# Oral Presentation Abstracts

## Schedule

**Wednesday, November 7**

7:10 –8:00 pm: Opening Keynote

**Thursday, November 8**

9:00 – 10:40 am: Tumor Heterogeneity

1:45 – 3:25 pm: Microenvironment and Metastasis

3:45 – 5:25 pm: Translational Systems Biology

**Friday, November 9**

9:00 – 10:40 am: Systems Immunology & Immunotherapy

1:45 – 3:25 pm: Systems Pharmacology

3:45 – 5:25 pm: Systems Biology: Bench to Bedside

7:15 – 8:45 pm: Systems Biology: Bench to Bedside

**Saturday, November 10**

9:00 – 10:40 am: Signaling Networks in Cancer

11:00 am – 11:50 pm: Closing Keynote

All talks will take place in the Lillie Auditorium.

Keynote talks are 50 minutes, including time for questions. Invited talks will be limited to 30 minutes, plus 10 minutes for questions. Selected talks will be limited to 12 minutes, plus 3 minutes for questions.

# Wednesday, November 7

## 7:10 –8:00 pm: Opening Keynote

**Christina Leslie (Memorial Sloan Kettering Cancer Center)**

# Thursday, November 8

## 9:00 – 10:40 am: Tumor Heterogeneity

Chaired by Tenley Archer (Boston Children's Hospital)

### Shannon Mumenthaler (University of Southern California)

***Unlikely suspects: deciphering the functional heterogeneity of fibroblasts in cancer***

Cancer is a complex adaptive system orchestrated by the interactions between tumor cells and their microenvironment. In particular, cancer-associated fibroblasts (CAFs), the dominant cellular component of the tumor stroma, are often associated with a poor prognosis and play an important role in tumor progression for a number of cancers. While significant literature has highlighted the influence of CAFs on cancer cell phenotypes including tumor cell proliferation and invasion, the role of CAF heterogeneity on treatment response remains largely understudied. Additionally, preclinical treatment studies often focus on drug-induced changes to tumor cells with little investigation into the impact on surrounding stromal cells. To advance our biological understanding of cancer and improve treatment efficacy, we are utilizing quantitative high-content imaging coupled with more physiologically-relevant patient-derived model systems to illuminate the dynamic interactions between cancer cells and their microenvironment. These studies are aimed at increasing our understanding of the functional and therapeutic utility of CAFs by leveraging expertise across disciplines. Our lab has developed several imaging-based workflows, combined with machine learning and other image analysis techniques, to rapidly and accurately classify cell types and cell behaviors within heterocellular populations. Using these approaches, we have identified cancer-associated fibroblasts as a source of environment mediated drug resistance in colorectal cancer. Specifically we discovered a novel mechanism by which drug treated CAFs render adjacent tumor cells resistant to anti-EGFR therapy.

### Paul Macklin (Indiana University)

***Open source software for studying 3D multicellular cancer systems biology in high throughput***

Key cancer processes – such as microenvironmental- and therapy-driven cell adaptations, invasion, and metastasis – take place not just within single cancer cells, but within multicellular systems. In these systems, multiple cell types live and communicate in complex biochemical and biomechanical environments. Therapies perturb these highly nonlinear systems, sometimes with unexpected results including side effects and treatment failure. To improve treatment success, we need to study not just

cancer cells in isolation, but also understand and control the multicellular system. Computational models can act as 'virtual laboratories,' where we can systematically study the multicellular systems biology of cancer. The ideal such laboratory would include cell and tissue biomechanics, biotransport of multiple chemical substrates including signaling factors, and many interacting cells. We recently developed and released PhysiCell (http://dx.doi.org/10.1371/journal.pcbi.1005991), an open source platform for 3-D multicellular systems biology. With this platform, desktop workstations can routinely simulate systems of ten or more cell-secreted chemical signals and tissue substrates, along with 10^5 to 10^6 cells that grow, divide, die, secrete chemical signals, move, exchange mechanical forces, and remodel their tissue microenvironment. We demonstrate PhysiCell in 2D and 3D simulation examples that examine (1) how mechanical interactions between cancer cells and the liver parenchyma can affect the successful seeding of colon cancer metastases, (2) how biochemical and biomechanical interactions between motile and non-motile breast cancer cells can impact tissue invasion, (3) potential designs for synthetic multicellular systems that transport cancer therapeutics, and (4) the critical role of stochastic migration in immune responses to tumors. We will briefly discuss how open source has accelerated PhysiCell's development, and we'll close with early results on using supercomputers to accelerate large-scale computational investigations. In the future, we aim to adapt these systems to drive high-throughput multicellular cancer systems biology.

## Christopher McFarland (Stanford University)

### *Traversing the fitness landscape of lung adenocarcinoma in vivo using tumor barcoding and CRISPR/Cas9-mediated genome editing*

Christopher D McFarland, Zoë N Rogers, Ian P Winters, Wen-Yang Lin, Dmitri A Petrov, and Monte M Winslow

The evolution of somatic cells into cancer is a rare event. To interrogate the evolutionary outcomes of early-stage tumors within their native environment, we combined tumor barcoding with lenti-Cre and CRISPR/Cas9-based mouse models of lung adenocarcinoma[1]. This technology allows us to precisely track the size of hundreds of tumors of programmable genotype within a single mouse. Strikingly, tumors initiated with the same genetic drivers, at the same time, within the same mouse vary in size by >1,000-fold after only ten weeks of tumor growth. Furthermore, different cancer genotypes exhibit categorically-different tumor size distributions. We propose two simple Markov models of tumor evolution to explain these different size distributions, whereby heavy-tailed tumor size distributions arise when a second transformative event is necessary for advanced tumor size. We then tested these two models by tracking the growth of hundreds of tumor growth trajectories over time using our quantitative barcoding approach in twelve different cancer genotypes. Our findings and model indicate that the likelihood of cancer depends on the variability in growth imparted by driver events, more than their mean effect, as malignancy represent an exceedingly-rare, exceptionally-advanced evolutionary state.

[1]Rogers, McFarland, Winters et al (2017). A quantitative and multiplexed approach to uncover the fitness landscape of tumor suppression in vivo. Nature Methods, 14:737-42.

## B. Bishal Paudel (Vanderbilt University)

### *Drug response epigenetic landscape of BRAF-mutated melanoma cells*

B. Bishal Paudel, Leonard A. Harris, Keisha N. Hardeman, Arwa A. Abugable, Corey E. Hayford, Darren R. Tyson, Christian T. Meyer, Joshua P. Fessel, and Vito Quaranta

Post-resistant tumor analyses or static measurements provide most of our current knowledge of tumor recurrence. Unfortunately, these studies neither elucidate the critical events that precede resistance, nor explain the dynamics of response in drug-treated cancer cells. Filling this knowledge gap may engender key advances in treatment of cancer. Therefore, we coupled single-cell time-lapse microscopy, bioinformatics, and mathematical modeling to study dynamics of drug response in BRAF-mutated melanoma cells. We recently reported that drug-treated cells entered a novel non-quiescent 'idling population state', in which cells continued to divide and die. We developed a mathematical model of drug-response dynamics to explain how drug-treatment may alter the 'epigenetic landscape' melanoma cells inhabit leading to a state of balanced division and death. Prior to convergence to 'idling state', BRAF-mutated melanoma cells exhibited complex short-term responses. Mathematically, the short-term response reflects an initial re-equilibration among distinct phenotypic basins, while idling constitutes the final equilibrated state. We speculate that idling populations, primed to acquire resistance, may constitute the bulk of residual disease. Thus, describing the molecular actors that define the basins in BRAF-mutated melanoma landscape, both drug-naïve and drug-induced, would, provide rational strategies to alter the landscape itself and enhance the clinical benefits. To this end, we utilized single-cell derived clones to find gene-signatures that correlate to drug-sensitivity and discovered that drug-naïve landscape could be defined with respect to the expressions of NOX5 and PGC1α. We show that altering activity and expression of both NOX5 and PGC1α modifies the initial landscape and enhances efficacy of BRAF-inhibitors. This strategy will maximize cell killing, and hence reduce the number of cells that reach the idling state. Taken together, our work provides a unifying view of how melanomas respond to BRAF-inhibition and suggest that 'targeted landscaping' provides rational strategies to suppress both an initial population and idling cells.

