability, equally increasing the relative and fractional viability in BT474 cells but primarily increasing fractional viability in AU565 cells. Fibroblasts delayed the onset of death and decreased the maximum death rate in a subset of cell lines. Modeling revealed heterogeneous death dynamics across cell lines in monoculture, with some cell lines exhibiting a constant death rate (HCC1954) and others exhibiting transient death rates (EFM192). Fibroblasts impacted the best-fitting death model of four cell lines and modified the growth and death rate in a cell line specific manner.

Conclusions:
We found that fibroblasts differentially impact the growth inhibition and cytotoxicity of lapatinib and separate intrinsic and extrinsic mechanisms of therapy resistance using mathematical modeling.

Poster #69

Single-cell and spatial transcriptomics analysis of clear cell ovarian carcinoma

Presenter: Ronaldo Francisco

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Introduction: Clear cell ovarian carcinoma (ccOC) is a rare and aggressive cancer with limited treatment options and poor survival rates. Unlike high-grade serous ovarian carcinoma (HGSOC), ccOC shares gene expression profiles with clear cell renal cell carcinoma (ccRCC), suggesting common mechanistic and microenvironmental traits among clear cell carcinomas. The factors driving responses to immune checkpoint inhibitors in ccOC remain unclear. In this study, we provide a comprehensive analysis of the ccOC tumor microenvironment, identifying phenotypic states and exploring their associations with clinical outcomes.

Methods: Data from ccOC patients were obtained from cohorts at Stanford Hospital and the University of Pennsylvania with IRB approval. Tumor microenvironment composition was characterized using in-house single-cell RNA-Seq data for ccOC (n = 10) and spatial transcriptomics data (n = 40). Comparative analyses included publicly available single-cell RNA-seq datasets for ccRCC (n = 6) and HGSOC (n = 14). Data were processed with the Seurat v5 workflow. Differential gene expression analysis was conducted using Seurat's FindMarkers function ($p < 0.05$, $|Log2FC| \geq 1$, and ≥ 30% minimum expression). A ccOC-specific reference signature matrix was generated from single nucleus RNA-Seq (n = 16) and applied with CIBERSORTx to deconvolute bulk RNA-Seq data from 239 samples from Memorial Sloan Kettering Cancer Center. Ecotypes specific to ccOC were identified using Ecotyper, and their clinical relevance evaluated with Cox proportional hazards models. Spatial transcriptomics analysis was performed on clear cell ovarian carcinoma tissue microarrays using the CosMx platform. Statistical analyses were performed in R, with significance thresholds set at p.adjust < 0.1.

Results: Although ccOC, ccRCC, and HGSOC exhibit similar cell type compositions, we identified a set of markers consistently and differentially expressed in both ccOC and ccRCC when compared to HGSOC. Markers in ccOC and ccRCC were primarily associated with ATP synthesis by chemiosmotic coupling, immune modulation pathways, such as regulation of PD-1 signaling, and VEGFR2-mediated cell proliferation. Pathways unique to HGSOC included those involved in immune signaling cascades and ECM remodeling, such as ECM proteoglycans and dermatan sulfate biosynthesis. By integrating tumor microenvironment composition and bulk RNA-Seq data from 239 ccOC patients, we identified 38 distinct cell states across nine ecosystems, including three significantly associated with poor prognosis and one linked to favorable outcomes. Three of these states were significantly associated with poor prognosis and one linked to favorable outcomes. Specifically, endothelial cells (state 1), pericytes (state 2), and fibroblasts (state 4) were associated with adverse prognosis, while epithelial cells (state 4) were linked to favorable outcomes. These survival differences were

further supported by the bulk expression levels of key genes within these states. Furthermore, distinct spatial organization patterns were observed in the ccOC microenvironment, with fibroblasts (state 4) strongly co-localizing with inflammatory communities enriched by myeloid cells.

Conclusion: We identified ccOC cell-type-specific transcriptomic profiles that can predict clinical outcomes for both poor and favorable prognoses. Our study presents the most comprehensive atlas to date of transcriptionally defined cell states in ccOC, providing a valuable resource for understanding the tumor microenvironment and its impact on clinical outcomes.