**Susanne Tilk (Stanford University)**

## *Effects of genome-wide mutation rates on the accumulation of deleterious mutations in cancer*

Susanne Tilk, Chris McFarland, Christina Curtis, and Dmitri Petrov

Cancer genomes exhibit far weaker signatures of negative selection than germline evolutionary processes [Weghorn 2017, Martincornera 2017]. This may be because there are reduced selective pressures on cancer genomes, or because cancers cells evolve under conditions that prevent the tumor population from weeding-out mildly deleterious passenger mutations. In asexual tumor evolution, recombination is absent and unable to purify deleterious passenger mutations from the tumor population over time. The challenge of weeding-out these mutations can be further exacerbated by elevated mutation rates that are prevalent in cancer cell populations. Consequently, the hundreds to thousands of mutational events that can accumulate during cancer progression may individually exert small effects, but collectively alter the overall dynamics of cancer progression. Here, we quantify the selective cost of deleterious passenger mutations, functionally characterize their effect in cancer, and interrogate differences in the accumulation patterns between fixed (clonal) and segregating (sub-clonal) passengers and drivers. We use a highly-conservative permutation-based null model of protein-coding substitutions to quantify the direction and magnitude of selection in cancer genomes by calculating the normalized ratio of non-synonymous to synonymous substitutions (dN/dS). We apply this method to 10,184 tumors across 33 cancer types and find that the mutation rate of tumors primarily dictates the threshold for moderately deleterious passenger accumulation. Furthermore, the accumulation rate of driver mutations depends heavily on overall mutation burden, suggesting that these mutations either contribute diminishing

benefits to tumor evolution, or that genetic linkage prevents drivers from accumulating at elevated mutations. Selective pressures are further reduced in sub-clonal drivers and passengers, across mutation rates, consistent with prior observations and with models of tumor progression where selection is reduced at later stages of progression. These findings suggest that elevated linkage effects may prevent selection from removing deleterious passengers or selecting for beneficial drivers in cancers with high mutation rates.

## 1:45 – 3:25 pm: Microenvironment and Metastasis

Chaired by Sara Gosline (Sage Bionetworks)

### Andrew Ewald (Johns Hopkins University)

### *Cellular and molecular mechanisms of epithelial metastasis*

The majority of cancer mortality is attributable to metastasis, the process by which cells escape from the primary tumor, access the systemic circulation, and colonize distant organs. Our work in normal development revealed that mammary epithelial cells have a high capacity for migratory behavior and can readily be induced to disseminate out of the epithelium through changes to either the microenvironment or the signaling state of the cell, without need for mutations. We next demonstrated that metastasis can be accomplished by cancer cells that retain an epithelial phenotype while transitioning between distinct phenotypic states specialized for either proliferation or migration. Our recent publications demonstrated that proliferative breast cancer cells acquire migratory and invasive potential through the expression of basal genes, such as keratin 14 (K14) and p63. This transition occurs specifically at the tumor stroma border. These K14+ cancer cells collectively invade and intravasate as adherent groups of cells, through microenvironments defined by aligned, fibrillar collagen I. Upon arrival at the distant site, these predominantly K14+ clusters transition to predominantly K14- growing metastases. We have developed 3D culture models of invasion past the myoepithelium, intravasation, and metastatic colony formation. We are currently exploiting these models to define the molecular drivers of transitions between proliferative and migratory epithelial states. We are also working on defining the role of epithelial cell adhesion programs in collective strategies for metastasis, on the role of the myoepithelium in restraining invasion, and on the mechanisms by which clusters of cancer cells gain access to the venous circulation. Our ultimate goal is to develop novel concepts for anti-metastatic therapies.

### Hunter Boyce (Stanford University)

### *Spatial analysis of multiplex immunohistochemistry data enables systems analysis of hypoxia and improved stratification of lung cancer patient outcomes*

Hunter B Boyce, Yunxia Sui, Jin Xia, Elizabeth McDonough, Alberto Santamaria-Pang, Anup Sood, Fiona Ginty, and Parag Mallick

A major challenge in early detection is not just knowing that disease is present, but also predicting the tumor's likely trajectory. Recent evidence has suggested that hypoxia, which occurs in a majority of cancers, may be a driver of aggressivity and poor outcomes. Understanding the complex interactions intrinsic to hypoxia is critical for understanding what molecular features drive tumor progression and stratifying patient risk for early stages of cancer. Here, we used a multiplexed immunohistochemistry

(IHC) platform to measure the single-cell expression of 29 proteins in a tissue microarray (TMA) of 252 formalin-fixed paraffin-embedded (FFPE) non-small cell lung carcinoma (NSCLC) samples. These data establish NSCLC histology-specific molecular subtypes that dominate tumor samples from patients with poor prognosis. Utilizing a density-based spatial clustering approach, we identified and characterized contiguous regions of hypoxia in tumor tissues. Hypoxic stress in the tissue results in a concurrent average increase of protein expression and decrease in protein pair correlations compared to normoxic tissue. Spatially derived features better predicted 5-year survival (AUC = 0.72) compared to non-spatially derived features (AUC = 0.49) in a held-out test set. Additionally, the total area of defined hypoxic regions was better able to separate the patient population in a Kaplan-Meier survival analysis (p=0.024) compared to standard percent positivity measures (p=0.268). Our results highlight the utility of highly multiplexed spatial analysis in understanding complex cancer phenotypes and demonstrate the clinical significance of the spatial organization of phenotypic cancer drivers.

## Aedin Culhane (Dana-Farber Cancer Institute)

### *Extracting the latent signals of tumor seed/soil using matrix factorization approaches*

Azfar Basunia and Aedin Culhane

Over 100 years ago, Stephen Paget's proposed the 'seed and soil' hypothesis, that the distribution of cancers metastasis are not coincidental. He proposed that the location tumor metastatic sites are the product of favorable interactions between metastasizing tumor cells (the 'seed') and the metastatic microenvironment (the 'soil'). With growing amounts of large scale gene expression studies of normal tissue (Gtex), primary tumors (TCGA) and metastases (MET500), we developed a study to extract signatures of tumor seed and soil. Tensor matrix factorization is well suited to discovering latent molecular features in complex molecular data (Meng et al. 2014, 2016) and we have applied it to extracting immune signatures of tumors (Thorsson et al. 2018). Whilst most matrix factorization approaches that couple multiple datasets extract the features with common structure either by maximizing the correlation (canonical correlation analysis) or the covariance (multiple coinertai analysis) (Meng et al. 2016, 2014), approaches that extract the contrasting variance between datasets have also been described (Abid et al. 2018). We apply integrated data tensor matrix factorization to the genes and pathway that are signatures of the primary tissue, primary tumor and metastatic sites. We apply this to extracting signatures of tumors that metastasize to bone, liver and lung.

References: Abid et al. 2018. Nature Communications 9 (1): 2134; Meng et al.. 2014. BMC Bioinformatics 15 (May): 162; Meng et al. 2016. Briefings in Bioinformatics 17 (4): 628-41; Thorsson et al. 2018. Immunity 48 (4): 812-30.e14.

## Andrew Gentles (Stanford University)

### *Pan-cancer analysis of time-to-distant metastasis in the context of node-positive and node-negative disease*

Almudena Espin Perez, Alborz Bejnood, Sophia L Sanchez, and Andrew J Gentles

Significant evidence exists that in some cancers, systemic permissiveness for metastasis of malignant cells from primary tumors to distant sites is mediated by interactions occurring in lymph nodes. We conducted a pan-cancer analysis of the association between gene expression levels and time-to-distant

metastasis (DMFS), comparing these to associations with overall survival (OS). Expression of MHC genes (particularly class II) showed favorable associations for time-to-metastasis as well as overall survival, with higher expression portending longer survival. However, a significant number of genes showed stronger association with DMFS than with OS, despite the fact that these would be expected to be strongly correlated given the clinical fact that metastasis is a strong driver of death from cancer. By performing these analyses separately in node-positive and node-negative disease, we further identified genes that were differentially related to DMFS. We further explored the influence of specific cell types on DMFS by applying the CIBERSORT algorithm to deconvolve bulk expression profiles. We found that specific immune populations were associated positively or negatively with time to metastasis, based on a previously validated signature matrix of 22 cell types. Notably, modulation of EMT-related genes, and changes in expression of immunosuppressive checkpoint pathways reflected earlier metastasis. Leveraging RNA-seq data generated on flow-sorted populations in head and neck squamous carcinomas, and melanoma, we also identified specific gene expression programs in fibroblasts that influenced DMFS. Overall, our results provide a map of the relationship between gene expression and cancer metastasis, in the context of lymph node involvement.

**Kevin Chen (University of California, San Diego)**

## *ECM-driven stress leads to cancer cell transdifferentiation and collective migration*

Daniel Ortiz, Brian Tsui, Sural K. Ranamukhaarachchi, Rishi N. Modi, Aditya Kumar, Tyler Goshia, Anthony Han, Adam J. Engler, Hannah Carter, and Stephanie I. Fraley

Cancer cells interact with a complex 3D extracellular matrix microenvironment. It is widely recognized that these interactions influence cellular phenotypes and that certain collagen architecture signatures are prognostic markers for metastatic disease. However, the mechanisms often remain unclear. The complexities of these interactions suggest that the integration of quantitative measurement techniques could lend a deeper understanding. Here, we integrate matrix engineering strategies with biophysical and biochemical analysis of cell response to study how ECM structure influences cancer cell migration. This systems approach revealed that ECM architecture modulates matrix degradability and thereby cell adhesion. Cells unable to degrade the matrix cannot stabilize adhesions or generate traction. As such, they also display several hallmarks of anchorage independent growth, including oxidative stress. We identified this state of stress as a trigger for a transition to collective migration and multicellular structure formation. Furthermore, we show that the transition to collective migration is preceded by two events: i) A motility switch from a persistent random walk to a highly persistent polarized migration and ii) Upregulation of a transcriptional module enriched for migration, vasculogenesis-associated genes and NOTCH signaling. Importantly, we show that this response is not mediated by hypoxia, stiffness, or bulk matrix density, but rather by matrix architecture-induced low adhesion and feedback through β1-integrin. At a translational level, we show that the identified migration and morphogenesis associated gene expression signatures are linked to a highly aggressive clinical phenotype known as vasculogenic mimicry (VM) and can predict survival in patient data across nine distinct tumor types. Using a systems approach to study cell-ECM interactions, we link a collagen architecture-induced cellular stress to the development of aggressive migration phenotype and morphogenesis program, which may represent a conserved metastatic response and lead to novel therapeutic targets.

## 3:45 – 5:25 pm: Translational Systems Biology

Chaired by Ayesha Shajahan-Haq (Georgetown University)

### Robert Gatenby (Moffitt Cancer Center)

### *Evolutionary dynamics in cancer therapy*

A number of successful systemic therapies are available for treatment of disseminated cancers. However, tumor response to these treatments is almost invariably transient and therapy fails due to emergence of resistant populations. The latter reflects the temporal and spatial heterogeneity of the tumor microenvironment as well as the evolutionary capacity of cancer phenotypes to adapt to therapeutic perturbations. Interestingly, although cancers are highly dynamic systems, cancer therapy is typically administered according to a fixed, linear protocol. Treatment is changed only when the tumor progresses but successful tumor adaptation begins immediately upon administration of the first dose. Applying evolutionary models to cancer therapy demonstrate the potential advantage of using more dynamic, strategic approaches that focus not just on the initial cytotoxic effects of treatment but also on the evolved mechanisms of cancer cell resistance and the associated phenotypic costs. The goal of evolutionary therapy is to prevent or exploit emergence of adaptive tumor strategies. Examples of this approach include adaptive therapy and double bind therapy. The former continuously alters therapy to maintain a stable tumor volume using a persistent population of therapy-sensitive cells to suppress proliferation of resistant phenotypes. The latter uses the cytotoxic effects of an initial therapy to promote phenotypic adaptations that are then exploited using follow-on treatment. In pre-clinical models, application of adaptive therapy permits indefinite tumor control with a single cytotoxic drug. Clinical results from studies using adaptive therapy and double bind therapy will be presented.

### Amy Brock (University of Texas at Austin)

### *Lineage-resolved analysis of chemoresistance strategies and clonal dynamics*

Benedict Anchang, Alborz Bejnood, Kara L. Davis, and Sylvia K. Plevritis

Heterogeneity across individual cancer cells and their descendants (clonally-derived lineages) impacts growth rate, tumor composition, and response to therapy. To improve treatment, new tools are required to measure and control the contributions of diverse cell subpopulations. Recent studies have demonstrated the utility of DNA-barcodes (consisting of random, unique, heritable sequences) in monitoring heterogeneous cell populations. Sequencing a barcode ensemble reveals clonal dynamics that may change with progression or treatment. Beyond observing and quantifying the lineage dynamics of a population, here we set out to manipulate specific cell lineages within the context of the whole cell population. To address this challenge, we developed a platform technology, Control of Lineages by Barcode Enabled Recombinant Transcription (COLBERT). We demonstrate that by tagging cells with a library of constitutively-expressed barcode guide-RNAs, this approach allows us to: 1) identify and quantify changes in the ensemble of lineages, 2) perform lineage-resolved genome and transcriptome analyses (scRNA-Seq), and 3) readily extract one or more specific lineages from the population by lineage-specific gene activation and FACS (using the expressed barcode gRNA with dCas9-VPR to drive expression of a fluorescent marker). Here we utilize the platform to quantify and isolate chemoresistant lineages from multiple cancer cell lines and from populations of primary patient derived cells. Using

COLBERT to analyze a CLL cell line with del(13q), we were able to define the trajectories of specific resistant lineages after treatment with first-line chemotherapy, fludarabine/mafosfamide, and second-line targeted BCL2 inhibitor, venetoclax. We report on clonal fitness dynamics and lineage-resolved transcriptome/genome signatures of cells that failed to respond to one or both of these treatments, and compare these responses across multiple evolutionary trajectories. COLBERT provides a means by which we can integrate diverse layers of genomic and functional data to study cancer cells on a lineage-by-lineage basis over the course of treatment.