Poster #70

Prediction of Colon Cancer Treatment Based on Microbiome Metabolism

Presenter: Annie Badenoch

University of Michigan

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Colon cancer affects 1 in 24 people during their lifetime, with 15-20% of patients experiencing drug resistance and 50-60% facing cancer recurrence. Combination therapy offers a promising approach to address these challenges by targeting multiple cancer pathways simultaneously, thus reducing the risk of resistance. However, to develop more effective treatments, the complex interactions between colon cancer and the gut microbiome must be better understood. The gut microbiome, particularly certain bacterial species, can significantly influence treatment outcomes, yet the mechanisms remain poorly understood. One such bacterium, Fusobacterium nucleatum, has been strongly associated with colon cancer progression and poor patient prognosis. Emerging evidence suggests that F. nucleatum exerts its harmful effects through metabolic mechanisms, as over 200 metabolic genes are differentially regulated in cancer cells exposed to this microbe. To address this challenge, we applied a machine learning algorithm, CARAMeL, which utilizes flux balance analysis to model metabolic flux and predict effective drug combination therapies. CARAMeL takes RNA sequencing data as input and maps it to a genome-scale metabolic model, generating simulated metabolic profiles. These profiles are then compared with training data to identify metabolic patterns associated with successful or failed drug combinations. By incorporating gut microbial influences into the model, we can better predict the impact of microorganisms on drug efficacy and resistance. Our results align with clinical observations regarding F. nucleatum's impact on colon cancer treatment, further validating the model's predictive power. Additionally, the model identified a novel potential therapeutic combination: synthetic progesterone paired with microtubule inhibitors. This finding opens the door to new treatment possibilities for colon cancer patients, particularly those affected by drug-resistant or recurrent disease. Integrating the gut microbiome into computational models like CARAMeL enables more accurate predictions of drug combinations, providing a powerful tool for overcoming the complexities of colon cancer treatment. By elucidating the metabolic mechanisms influenced by microbes such as F. nucleatum, we can develop more targeted and effective therapies, ultimately improving patient outcomes.

Poster #71

Integrating Language-Vision Models and Spatial Transcriptomics to Study Metastatic Cancer Microenvironments

Presenter: Fangyang Wang

MD Anderson Cancer Center

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Metastatic cancers are aggressive and complex to treat as the disease can co-exists in multiple unique microenvironments and organ systems. Future therapeutic strategies for metastatic cancer will require mechanistic insights build on a mix of quantitative and high-throughput measurement in pre-clinical models and real-world data. Spatial transcriptomics has emerged as a powerful tool for studying the intricate architecture of tumor tissues, enabling researchers to unravel molecular heterogeneity and tissue organization. An essential part of spatial data analysis is cell type annotation, which often involves analyzing RNA expression data and pathology review. While pathology insights provide essential context for tissue structure, access to expert pathologists is often limited.

This study investigates the application of foundational language-vision pathology model (CONCH) for cell type annotation in brain metastatic cancer spatial transcriptomics (Visium platform). By leveraging these models, we generate similarity scores for a set of text description prompts across all spots in spatial transcriptome slides. These similarity scores enable tissue segmentation and provide a basis for annotation. Furthermore, we exploit the model's embedding space to perform unsupervised clustering and using similarity scores to annotated unbiased clusters.

Colocalized ligand-receptor pair of adjacent cells, which indicate cell-cell communication, can be modeled as spatially weighted cosine similarity. Joint non-negative matrix factorization (NMF) was employed to integrate the colocalization score and CONCH predicted class score enables the identification of ligand-receptor colocalization pattern and their underling cell type pattern. Our analysis revealed ligand-receptor colocalization at tumor-normal and tumor-stroma interfaces, providing insights into intercellular signaling dynamics within the metastatic microenvironment.

Our approach demonstrates the potential of foundational models to enhance tissue type annotation, uncovering biological insights in spatial biology. These findings contribute to scalable methodologies for studying the complex interplay of cells in metastatic cancer.

Poster #72

Evolving Data Application and Systems: Supporting Patient Derived Model Research for Over 10 Years.

Presenter: Christopher A Bristow

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Patient Derived Model (PDM) systems are valuable tools for cancer therapeutics discovery. While large scale characterization and biobanking efforts have provided best-practices roadmaps (MI-PDX, PDMR, PDX-NET), harnessing the value of PDMs can be challenging to navigate for individual organizations, as they have unique technology constrains that are shaped by risk-governance processes and shifting compliance landscapes. Also, the execution of PDM research is complex, requiring extensive coordination across multiple data disciplines and interactions with a network of clinical and basic research teams. In exploring PDMs for cancer discovery, roadmaps are valuable, however, each organization must add large-scale features, and this transforms these roadmaps into something more like an atlas. As institutions navigate these challenging terrains, leveraging data application and system technologies becomes critical to achieving scalable, compliant, and impactful research outcomes.