## Sagar Chhangawala (Memorial Sloan Kettering Cancer Center)

### *Chromatin accessibility maps of Recurrence in Pancreatic Cancer*

Chhangawala S, Dhara S, Askan G, Zhang L, Makohon-Moore A, Sinha S, Glassman D, Yu K, Iacobuzio-Donahue C, Moffitt R, Chandwani R, Balachandran V, Leslie CS, and Leach SD

Pancreatic cancer is expected to become the 2nd deadliest cancer by 2020, and few therapeutic options are currently available. Additionally, 50% of pancreatic cancer patients recur within just one year. This difference in recurrence is still unexplored. Previous genomic analyses of pancreatic tumors, including somatic mutation mapping and gene expression profiling, have revealed genetic and transcriptomic heterogeneity. However, the source of this heterogeneity is unclear. We hypothesized that epigenetic heterogeneity underlies previously described difference in recurrence and can be dissected by mapping the chromatin accessibility landscape in pancreatic cancer cells. In collaboration with the lab of Dr. Steven Leach, we sorted fresh patient tumor samples based on EpCAM (an epithelial cell marker) to enrich for tumor cells and subjected them to ATAC-seq. ATAC-seq is a sequencing method that maps regions of open chromatin and enables the computational analysis transcription factor (TF) binding at chromatin accessible sites. After optimizing the ATAC-seq analysis pipeline for improved peak calling in fresh human tumor samples, we assembled a patient cohort of 54 samples; each used to generate replicate ATAC-seq libraries. We used Irreproducible discovery rate (IDR) framework to find reproducible peaks in same-patient replicates and created a peak atlas across all patients. Using supervised learning and generalized linear modeling, we were able to characterize the changes in RNA-seq and ATAC-seq between recurrent vs non-recurrent patients. We characterized TF motifs in the atlas peaks and used ridge regression to identify differential TF activity enriched in recurrent patients. Two TF hits, ZSCAN1 and HNF1b, were experimentally validated to predict recurrence in our cohort and an independent cohort. These results reveal novel regulatory programs in recurrent patients of pancreatic cancer and support the development of individualized therapies.

## Luciane Kagohara (John Hopkins University)

### *Integrated time course omics analysis distinguishes immediate therapeutic response from acquired resistance*

Genevieve Stein-O'Brien, Luciane T Kagohara, Sijia Li, Manjusha Thakar, Ruchira Ranaweera, Hiroyuki Ozawa, Haixia Cheng, Michael Considine, Sandra Schmitz, Alexander V Favorov, Ludmila V Danilova, Joseph A Califano, Evgeny Izumchenko, Daria A Gaykalova, Christine H Chung, and Elana J Fertig

Background: Targeted therapies specifically act by blocking the activity of proteins that are encoded by genes critical for tumorigenesis. However, most cancers acquire resistance and long-term disease remission is rarely observed. Understanding the time course of molecular changes responsible for the development of acquired resistance could enable optimization of patients' treatment options. Clinically,

acquired therapeutic resistance can only be studied at a single time point in resistant tumors. To determine the dynamics of these molecular changes, we obtained high throughput omics data weekly during the development of cetuximab resistance in a head and neck cancer in vitro model. Results: An unsupervised algorithm, CoGAPS, was used to quantify the evolving transcriptional and epigenetic changes. Applying a PatternMarker statistic to the results from CoGAPS enabled novel heatmap-based visualization of the dynamics in these time course omics data. We demonstrate that transcriptional changes result from immediate therapeutic response or resistance, whereas epigenetic alterations only occur with resistance. Integrated analysis demonstrates delayed onset of changes in DNA methylation relative to transcription, suggesting that resistance is stabilized epigenetically. Conclusions: Genes with epigenetic alterations associated with resistance that have concordant expression changes are hypothesized to stabilize resistance. These genes include FGFR1, which was associated with EGFR inhibitor resistance previously. Thus, integrated omics analysis distinguishes the timing of molecular drivers of resistance. Our findings provide a relevant towards better understanding of the time course progression of changes resulting in acquired resistance to targeted therapies. This is an important contribution to the development of alternative treatment strategies that would introduce new drugs before the resistant phenotype develops.

## Jason Sheltzer (Cold Spring Harbor Labs)

### *Genetic determinants of cancer patient outcome*

Joan C. Smith and Jason M. Sheltzer

Successful treatment decisions in cancer depend on the accurate assessment of patient risk. To improve our understanding of the molecular alterations that underlie deadly malignancies, we collected and analyzed the genomic profiles of 35,946 solid tumors from patients with known outcomes. To our knowledge, this is the largest such study that has ever been conducted, and the first study to perform a pan-cancer, exome-wide analysis of prognostic mutations, copy number alterations, CpG methylation sites, and more. Our analysis finds that mutations in cancer driver genes are almost never associated with patient survival time. At the same time, copy number changes in these same genes exhibit significant prognostic power, independent of their mutation status. Focal copy number alterations are associated with worse outcomes than broad alterations, and driver gene CNAs remain strongly prognostic when controlling for tumor stage, grade, TP53 status, and total tumor aneuploidy. Analysis of methylation, microRNA, mRNA, and protein expression in primary tumors define several additional prognostic patterns, including signatures of tumor mitotic activity and tissue de-differentiation, that remain strongly associated with patient outcome after correcting for common clinical parameters. By combining data across independent patient cohorts, we further identify robust genetic biomarkers of patient outcome, and demonstrate that a subset of these alterations also confer specific therapeutic vulnerabilities. In total, our analysis establishes a comprehensive resource for biomarker identification, underscores the importance of gene copy number profiling in assessing clinical risk, and provides a precision-medicine approach to treating cancer patients with the worst likely outcomes.

# Friday, November 9

## 9:00 – 10:40 am: Systems Immunology & Immunotherapy

Chaired by Kathleen Wilkie (Ryerson University)

### Ilya Shmulevich (Institute for Systems Biology)

***The immune landscape of cancer***

I will present the results of an extensive immunogenomic analysis of more than 10,000 tumors, comprising 33 diverse cancer types by utilizing data compiled by The Cancer Genome Atlas. The analysis identified six immune subtypes that encompass multiple cancer types and are hypothesized to define immune response patterns impacting prognosis. Across these immune subtypes, we identified and characterized multiple control modalities of the intracellular and extracellular networks (transcription, microRNAs, copy number, and epigenetic processes) that are involved in tumor-immune cell interactions. I will describe the companion web portal, CRI iAtlas, which allows for interactive exploration of the analyses and data and serves as a resource for future targeted studies to further advance the immuno-oncology field. I will also briefly describe the ISB Cancer Genomics Cloud (ISB-CGC) resource and how it was used to perform very large scale immunogenomic analyses.