In this poster we highlight our approach to operationalize PDM data streams and to support over a decade of collaborative projects and strategic initiatives. This overview will provide a data technology focus on five areas that influence the execution of PDM research. First, we will introduce the team composition and interactions. Second, we discuss the evolving model development toolbox and how this influences operations. Third, we focus on model annotations, how we build consensus from public efforts and how we maintain a derivation graph. Fourth, we share our experience with digitalization of key data streams and supporting metadata curation with applications. Fifth, we showcase the model characterization workflows and the visualization engines that support access and discovery.

Each highlighted area requires coordinating information across large peer-peer networks and herding data into an integrative datastore. In assembling our FAIR data support ecosystem for PDM research, we have leveraged a range of technologies (including no-code, cloud, containerized, k8s) to adapt with changes in our compute service landscape and ensure the data flow are safe and synchronized. The exploration of data technologies and application building can both enhance the accuracy and reproducibility of PDM research and accelerate discoveries by leveraging advanced analytics and machine learning capabilities. Over the decade of exploring PDM landscapes, we would like to emphasize the importance of multidisciplinary collaboration and continuous technological advancement. Together, we can make technology and data drive impactful discoveries for cancer research.

Poster #73

Transcriptional trajectory inference of image-localized, multi-regional high-grade glioma biopsies reveals distinct population ecologies and sex-associated enriched pathways

Presenter: Kristin Swanson

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The intra- and inter-patient heterogeneity in high-grade glioma (HGG) continues to contribute to its poor prognosis. Clinical biopsies are often harvested from limited regions and typically are not image localized. Thus, they fail to capture the diversity within tumor regions, immune expression or normal cell abundances that play key roles in tumor development. It is important to gain an understanding of these subpopulation ecologies, their spatial resolution, and interactions between them that may then be exploited for future therapeutic benefit. Further, these may differ by patient characteristics such as sex, age at diagnosis and

treatment status. Using an ongoing image-localized biopsy collection protocol, we have so far evaluated the bulk transcriptomics of 202 multi-regional biopsies from 58 patients to characterize HGG heterogeneity. These samples were processed through Monocle, a reverse graph embedding algorithm that groups samples into states and orders them along developmental trajectories. Deconvolution methods were previously used to predict relative abundances of 7 normal, 6 glioma, and 5 immune cell subpopulations for each sample, which we have now overlaid on the Monocle graph. Monocle classified HGG into 4 main states along a three-pronged trajectory. These states reveal distinct population ecologies with associated enriched gene pathways. We also note significant immune pathway enrichments that differ between state and patient-reported sex. Together, these algorithms reveal a simple transcriptomic trajectory that helps us understand the development and evolution of HGG. Characterizing the in vivo diversity within and between high grade gliomas is important for understanding prognosis, stratifying future treatments and ultimately improving patient outcome.

Poster #74

Personalized cancer treatment strategies incorporating irreversible and reversible drug resistance mechanisms

Presenter: Wei He

Georgetown University

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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.

Poster #75

A Comprehensive Meta-Analysis of Breast Cancer Gene Expression Data: Understanding the Molecular Complexity of a Silent Epidemic

Presenter: Ifeanyichukwu Nwosu

Brigham Young University

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Triple-negative breast cancers (TNBC) occur more frequently in African Americans and are associated with worse outcomes when compared to other subtypes of breast cancer. These cancers lack expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) and have limited treatment options. To shed light on mechanisms behind these differences and suggest novel treatment mechanisms, we used a meta-analytic approach to identify gene expression differences between African Americans and European Americans with breast cancer; additionally we compared gene expression levels based on ER, PR, HER2 and TNBC status. After gathering and standardizing gene expression data and metadata from 85 datasets (representing 23,000 samples), we identified genes that were consistently expressed differently between these groups via a meta-analysis. To evaluate the robustness of these gene lists, we devised a novel computational methodology that uses cross validation and classification. We also computed overlaps between the most significant genes and known pathways. Using a random effects model and a false discovery ratio of 0.05, we identified genes that confirm the validity of our methodology because they are known to play a significant role in their respective breast cancer subtypes (e.g., ESR1 for ER status and ERBB2 for HER2 status). We also discovered genes that have not been identified previously in the literature and may be new potential targets for breast cancer therapy. GATA3, CA12, TBC1D9, XBP1 and FOXA1 were among the top 100 significant genes for ER, PR, and TNBC. However, none of these genes overlapped with HER2 status, supporting prior research that HER2 tumors are mechanistically different from endocrine breast cancers. The genes identified from the race meta-analysis—including DNAJC15, HLA-DPA1, STAP2, CEP68, MOGS—have not been identified in presently known pathways, highlighting a potential area of further research.

Poster #76

A Translation-Oriented Pipeline for Analyzing Drug Combination Screens

Presenter: Christian Meyer

University of Colorado Boulder

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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.

Poster #77

A morphology and secretome map of pyroptosis

Presenter: Michael J. Lippincott

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Pyroptosis represents one type of Programmed Cell Death (PCD). It is a form of inflammatory cell death that is canonically defined by caspase-1 cleavage and Gasdermin-mediated membrane pore formation. Caspase-1 initiates the inflammatory response (through IL-1β processing), and the N-terminal cleaved fragment of Gasdermin D polymerizes at the cell periphery forming pores to secrete pro-inflammatory markers. Cell morphology also changes in pyroptosis, with nuclear condensation and membrane rupture. However, recent research challenges canon, revealing a more complex secretome and morphological response in pyroptosis, including overlapping molecular characterization with other forms of cell death, such as apoptosis. Here, we take a multimodal, systems biology approach to characterize pyroptosis. We treated human Peripheral Blood Mononuclear Cells (PBMCs) with 36 different combinations of stimuli to induce pyroptosis or apoptosis. We applied both secretome profiling (nELISA) and high-content fluorescence microscopy (Cell Painting). To differentiate apoptotic, pyroptotic and healthy cells, we used canonical secretome markers and modified our Cell Painting assay to mark the N-terminus of Gasdermin-D. We trained hundreds of machine learning (ML) models to reveal intricate morphology signatures of pyroptosis that implicate changes across many different organelles and predict levels of many pro-inflammatory markers. Overall, our analysis provides a detailed map of pyroptosis which includes overlapping and distinct connections with apoptosis revealed through a mechanistic link between cell morphology and cell secretome.

Poster #78

eIF3e and eIF3d selectively regulate the acute hypoxic translational response

Presenter: Kate Matlin

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Cancer cells are well-equipped to respond to environmental stress, including hypoxia, through the tight control of gene expression. In hypoxia, gene expression changes enable cells to survive, gain an invasive phenotype, and metastasize more efficiently. While the transcriptional response to hypoxia has been well-defined, the translational response to hypoxia is less understood but likely contributes to metastasis. In many cellular stresses, canonical translation is diminished, and to mount a proper stress response, cells rely upon specialized non-canonical translation mechanisms. The human eukaryotic initiation factor (eIF)3 complex consists of 13 proteins essential for canonical translation initiation. Recently, one of the complex members, eIF3d, was found to promote translational initiation on selective mRNAs, particularly under cellular stress, through its function as an alternative mRNA cap-binding protein. While canonical translation is attenuated in hypoxia, the identity and impact of specialized non-canonical translation is poorly understood.

We hypothesized that eIF3d and its binding partner eIF3e may drive non-canonical translation in hypoxia. To test this model in an unbiased manner, we measured transcriptome-wide changes in translation using ribosome profiling and RNA-seq in normoxic and hypoxic conditions in a metastatic breast cancer cell line (MCF7-SIX1) with or without depletion of eIF3d or eIF3e. Specifically, we calculated changes in translational efficiency (TE ~ the per transcript ratio of ribosome-protected footprints vs RNA levels) to account for mRNA expression changes across conditions (e.g. normoxia vs hypoxia, siCtrl vs si3e or si3d). We observed hundreds of changes in TE after acute 1% hypoxia (1 hr) almost completely driven by changes in ribosome occupancy and nearly no changes in RNA levels. Remarkably, depletion of eIF3e or eIF3d ablated the acute hypoxic translational changes. We also found that eIF3e and eIF3d regulated the translation of more mRNAs in hypoxia than in normoxia.

We next explored the role of eIF3e and eIF3d in hypoxia and invasion phenotypes. We found that eIF3e and eIF3d are required for upregulating HIF1alpha in 2D cell culture. Further, depletion of eIF3e or eIF3d suppressed the formation of the hypoxic core in tumorspheres and decreased invasion. To query the clinical relevance of targeting eIF3e or eIF3d in breast cancer, we generated signatures for RNAs differentially expressed in our control cells versus eIF3e/eIF3d depleted cells. Using RNA microarray and RNA-seq data from breast cancer patients in METABRIC and TCGA patient cohorts, we calculated the enrichment of eIF3e/d signatures in patient samples and how enrichment correlated with patient survival. We found that high enrichment of the eIF3e signature correlated with worse survival rates, while the eIF3d signature did not predict survival. Finally, we identified a compound that binds eIF3e, which also ablated the acute hypoxic translational response, attenuated HIF1alpha expression, and reduced the formation of the hypoxic core in tumorspheres. Our findings suggest that inhibiting non-canonical translation through eIF3e/d could be a means to target metastasis in a cancer-cell-specific manner.