### Philipp Altrock (Moffitt Cancer Center)

***Evolutionary dynamics of non-Hodgkin's lymphoma CAR T cell therapy***

Non-Hodgkin Lymphoma (NHL) is the most common hematologic malignancy in the United States with an estimated 72,000 new cases (4.3% of all cancer cases) and 20,000 deaths (3.4% of all cancer deaths) in 2017; the median 5-year survival rate is 71%. Despite a possible cure, with front-line chemotherapy, there exist patients that do not response or relapse and develop refractory disease. These patients have a median overall survival of less than seven months. Chimeric antigen receptor (CAR) T-cell therapy for refractory NHL relies on expansion of engineered T- cells that specifically target tumor cells expressing CD19. Here we combine mathematical modeling with statistical data-analysis based on recent results of clinical studies of CAR T-cell dynamics in individual patients. We use statistical and mathematical modeling to elucidate the key mechanisms that drive evolutionary dynamics of anti-CD19 CAR T-cell therapy. To this end, we integrate patient specific tumor burden profiles, inflammatory cytokine profiles, and CAR T cell population dynamics into our model. We find that the success of therapy may depend on dynamic regulation of inflammatory cytokines in the tumor microenvironment, as well as on specific kinetic properties of the heterogeneous CAR T-cell population. Relative abundances of juvenile and effector T cells can be key factors that drive the duration of treatment response, and the tumor-killing rate of this CD19-specific immunotherapy. Our modeling framework elucidates disease and treatment specific coevolution of T cells and tumor, and can quantify the impact of between patient variability on the levels of tumor burden, tumor growth rate prior to treatment, and therapy related inflammatory response.

## Jennifer Oyler-Yaniv (University of California, Los Angeles)

### *Catch and release of cytokines mediated by tumor phosphatidylserine converts transient exposure into long-lived inflammation*

Jennifer Oyler-Yaniv, Alon Oyler-Yaniv, Mojdeh Shakiba, Nina K. Min, Ying-Han Chen, Sheue yann Cheng, Oleg Krichevsky, Nihal Altan-Bonnet, and Grégoire Altan-Bonnet

Immune cells constantly survey the host for pathogens or tumors and secrete cytokines to alert surrounding cells of these threats. In vivo, activated immune cells secrete cytokines for several hours, yet an acute immune reaction unfolds over days. Given these divergent timescales, we addressed how cytokine-responsive cells translate brief cytokine exposure into phenotypic changes that persist over long timescales. We studied melanoma cell responses to transient exposure to the cytokine Interferon γ (IFNγ) by combining a systems-scale analysis of gene expression dynamics with mathematical modeling and experiments. We discovered that IFNγ is captured by phosphatidylserine (PS) on the surface of viable cancer cells both in vitro and in vivo, and then slowly released to drive long-term transcription of cytokine-response genes. In addition, PS-mediated capture and release was relevant to a select group of inflammatory cytokines aside from IFNγ. This mechanism introduces an additional function for PS in dynamically regulating inflammation across diverse cancer and primary cell types and has potential to usher in new immunotherapies targeting PS and inflammatory pathways.

## Nathan Reticker-Flynn (Stanford University)

### *Lymph node colonization promotes distant tumor metastasis through the induction of systemic immune tolerance*

Nathan E. Reticker-Flynn, Maria M. Martins, Pamela A. Basto, Weiruo Zhang, Alborz Bejnood, Justin A. Kenkel, Andrew Gentles, Sylvia K. Plevritis, and Edgar G. Engleman

Metastasis is the primary cause of cancer-associated deaths, yet the mechanisms underlying this phase of the disease remain poorly understood. For most cancers, metastasis to distant organs is typically preceded by spread to regional and distant lymph nodes (LNs). While LNs represent sites of drainage from tissues and tumors, they are interaction hubs of the adaptive immune system and are composed of large quantities of potentially tumor-reactive lymphocytes. In this study, we use mouse models and systems approaches to reveal that LN metastasis represents an enabling step in the formation of distant organ metastases, resulting from the capacity of LN tumors to induce systemic tumor immune tolerance. Through serial in vivo passaging, we have generated a panel of nearly 300 unique cell lines exhibiting varying degrees of LN-metastatic potential. Transcriptional and epigenetic profiling of the lines reveals a pattern of immune-related alterations conferred by constitutive activation of an interferon signaling axis, which is conserved in the human disease. Using CRISPR/Cas-9, we demonstrate that the identified gene networks are both necessary and sufficient for the enhanced metastatic seeding of LNs. We show that the LN metastatic populations are capable of facilitating enhanced distant seeding of tumors in the lungs. Organism-wide immunophenotyping by mass cytometry reveals broad systemic changes in the immune repertoire of mice bearing the LN tumors. These LN metastases are capable of both evading NK cells and inducing regulatory T cells. Egress of tolerized lymphocytes from involved LNs results in systemic immune tolerance, and blockade of this egress prevents the formation of distant metastases. Finally, we investigate the evolution of these metastatic traits through the use of single-cell sequencing, fluorescent protein multiplexing, and DNA barcoding and establish clonal relationships between metastases. These findings demonstrate a critical role for LN metastasis in promoting tumor immune tolerance.

## Siranush Sarkizova (Harvard University/Broad Institute)

### *More accurate prediction of epitope presentation in tumors for cancer vaccines based on large datasets of HLA-associated epitopes*

Siranush Sarkizova, Susan Klaeger, Derin B Keskin, Karl R Clauser, Hasmik Keshishian, Christina R Hartigan, Nir Hacohen, Catherine J Wu, and Steven A Carr

Cancer vaccine therapies rely on accurate selection of immunizing peptides to potentiate tumor-specific immune responses against neoepitopes. Given the unique accumulation of mutations in each tumor as well as the patient's particular complement of HLA alleles, the ability to predict which epitopes will be presented by the tumor is a fundamental prerequisite for successful vaccine design. We recently showed that prediction of endogenous antigen presentation is greatly improved when models utilize single HLA allele ligandome datasets and integrate intracellular processes such as proteasomal processing and gene expression. We now extend our approach to many more HLA alleles, address limitations in model performance and test the utility of these approaches in predicting antigen presentation in primary tumors. We utilized a single-allele method to profile naturally presented peptides on HLA molecules via mass spectrometry to collect endogenous ligandome data for 92 HLA- A, B, C and G alleles, identifying >190,000 peptides. In addition, endogenously presented antigens on primary tumor-derived cell lines from 4 melanoma patients were also identified by MS. To extract knowledge from this vast dataset, we created analytic and predictive computational tools. Furthermore, we evaluated the utility of the models to predict HLA ligands on primary tumors and learn tumor-specific presentation biases. Based on our observations that certain alleles present non-9-mer peptides with high frequency, we built length-specific models that often outperform length-unspecific predictors currently used. By clustering allele-specific peptides into sub-motifs, we derived an approach to delineate allele similarity at finer granularity. Notably, deconvolution of tumor-presented peptides revealed a skewed distribution of allele utilization and showed that ~10% of peptides are presented on HLA-C, which has been historically understudied. We show that our allele-specific neural network models are better at discriminating tumor-presented epitopes than state-of-the-art algorithms and develop an approach to dissect the basis for differences in prediction accuracy.