Poster #79

Characterizing and Targeting Multi-Gene Dependencies in Cancer

Presenter: Julia Curd

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Precision oncology customizes cancer treatment by targeting the specific genetic characteristics of a patient's tumor, offering effective alternatives where conventional one-size-fits-all treatments may fail. Despite its promise, precision oncology currently benefits only 5-8% of cancer patients, and only about 15% of proteins are considered druggable. Publicly available cancer resources, such as the The Cancer Dependency Map Project (DepMap), have characterized gene dependencies - genes whose inhibition can result in cancer cell death - uncovering potential new therapeutic targets for precision medicine. However, a major limitation remains: current analyses focus on single gene dependencies, which may be challenging to target precisely and are inflexible to the polypharmacological nature of small molecules. Expanding this focus to analyze groups of genes as dependencies can reveal higher-order biological functions, such as signaling pathways, which could offer more robust, higher-order targets for precision oncology.

We applied BioBombe, an artificial intelligence and machine learning (AI/ML) framework, to uncover these multi-gene dependencies. We initially developed BioBombe for RNAseq analysis, utilizing several dimensionality reduction algorithms, including Principal Component Analysis, Independent Component Analysis, Non-negative Matrix Factorization, Variational Autoencoders (VAE), Beta VAEs, and Beta Total Correlational VAEs to compress input data that reveal hidden patterns. The core innovation of BioBombe is that it trains many different models to compress input data into several different latent dimensionalities, which we have shown optimizes hidden pattern discovery. Preliminary BioBombe analysis characterized the landscape of multi-gene dependencies across cancer types. We applied gene set enrichment analyses (GSEA) using Reactome gene sets to interpret these multi-gene dependencies. We identified generic processes like "Mitotic Metaphase and Anaphase" (R-HSA-2555396) and "Mitotic Anaphase" (R-HSA-68882), along with metabolic pathways such as "Citric Acid (TCA) Cycle and Respiratory Electron Transport" (R-HSA-611105) highlighting essential processes required for cancer cell growth. Other multi-gene dependencies showed significant enrichment for certain cancer types. In glioma for example, the most enriched pathways include TP53-related processes, such as "Transcriptional Regulation by TP53" (R-HSA-3700989), "Regulation of TP53 Activity Through Methylation" (R-HSA-6804760), and "TP53's influence on metabolic genes" (R-HSA-5628897). Other critical pathways for glioma involve mitochondrial function and biogenesis, like "Mitochondrial Biogenesis" (R-HSA-1592230) and "Transcriptional Activation of Mitochondrial Biogenesis" (R-HSA-2151201), suggesting potential vulnerabilities in cancer cell energy production and metabolic regulation.

We also tested the correlation of multi-gene dependencies to the cell-killing efficacy of specific drugs. This analysis identified drugs with strong correlations and high variance across specific cancer types, indicating promise of our method to repurpose drugs. In gliomas, drugs like osimertinib, ibrutinib, and gefitinib (all EGFR inhibitors) are among those with the highest correlations, aligning with previous evidence of their efficacy in clinical trials or in vitro testing. We also pinpointed unique drugs, previously untested in gliomas, for repurposing consideration, such as Ro-4987655 (MEK inhibitor), EVP4593 (NFkB inhibitor), and BAY-87-2243 (HIF inhibitor), which show potential for broader application beyond their original indications. These findings not only underscore BioBombe's capacity to reveal novel mult-dependencies and druggable pathways but also to support the advancement of novel, more targeted therapeutic approaches across diverse cancer types.

Poster #80
Characterizing interactions between the peripheral nervous system and human bladder tumors

Presenter: Cailin Deiter

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The progression of solid tumors depends on interactions with the surrounding tissues, known as the tumor microenvironment (TME). The TME includes immune cells, blood vessels, connective tissue, and various organ-specific non-tumor cell types, including peripheral neurons. Many TME interactions are well known, but until recently, interactions between peripheral neurons and tumor cells were largely ignored. Akin to tumor angiogenesis, tumors can build up local autonomic and sensory nerve networks. Growing evidence demonstrates that signals from these neurons can modulate therapeutic responses and all stages of tumor progression (initiation, proliferation, survival, metastasis) in many cancers. Nerves have been reported in bladder tumors, but the impact of tumor-nerve interactions in bladder cancer (BC) remains unknown. Our preliminary bioinformatics results revealed that the upregulation of genes involved in innervation is strongly associated with mortality in BC. However, the bladder contains several types of nerves, so further studies are required to understand how each type influences BC. Here, we use multispectral imaging to characterize the types and abundance of nerves in human bladder tumors. We then integrate the innervation profiles with clinical, genomic, and transcriptomic data to elucidate the relationships between tumor innervation, patient outcomes, and underlying tumor biology in BC. By characterizing the neural microenvironment of bladder tumors, we aim to identify novel therapeutic targets and prognostic markers, ultimately improving patient outcomes in bladder cancer.