# 1:45 – 3:25 pm: Systems Pharmacology

Chaired by Stacey Finley (University of Southern California)

## Saroja Ramanujan (Genentech)

### *Mechanistic systems modeling in oncology drug development - from pathway inhibitors to cancer immunotherapy*

Genentech is actively applying quantitative systems pharmacology (QSP) modeling approaches to support the development of various anti-cancer agents with diverse mechanisms of action, from signaling pathway inhibition to immune system stimulation. In an example of the application of systems modeling in the clinical development of a signaling pathway inhibitor, we developed a mechanistic computational model to link EGFR activation, MAPK signaling, and tumor growth data based on in vitro and in vivo studies of EGFR, BRAF, MEK, and ERK inhibitors and limited clinical data on Phase I combination therapy trials of the former three agents in BRAF V600E -mutant colorectal cancer. Using this model, we

were able to accurately forecast the clinical response to the concurrent Phase I trial of an ERK inhibitor and to assess the likely response to combination MEKi + ERKi, including the effect of different dose/regimens. In the cancer immunotherapy space, we have used systems modeling to improve translation and early clinical trial design of an anti-CD20/CD3 T-cell dependent bispecific (TDB) antibody in Non-Hodgkins Lymphoma (NHL). We developed a quantitative systems model of the dynamics and interactions of B- and T-lymphocytes in circulation, lymphoid tissues, and B-cell tumor, incorporating the effects of anti-CD20/CD3 TDB on these mechanisms. The model was developed using in vitro potency data and PKPD data from cyno studies. A "humanized, tumor-bearing" version of the model was then used to predict the effect of alternate dose regimens of the TDB on T and B cell dynamics, T cell activation, systemic cytokine (IL6) levels, and tumor regression in "virtual" NHL patients. Results supported a modified, fractionated step-up Phase Ib dose-regimen, which successfully improved tolerability and enabled the administration of higher, more efficacious doses. This model is being further developed and modified to investigate questions such as optimal dose, combination therapies, and biomarkers of response. These examples illustrate the power of mechanistic systems modeling in development of cancer drugs, both in more traditional cancer-cell targeted therapies, but also in the growing area of cancer immunotherapy.

**Arvind Singh Mer**

**(Princess Margaret Cancer Centre/University of Toronto)**

## *Systematic assessment of genomic biomarkers for drug sensitivity prediction in patient derived xenografts*

A. S. Mer, B. Brew, J. Ortmann, D. Cescon, A. Goldenberg, and B. Haibe-Kains

The key challenge in cancer precision medicine consists in finding robust biomarkers for drug response prediction. Patient-derived tumor xenografts (PDXs) have emerged as reliable preclinical models, since they better recapitulate tumor response to chemo- and targeted therapies. However, the published molecular and pharmacological profiles of PDXs lack standards and are scattered throughout. Efficient storage, access and analysis is key to the realization of the full potential of in vivo pharmacogenomic data. To address this, we have developed Xeva (XEnograft Visualization & Analysis), an open-source software package for processing, visualization and integrative analysis of a compendium of in vivo pharmacogenomic datasets. The Xeva platform follows PDX minimum information (PDX-MI) standards and can handle both replicate-based and 1x1x1 experimental designs. We used Xeva to characterize the variability of gene expression and pathway activity across passages. We found that the vast majority of the genes and pathways were consistent across passages (median intraclass correlation=0.53 for genes and positive enrichment score for 97% pathways); however, the activity of the regulation of DNA templated transcription elongation, lysine trimethylation and histone H2A acetylation pathways pathways were strongly affected by model passaging (gene set enrichment analysis false discovery rate [FDR] <0.05). We then leveraged our platform to link the drug response and the pathways whose activity is consistent across passages by mining the large Novartis PDX Encyclopedia (PDXE) data containing 1,075 PDXs spanning 5 tissue type and 62 anticancer drugs. We identified 1035 pathways significantly associated with response to 34 drugs (FDR < 0.10), including known predictive associations such as the PI3K pathway activity with binimetinib, and ERBB signaling pathway with tamoxifen. Among the significant pathway-drug associations, we found novel biomarkers based on gene expressions, copy number variations (CNV) and mutations which can robustly predicts drug response. Xeva provides a flexible platform for integrative analysis of preclinical in vivo pharmacogenomics data to identify robust biomarker predictive of drug response, a major step toward precision oncology.

## Beril Tutuncuoglu (University of California, San Francisco)

### *CRISPR-Cas9 based platform reveals PARP-inhibitor hypersensitivity of cells deficient in novel BRCA1-interactors*

Beril Tutuncuoglu, Minkyu Kim, and Nevan Krogan

Determination of the increased sensitivity of BRCA1-deficient cells to poly (ADP-ribose) polymerase (PARP) inhibitors resulted in development of effective and targeted cancer therapies. Approval of PARP inhibitors as mono- or combination- therapies for breast and ovarian cancers demonstrate the importance of identifying specific genes and pathways that mediate pathway alterations in cancer cells. With about 450 proteins participating in double-stranded damage repair (DDR) pathway, it is expected that some of the synergistic interactions between DDR genes are yet to be discovered. Our lab conducted a comprehensive affinity purification mass spectrometry (AP-MS) study using about 20 genes, including kinases, tumor suppressors, and DNA repair proteins that are involved in breast cancer progression, as baits in three different cell lines. This curated large scale interactome data provided a list of genes that are likely to be involved in DSB repair and offered an advantage to conduct a targeted CRISPR-Cas9 screen to test genetic interactions upon PARP1 inhibition. In this study, we used arrayed CRISPR-Cas9 gene editing by nucleofection platform to knockout 92 BRCA1-interacting genes and screened for cell growth in olaparib and cisplatin treatment conditions. Besides the known HRR factors, we identified four new factors (GPS1, UBE2N, RAB3GAP, THOC1) whose absence resulted in increased olaparib or cisplatin sensitivity. Interestingly, the data points to knockouts of a few genes that respond differently to olaparib and cisplatin treatments. These results indicate an important role for neddylation in DDR pathway, mechanistic details of which are currently under investigation. These results point to the importance of testing genes with different classes of chemotherapy drugs for discovery of new therapeutic targets and for increasing the number of patients that could benefit from already FDA-approved agents.

## David Wooten (Vanderbilt University)

### *Quantifying drug combination synergy along axes of potency and efficacy*

David Wooten, Christian Meyer, Bishal Paudel, Leonard Harris, Darren Tyson, Carlos Lopez, and Vito Quaranta

Two goals motivate treating diseases with drug combinations: reduce off-target toxicity by minimizing doses (synergistic potency), and improve outcomes by escalating effect (synergistic efficacy). Surprisingly, established drug synergy frameworks obscure such distinction, failing to harness the full potential of modern chemical libraries. We therefore developed Multidimensional Synergy of Combinations (MuSyC), a formalism based on generalized, multidimensional Hill-kinetics with parameters that decouple synergistic potency and efficacy. In mutant-EGFR driven lung cancer, MuSyC provides the insight that combining a mutant-EGFR inhibitor with inhibitors of other kinases may only result in synergistic potency, whereas synergistic efficacy can be achieved by co-targeting epigenetic regulation or microtubule polymerization. In mutant-BRAF melanoma, MuSyC validates a synergistically efficacious combination identified by differential expression analysis. Additionally, we show that the dominant alternative synergy metrics, Loewe Additivity, Combination Index, and Bliss Independence emerge as special cases of MuSyC, and we highlight cases where these alternative metrics cannot be applied or lead to biased results. These findings showcase MuSyC's potential to transform the enterprise

of drug-combination screens by precisely guiding translation of combinations towards dose reduction, improved efficacy, or both.