Poster #81

Improving Response to Immunotherapy in HER2+ Breast Cancer

Presenter: Rebecca A. Bekker

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HER2+ breast cancer is aggressive and has historically had poor outcomes. Despite therapeutic advances, such as the development and approval of HER2 targeted therapies and the associated improved outcomes, resistance invariably develops. However, recent preclinical and clinical evidence suggest patients may benefit from combination therapies that include immunotherapy. The PANACEA trial, which investigated the efficacy of the combination of the HER2 monoclonal antibody trastuzumab and the PD-1 inhibitor pembrolizumab in HER2+ breast cancer, reported a 15% response rate in patients with PDL1+ tumors. This unexpectedly low response rate may be a result of a highly tumor-engineered immune-suppressive niche, containing myeloid derived suppressor cells (MDSCs), FoxP3+ T reg cells, and tumor-associated macrophages, amongst others. Understanding the interactions between these pro- and anti-tumor immune subtypes, and their interactions with HER2+ breast cancer cells, may be instrumental to improving response to immunotherapies. To this end, we have developed an agent-based model (ABM) of tumor-immune interactions, initialized with and calibrated

to digitized multiplex immunohistochemistry (mIHC) slides of untreated spontaneous lung metastases from the NT2.5LM HER2+ murine model. Modeling in silico tumors treated with chemotherapy alone or chemo-immunotherapy combinations reveals the properties of MDSCs and CD8+ T cells, and their interactions with cancer cells, which determine and impact response to treatment. By uncovering the cellular dynamics and immune interactions that shape treatment outcomes, our model offers a pathway to tailor immunotherapy strategies to improve patient outcomes in advanced HER2+ breast cancer.

Poster #82

GRACKLE: Graph Regularization Across Contextual KnowLedgE: An interpretable matrix factorization approach for clinical subtyping

Presenter: Lucas Gillenwater

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Non-negative matrix factorization (NMF) is a common molecular and clinical subtyping approach wherein an input matrix with zero or positive values (e.g., gene expression counts) is decomposed into two matrices of latent components (e.g.,samples by latent components and transcripts by latent components) that approximate the reconstruction of the input matrix. NMF is advantageous in cancer research for two reasons. First, NMF does not assume independence of each component after decomposition, which reflects observed molecular biology, for example, where genes are involved in mechanisms across multiple pathways. Second, the decomposed matrices are interpretable since the values of components correspond between molecular features and samples, thus appreciating the combinatorial patterns of molecular differences across phenotypic contexts. However, NMF is an unsupervised approach that does not consider additional sample information (e.g., patient demographics, previous subtypes) or biological plausibility in the decomposition. There is no guarantee that the identified components represent shared and distinct mechanisms in context of prior knowledge about the tumors.

Here we present GRACKLE (Graph Regularization Across Contextual KnowLedgE), a novel NMF method that considers known molecular mechanisms and is flexible to incorporate additional information about the samples. By incorporating graph regularization based on a sample affinity matrix and a prior tissue-specific gene regulatory network into model training, GRACKLE produces latent components that are interpretable in context. The sample affinity matrix may be based on a single source or combination of sample metadata, including previous subtypes, clinical diagnoses, or other omic data (e.g.,methylation, miRNA, etc.) profiles that may affect gene regulation. Since the decomposed components share information in the training process, this additional regularization ensures that resulting sample components are informed by known interactions of gene regulation and that the gene components represent context specific molecular mechanisms.

We demonstrate the efficacy of GRACKLE in comparison to other matrix decomposition approaches with simulated experiments using gene regulatory networks and in silico predicted gene expression data from the DREAM4 challenge. In addition, we apply GRACKLE to the multiomic breast cancer profiles from the TCGA cohort, assessing the defined components in relation to known breast cancer subtypes. Finally, we appraise the potential of treatments by comparing the GRACKLE defined components against mechanisms of action profiles defined by the LINCS drug screening consortium. All together, we show that GRACKLE is a methodological advance over previous NMF approaches in identifying clinical subtypes with corresponding inference of context-specific molecular mechanisms. GRACKLE is available at: https://github.com/lagillenwater/GRACKLE.

Poster #83

Investigating the Effects of Axl Inhibition: Reprogramming the Myeloid Compartment of the Tumor Microenvironment

Presenter: Anisha Datta

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The success of checkpoint blockade has revolutionized cancer therapeutics, underscoring the power of immunotherapy. However, this success is limited to patients with "hot" tumors, i.e., tumors characterized by T cell infiltration prior to treatment. In contrast, patients with "cold" tumors, i.e., tumors which lack T cell infiltration, do not respond to this treatment modality. There is thus a need to develop therapies that convert "cold" tumors to "hot" ones. Given the prerequisite to T cell infiltration and activation is myeloid cell activation, developing methods to boost macrophage and dendritic cell activation has become an area of intense study. The receptor tyrosine kinase family comprised of the TYRO3, AXL, and MERTK receptors (TAMRs) is an attractive target for activating macrophages and dendritic cells because the TAMRs are known to be innate immune checkpoints. Furthermore, AXL specifically has been extensively studied in cancer biology due to its role in promoting tumor cell survival, invasion, metastasis, drug resistance, and immune evasion. Thus, AXL is a dual target, and many therapies that have been developed to target this receptor are in clinical trials. However, the effects of AXL inhibition on the tumor microenvironment – specifically the immune compartment – are incompletely understood. In this work, we developed a human in vitro model system to elucidate the effects of AXL inhibition on tumor cells, macrophages, dendritic cells, and the crosstalk between these cell types. We selected the A375 melanoma cell line as the tumor cell model in our system due to its overexpression of AXL and relevance to ongoing clinical trials. We generated primary human monocyte-derived macrophages (HMDMs) or dendritic cells (HMDDCs) from monocytes isolated from healthy donors. A375 conditioned media yielded an increase in AXL and MERTK expression by HMDMs. In A375-HMDM co-culture experiments, AXL inhibition via the small molecule inhibitor Bemcentinib, resulted in increased HMDM expression of HLA-DR, CD86, and CD80, meaning the macrophages have increased antigen presentation and are in an immunostimulatory state. Likewise, in A375-HMDM-HMDDC tri-culture experiments, Bemcentinib treatment resulted in increased HMDDC expression of HLA-DR, CD40, and CCR7, meaning the dendritic cells have increased antigen presentation and are in a migratory state. Interestingly, in these tri-culture experiments, while HMDM expression of HLA-DR and CD80 was maintained at the level observed in the A375-HMDM co-culture experiments, the macrophage expression of CD206 and CD40 significantly decreased. These results not only demonstrate that AXL inhibition via Bemcentinib affects myeloid cells in the tumor microenvironment but also underscore the importance of studying AXL inhibition at a systems level to understand the nuances of AXL's role as a dual target. As such, we intend to investigate changes in cell-cell communication that result from AXL inhibition by conducting ligand-receptor interaction analysis of single cell RNA sequencing data. Our ongoing work also includes assaying the media from the co-culture and tri-culture experiments to characterize changes in the secretome resulting from Bemcentinib treatment. Altogether, our work supports the hypothesis that AXL inhibition reprograms the myeloid compartment of the tumor microenvironment, a crucial step towards generating a "hot" tumor.

Poster #84
Fibroblast Dynamics in a Quantitative Systems Pharmacology Model for Combination Therapy in Hepatocellular Carcinoma

Presenter: Shuming Zhang

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Introduction:
Hepatocellular carcinoma (HCC), the most prevalent form of primary liver cancer, has a five-year survival rate of roughly 20%. Currently, nivolumab (anti-PD1 therapy) and atezolizumab (anti-PDL1 therapy) are standard treatments, but overall response rates remain low. To improve the efficacy of systemic therapies for advanced HCC, novel combination therapies are being explored. In this study, we created approximately 500 virtual patients resembling actual HCC cases and simulated tumor progression treated with cabozantinib (VEGFR inhibitor) and nivolumab (anti-PD1 therapy) using a quantitative systems pharmacology (QSP) model.