## Jorge G.T. Zanudo
## (Pennsylvania State University/Dana-Farber Cancer Institute)
### *Network modeling of drug resistance mechanisms and drug combinations in breast cancer*

Jorge G. T. Zanudo, Reka Albert, and the SU2C-The V Foundation-NSF Drug Combinations Convergence Team

Durable control of invasive solid tumors is thwarted by the lack of knowledge of effective drug combinations and of the acquired and intrinsic resistance mechanisms of drugs. In an effort to tackle this problem, the SU2C-NSF-TVF Drug Combination Convergence Team is using mechanistic models of cancer cell signaling based on therapeutic and cell line data in order to identify elements within cancer cells that might eventually be exploited through therapeutic combinations. Here we present a comprehensive mechanistic network model of signal transduction in ER+ PIK3CA-mutant breast cancer. Focusing on PI3K inhibitors, the model recapitulates known resistance mechanisms and predicts other possibilities for resistance: loss of RB1, FOXO3, P27, or PRAS40. To test these predictions, we analyzed genome-wide CRISPR screens of two breast cell lines in the presence of PI3K inhibitors and found that the predicted genes were significantly enriched in the screens. Some of these resistance genes (e.g. loss of RB1) were found to be cell-line specific and follow-up experiments in RB1-KO cells confirmed the cell-line-specific nature of PI3K-inhibitor resistance. The model also reveals known and novel combinatorial interventions that are more effective than PI3K inhibition alone. For example, the model predicts that the combination of PI3K inhibitors with inhibitors of anti-apoptotic proteins MCL1 or BCL2 would be effective. Follow up experiments in cell lines confirmed that MCL1 inhibitors enhance the effect of PI3K inhibitors and that this combinatorial effect is cell-line-specific, similarly to what was found in the resistance genes case. In conclusion, the model predicted drug resistance mechanisms and effective drug combinations, some of which were verified experimentally and found to be cell-line-specific. Next iterations of the model will incorporate the identified discrepancies, the newly identified resistance mechanisms to drugs of clinical interest, and the results from cell death and BH3 profiling experiments in response to these drugs.

# 3:45 – 5:25 pm: Systems Biology: Bench to Bedside

Chaired by Doug Lauffenburger (Massachusetts Institute of Technology)

## Garry Nolan (Stanford University)
### *Pathology from the ~~molecular~~ atomic scale on up*

## Louis Weiner (Georgetown University)

### Employing systems biology approaches to uncover a new mechanism of resistance to antibody targeted immune attack

Dalal S. Aldeghaither[1,3], Joseph C. Murray[2], David J. Zahavi[1], Elana J. Fertig[2], Garrett T. Graham[1], Yong-Wei Zhang[1], Allison O'Connell[1], Junfeng Ma[1], Sandra A. Jablonski[1], and Louis M. Weiner[1]

1 Department of Oncology and Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC

2 Johns Hopkins University School of Medicine, Baltimore, MD.

3 King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

Antibody-dependent cell-mediated cytotoxicity (ADCC) provides a model for uncovering immune resistance mechanisms. We continuously exposed epidermal growth factor receptor (EGFR)+ A431 cells to KIR-deficient NK92-CD16V effector cells and the anti-EGFR mAb cetuximab. Persistent ADCC exposure yielded ADCC-resistant cells (ADCCR1) that, compared with control ADCC-sensitive cells (ADCCS1), exhibited reduced EGFR expression, overexpression of histone- and interferon-related genes, and failure to activate NK cells, without evidence of epithelial to mesenchymal transition. These properties gradually reversed following withdrawal of ADCC selection pressure. Remarkably, the development of resistance was strongly associated with lower expression of multiple cell surface molecules that contribute to cell:cell interactions and immune synapse formation. Classic immune checkpoints did not modulate ADCC in this unique model system of immune resistance. We show that the induction of ADCC resistance involves genetic and epigenetic changes that lead to a general loss of target cell adhesion properties required for the establishment of an immune synapse, killer cell activation, and target cell cytotoxicity. This work illustrates the importance of system biology tools to unravel cancer-related complexity.

## 7:15 – 8:45 pm: Systems Biology: Bench to Bedside

Chaired by Dan Gallahan (National Cancer Institute)

## Rosalie Sears (Oregon Health & Science University)

### *Mechanism that confer therapeutic resistance in triple-negative breast cancer*

Ellen Langer, Tyler Risom, Meghan Turnidge, Andrew Adey, Kristof Torkenczy, Joe Gray, and Rosalie Sears

Intratumoral heterogeneity resulting from cell-intrinsic and cell-extrinsic factors is a major cause of therapeutic resistance in breast cancer. Using high-content imaging, we have characterized the expression of luminal, basal, and mesenchymal markers in triple-negative, basal-like breast cancers and have shown that this subtype of breast cancer has high differentiation-state heterogeneity and that distinct classes of targeted therapeutics eliminate or enrich specific differentiation-state subpopulations resulting in increased homogeneity of drug-tolerant persister cells. We demonstrate that BET inhibitors are effective combination therapies with these state-aggregating drugs, and using single cell ATAC-seq we show that BET inhibition prevents the epigenetic changes necessary for transition to a resistant differentiation state. In addition to cell-intrinsic mechanisms that influence heterogeneity, plasticity, and

therapeutic efficacy, we are interrogating cell-extrinsic factors and stromal cell types that affect differentiation state heterogeneity and therapeutic response. For this, we are generating complex, heterotypic, scaffold-free and highly manipulable in vitro tumor tissues in which breast cancer cells are surrounded by stromal cell types including fibroblasts, endothelial cells, and mesenchymal stem cells using a 3D tissue bioprinter. As these printed tissues mature over 1-3 weeks, the cells self-organize, lay down matrix, and respond to extrinsic signals. Together with xenograft and genetically engineered mouse models, these tissues are being used to understand the effects of individual stromal cell types and cell-extrinsic factors on the differentiation state heterogeneity and therapeutic response of triple-negative breast cancer to a variety of targeted drugs.

### Victoria Seewaldt (City of Hope)

### *Systems approach to distinguish aggressive cancer vs. benign breast lesions*

The precursor lesions for biologically aggressive breast cancers are not well understood. The biologic potential of precancerous breast lesions is primarily determined by morphology (normal < hyperplasia < atypia < ductal carcinoma *in situ* (DCIS) < invasive cancer). But morphology alone, does not always account for the biological potential of a precancerous lesion; precancerous lesions are assessed at a single time point and the metastatic potential of a precancerous lesion takes second place to morphology. Here we aim to take a systems biology approach to understand temporal/spatial events that drive the transition of biologically aggressive premalignant lesions to invasive cancers. Our team integrates cutting edge technology including *in situ* single cell analysis and photoacoustic real-time imaging.

# Saturday, November 10

## 9:00 – 10:40 am: Signaling Networks in Cancer

Chaired by Brian Joughin (Massachusetts Institute of Technology)

### Ursula Klingmüller (DKFZ, German Cancer Research Center)

### *Deciphering molecular mechanisms regulating cellular decisions in the erythroid system*

Cellular signal transduction is governed by multiple feedback mechanisms to elicit robust cellular decisions. The specific contributions of individual feedback regulators, the impact of single cells heterogeneity and integration of signals with opposing effects by intracellular signaling network remain unclear. As biological model system we study the link of signal transduction and cellular decisions in erythroid progenitor cells since these cells are readily accessible and cellular decisions such as proliferation and survival depend on a single factor, the hormone erythropoietin (Epo). The key pathway connected with cell survival is Epo-induced activation of the JAK2-STAT5 signaling cascade. With a dynamic pathway model based on extensive time-resolved data sets in primary erythroid progenitor cells, we show that the two transcriptional negative feedback regulators CIS and SOCS3 divide the labor to control pathway activation. They are most effective at different ligand concentration ranges due to their distinct inhibitory mechanisms. This characteristic systems property enables the system to effectively control STAT5 responses for Epo concentrations that in vivo can vary 1000-fold. To uncover the

threshold that determines survival of erythroid progenitor cells and to identify the sources of single cell heterogeneity in STAT5 activation, we combined a population level model with mixed-effect modelling of single cells and linked the model to survival data. With this approach we showed that there is a low critical threshold amount of pSTAT5 in the nucleus of a single cell that has to be exceeded to ensure survival. As a model system to study cellular decisions upon exposure to factors with opposing effects, Epo as proliferation stimulating factor and transforming growth factor β (TGFβ) as an anti-proliferative factor, we studied dynamic responses in the factor dependent cell line BaF3 expressing the Epo-receptor (BaF3-HA-EpoR). A multi-level analysis including time-resolved proteomics data identified the glucose metabolism as key integrator of pro- and anti-proliferative stimuli. Taken together, data-based mathematical modeling provided unexpected insights into molecular mechanisms that enable the system to control responses over a broad range of ligand concentrations and integrate the impact of multiple factors.