Methods:
We introduced a new module to our existing QSP model, where cancer-associated fibroblast (CAF) activation is controlled by TGF-β concentration. CAFs secrete extracellular matrix (ECM), impeding physical contact between immune and cancer cells, thus reducing cancer cell killing rates. The CAF activation rate was calibrated based on the CAF/stellate cell ratio from single-cell RNA sequencing data, while ECM secretion rates were fitted using experimental data. We recalibrated the pharmacodynamic (PD) parameters of nivolumab and cabozantinib based on response rates from phase 3 clinical trials (1,2). The model was validated using data from a phase 2 combination therapy trial (3). Random variations were applied to baseline model parameters to reflect inter-patient variability. Finally, we simulated tumor progression under nivolumab and cabozantinib treatment and projected clinical responses for the virtual patient population.

Results:
After introducing the fibroblast module, we calibrated the parameters using phase 3 nivolumab monotherapy data (Checkmate 459), achieving an objective response rate (ORR) of 17.1% (95% CI: 12.0–25.0%), similar to the trial ORR of 15%. Cabozantinib PD parameters, calibrated based on Abou-Alfa et al., yielded a 3% ORR (95% CI: 0–6%), comparable to the 4% ORR observed in the clinical trial. The combination therapy simulation predicted a 21.6% ORR (95% CI: 13.0–30.0%) for the virtual cohort, close to the 18.1% ORR observed in CheckMate040. By varying CAF activation rates among virtual patients, we found that ECM and CAF densities were negatively associated with treatment outcomes, aligning with observations from clinical trials and single-cell RNA sequencing data (4,5). The model also demonstrated that TGF-β regulates immune activation through macrophage polarization and CAF activation. Finally, our model identified CAF density, CD8/Treg ratio, and TGF-β concentration as predictive biomarkers of treatment outcomes.

Conclusion:
This study mechanistically modeled the role of CAFs in the tumor microenvironment (TME). Supported by genomic data and clinical results, we showed that dense fibrotic tissue in the TME correlates with poorer treatment outcomes. This validated model provides a platform for biomarker identification and future clinical trial design.

Poster #85

Estrogen Sensitization for Combating Aggressive Phenotypes in Estrogen-Receptor Negative Breast Cancer (ESCAPE)

Presenter: Harley Richker

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Breast cancer subtypes vary significantly in their prognosis and available treatments. Among those subtypes with a characteristically poor prognosis is Triple Negative Breast Cancer (TNBC). In contrast, Estrogen Receptor (ER) positive breast cancer is significantly more treatable and has a vastly higher 5 year survival. While TNBC tumors are characterized as such because of the low number of cancer cells with hormone sensitivity, the number of those cells in a TNBC tumor at any given time is not zero. Drawing inspiration from evolutionary game theory and the replicator equation, we explore the potential impact of estrogen supplementation on the phenotypic composition of TNBC tumors. This theoretical framework suggests that supplemental estrogen might induce substantial changes in the tumor's evolutionary trajectory. Here we test this potential on MDA-MB-231 cells in order to assess the ability to modulate the number of estrogen receptor positive cells via the addition of supplemental estrogen. Beyond the clinical implications, this project also serves to address fundamental questions on how free estrogen interacts with tumor cells and impacts selection dynamics.

Poster #86

Discovery of Novel Ecotypes for Immunotherapy Response Prediction in Non-small Cell Lung Cancer

Presenter: Ilayda Ilerten

Stanford University

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths globally. While immune checkpoint inhibitors (ICIs) have recently improved outcomes for patients with advanced stages of NSCLC, their effectiveness remains limited, with only about 20% of patients responding to treatment. Existing FDA-approved biomarkers, such as PD-L1 expression and tumor mutational burden (TMB), have shown limited predictive accuracy for ICI outcomes. In this study, we propose integrating the EcoTyper framework with bulk and single-cell sequencing data, alongside advanced single-cell imaging technologies, to identify novel biomarkers in NSCLC patients. These biomarkers may provide insights into resistance mechanisms to ICIs and suggest potential therapeutic targets. EcoTyper is a deconvolution and machine learning-based framework that has been previously applied to thousands of tumor transcriptomes to uncover transcriptional state networks across various cell types, including epithelial, immune, and stromal cells, within the tumor microenvironment (TME). Preliminary findings indicate that lung-specific ecotypes have better prognosis on LUSC patients in TCGA cohort (N=424) when compared to pan-cancer ecotypes which has been discovered previously. Additionally, lung-specific ecotype 6 (E6) and the pan-cancer CE9 significantly predict responders within an ICI-treated cohort of 110 LUAD patients. We are in the process of validating the cell states found from bulk RNA-seq in matched sc-RNA and spatial transcriptomics datasets. We anticipate that our approach will deepen our understanding of cell state diversity and biology in NSCLC, potentially leading to new biomarkers for predicting responses to ICI therapies.