## Eric Batchelor (National Cancer Institute, NIH)

### *Defining the network architecture coordinating double strand break repair and p53 dynamics*

Eric Batchelor, Marie Harton, and Ryan Hanson

The dynamics of the tumor suppressor p53 are an important part of the DNA damage response. The pulses of accumulation and degradation of p53 change in response to different forms of DNA damage: double strand breaks generate sustained p53 oscillations, while UV damage generates a single p53 pulse. Altering p53 dynamics changes cell fate in response to stress; however, it is not well understood how p53 dynamics affect the dynamic expression of the numerous target genes regulated by p53 to generate such changes. I will present recent work from my lab focused on systems-level analysis of p53 target gene expression dynamics and cell fate regulation. Using high-throughput gene expression profiling and single cell transcriptional analysis, we determined that p53 dynamics generate a spectrum of target expression profiles, from strongly oscillating to monotonically increasing gene expression. We determined that p53 dynamics affect not only direct p53 target genes but also the entire transcriptome due to indirect effects mediated by p53's repression of the proto-oncogene MYC. Using microfluidic delivery of a small molecule to control p53 dynamics in individual cells, we show that specific features of p53 pulses (amplitude, duration, or frequency) are decoded by p53 target promoters to generate distinct probabilities of firing and rates of activation. Finally, focusing on DNA damage repair mechanisms, we show that p53 dynamics are shaped by specific DNA repair pathways. Common cancer-associated mutations and small molecule inhibition of the pathways alter p53 dynamics and p53-dependent cell fate regulation. These studies suggest novel therapeutic strategies based on the timing of drug-delivery to generate specific p53 dynamics-mediated cell fate outcomes in cancer cells.

## Sean Gross (Oregon Health & Science University)

### *Accurate transmission of IGF-I into AKT signaling activity in individual cells*

Sean M Gross, Mark A Dane, Elmar Bucher, and Laura M Heiser

Cells sense and respond to their environment by activating distinct intracellular signaling pathways, however signal transmission in individual cells is not well understood. To assess the accuracy of signal transmission in individual cells, we developed an optimized genetically encoded sensor for IGF-I

signaling. We stably expressed this sensor in HeLa cells and used live-cell imaging to monitor dynamic responses to IGF-I in individual cells. Across the population, signaling responses overlapped between different IGF-I doses, suggesting limited transmission accuracy. However, analysis of individual cell traces revealed relatively constant responses over time. An information theoretic approach to calculate the channel capacity using variance of the single cell time course data--rather than population data--predicted that cells were capable of discriminating multiple growth factor doses. We validated these predictions by tracking individual cell responses to multiple IGF-I doses and found that cells can accurately distinguish at least four different IGF-I concentrations, and also that the input-output relation varies across the population of individual cells. Furthermore, by monitoring responses to the PI3K inhibitor alpelisib we found a similar discriminatory ability to pathway inhibition. Our studies indicate that heterogeneous responses to IGF-I arise from cells encoding the growth factor input into different signaling outputs and that individual cells can accurately sense and respond to a range of stimuli of varying strengths. These observations reveal the importance of viewing each cell as having its own communication channel and underscore the importance of understanding responses at the single cell level.

## James Joly (University of Southern California)

### *PKA-mediated glycogen catabolism promotes cancer cell resistance to glucose deprivation*

James H Joly, Nicholas A Graham

Oncogene-directed metabolic reprogramming can render cancer cells dependent on metabolic substrates including glucose for survival. As such, the vulnerability of cancer cells to glucose deprivation presents an attractive opportunity for therapeutic intervention. However, some cancer cells, even highly glycolytic ones, are resistant to glucose deprivation. The molecular mechanisms by which cancer cells evade glucose deprivation-induced cell death remain unknown. Here, using mass spectrometry-based metabolomics and phospho-proteomics, we identified cyclic-AMP levels and phosphorylation of the activation site of PKA, Thr198, to be molecular markers of survival upon glucose deprivation. Consistent with these observations, treatment of glucose-deprivation insensitive cancer cells with PKA inhibitors rendered cells sensitive to glucose deprivation, and expression of a constitutively active mutant of PKA rescued a glucose-deprivation sensitive cell. To understand the mechanism by which PKA promotes survival of cancer cells under glucose deprivation, we profiled the phospho-proteome in the presence and absence of PKA inhibitors and found phosphorylation of phosphoglucomutase-1 (PGM1), a critical regulator of glycogen catabolism, to be a marker of cancer cell resistance to glucose deprivation. Taken together, these findings demonstrate the critical role of PKA signaling to promote resistance to metabolic stress in cancer. These findings will serve as the basis for rational design of novel anti-metabolic treatment strategies co-targeting glucose restriction and glycogen catabolism.

## Manu Kumar (Massachusetts Institute of Technology)

### *Computational analysis of single-cell RNA-sequencing identifies tumor microenvironment cell-cell communication*

Manu P. Kumar, Eliot T. McKinley, Ken S. Lau, and Douglas A. Lauffenburger

Tumors are composed of multiple cell types, including malignant, immune, and stromal cells, that communicate by ligand-receptor interactions. Cell-cell communication between these cell-types plays an

important role in many aspects of tumor behavior, including tumorigenesis, tumor progression, therapeutic resistance, and immune infiltration. However, our knowledge of this complex network of cell-cell interactions in a tumor microenvironment is still incomplete. Therefore, there is a need to more systematically understand the spectrum of cell-cell interactions occurring in the tumor microenvironment. Here, we developed an approach to computationally characterize cell-cell communication mediated by ligand-receptor interactions and its effect on intracellular signaling using single-cell RNA sequencing (scRNA-seq) data. We measured scRNA-seq profiles of more than 10,000 single cells collected from APC and azoxymethane (AOM) mouse models of colorectal cancer as well as normal colon tissue. To identify cell-types present in each tumor, we first created a training data set of high-confidence cell-types that express multiple known marker genes and then trained a decision-tree classifier to predict cell-types from full scRNA-seq expression profiles. We then screened for expression of known ligand-receptor interactions using a published list of approximately 1,800 interactions to infer cell-type specific interactions (Ramilowski et al. 2015). In addition, we used a previously published approach to infer the activity of 14 different signaling pathways based on the expression levels of transcripts that are predictive of perturbations of the respective signaling pathway (Schubert et al. 2018). Finally, to connect inter-cellular signaling via ligand-receptor interactions with intra-cellular pathway activation, we correlated cell-type specific pathway activation scores with inferred ligand-receptor interactions. In this manner, we can begin to identify receptor-ligand mechanisms by which cell-types in a tumor can alter the signaling behavior of surrounding cell-types. Altogether, this work advances methodological approaches for uncovering cell-cell communication in tumor microenvironments using single-cell sequencing studies.

## 11:00 am – 11:50 pm: Closing Keynote

### Joe Gray (Oregon Health & Science University)